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NATURE

A WEEKLY JOURNAL OF SCIENCE

"To the solid ground
Of nature trusts the Mind that builds for aye"—WORDSWORTH

199

Volume 195

JULY 7, 1962, to SEPTEMBER 29, 1962

LONDON
MACMILLAN AND CO., LTD.
NEW YORK: ST. MARTIN'S PRESS, INC.

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Printed in Great Britain by Fisher Knight & Co Ltd, The Gainsborough Press, St Albans,
and published by Macmillan & Co Limited, at St Martin's Street, London, W C 2,
and St Martin's Press, Inc, 103 Park Avenue, New York 17, N Y, U S A
Saturday, December 8 1962

[illegible] Library. [illegible] GE. 92, [illegible] Road. CALCUTTA

22.8.62

PARTNERSHIP BETWEEN SCIENCE, TECHNOLOGY AND THE HUMANITIES

SIR WILLIS JACKSON'S inaugural lecture at the Imperial College of Science and Technology on the partnership between science and electrical engineering (see p. 7 of this issue) is of unusual interest, not merely because of the importance of a number of points he makes but no less because of the way it falls into the pattern of several such recent addresses or papers. Lord Hailsham, for example, has repeatedly emphasized the importance of locating institutes for fundamental research in close relation with universities and the danger otherwise of such institutes stagnating within one or two decades, and in addressing the Parliamentary and Scientific Committee last March he referred specifically to the report of the Zuckerman Committee on the organization and management of research, although without, as does Sir Willis Jackson, considering the mobility of scientists in this connexion. To indicate the extent to which the organization of scientific effort is receiving initial discussion, it should suffice to note Dr. W. R. Brode's review of the growth of science in the United States and its relation to the national science programme which appeared in *The American Scientist* of March, and Prof. C. Holton's penetrating analysis of scientific research and scholarship contributed to the *Journal of the American Academy of Arts and Sciences* (*Daedalus*) for Spring, 1962.

Both these articles, like Sir Willis Jackson's lecture, are concerned with factors which encourage creative work in science and, as might be expected, provide strong support for a good deal of Sir Willis Jackson's argument. In particular, Dr. Brode emphasizes the necessity for an adequate supporting body of technicians if we are to utilize effectively the services of scientists and technologists, and Prof. Holton examines carefully the functioning of the group system and its relation to the rate of scientific advance. Both papers distinguish factors which hinder the conduct and organization of research and should contribute to that clearer public understanding of its nature which is an essential step both in securing adequate financial support as well as the conditions which will attract really competent investigators.

Although Sir Willis gives some fascinating glimpses of the opportunities for research in his chosen field and of the interaction between science and technology, and especially of the way the development of new techniques opens up new fields, the core of his lecture is in his reiteration of conditions which determine effective scientific work. First, however, after stressing the need for full freedom of investigation, he delivers a timely attack on the tendency to disparage technological or applied research. This is quite independent of the appropriate balance between fundamental and applied research. The intellectual or professional effort involved in industrial research and development is in no way intrinsically inferior to that required in research into purely scientific problems; both can on occasion be trivial, but the nature of the problem determines the effort required, and if our colleges of technology, for example, are to gain their rightful place in the educational structure, the value of the applied research which they undertake must be properly recognized.

If Sir Willis had done no more than give this timely rebuke to the attempt to disparage applied research, as such, wherever conducted, his inaugural lecture would have been of real service. He could scarcely have struck a more effective blow in support of the research activity of the engineering schools than he has here done by putting engineering research, and applied research generally, as part of research activity in general, and as an essential element in productive activity. For that attitude to be widely and effectively dissociated, however, much wider mobility of staff will be required and it is in this connexion that Sir Willis welcomes the observations of the Zuckerman Report.

Sir Willis sees clearly that useful as will be some improvement in mobility within the Scientific Civil Service, what is really required is greater mobility in industry and in educational institutions generally. The Zuckerman Report merely displays the possibility of systematically encouraging and deliberately arranging movement of staff, and even so far as the Civil Service is concerned, it must be admitted that Government handling of staff relations has not exactly been designed to discourage suspicions whether a policy of mobility will not be allowed to affect adversely the careers of scientists. The Zuckerman Report was firm on this point, but some assurances may well be required when attempts are made to implement such a policy. Meanwhile, however, it is also to be noted that such a policy of mobility, at least on a part-time basis, offers a reasonable prospect of overcoming the staffing difficulties in some departments of universities or colleges of technology by the use of qualified senior men from industry in readerships or lectureships in applied science or management.

Sir Willis himself lays some stress on exchanges of this kind and suggests that Britain has been somewhat backward compared with other countries. He does not believe that the teaching of technology should be limited to full-time staff but believes that the raising of the level of technological teaching in Great Britain demands a much greater mobility of staff between educational institutions, industry, Government establishments and the public services, and points to the example already set by medicine. Obviously, here again there is no room for the intellectual snobbery which looks down on applied research as inferior to fundamental research.

Sir Willis Jackson has two fields particularly in mind: one is the foundation and teaching of the principles of design, and the other is the interplay of scientific, technical, economic and human considerations in pursuit of new developments and in handling major national and international projects Britain's arrangements, he comments, are much too compartmented, leading to absence of mutual understanding, sympathy and respect, to delays in implementing in one domain what is emerging from another, and to failure to produce enough men capable of rising above particular loyalties to resolve the increasingly complex problems which face the community The great danger is that, at critical points, tradition will delay decisions on policy too long.

Sir Willis is particularly anxious as to the effective co-ordination of the national effort both in research and in production—not so much the existence of duplication of research, development or design effort, as the waste of manpower, particularly trained manpower, through the absence of supporting technicians or craftsmen, inefficient and unimaginative direction, and the lack of appropriate co-operation arrangements While he recognizes that the Government has its own responsibilities, particularly on the educational side and in stimulating the conditions which, for example, through interchangeable pension systems could encourage mobility of staff, he is clear that, quite apart from its bearing on the maintenance of a free society, a major responsibility for initiative must rest with industry itself He too sees the use of civil research and development contracts as providing an important contribution, but insists that the right place for research and development is as close as possible, organizationally and in location, to design and industrial production if the difficulties of transfer are to be kept to a minimum and the maximum use made of industrial experience

It is at this point that Sir Willis refers to the importance of the wise use of skilled manpower and the need not merely to see that the scientist and technologist is fully supported with technicians but also that highly trained men and women are not used carelessly in work for which men and women of lesser qualifications would serve. It is here also that he points out the wide opportunities for inquiry that confront us we have not yet seriously attempted to relate the advance of scientific knowledge and of its technological application to the rate of growth of the Gross National Product. The full authority of this lecture is put behind what Dr. B V. Bowden urged before the British Association last year in regard to the use of the computer and like methods in administration

Even more seriously Sir Willis Jackson is concerned over the neglect of the sociological problems which Britain has encountered It is not simply a matter of education for management in the narrowest sense, of providing industry and Government alike with managers competent to use the new techniques that technological advance has put at our disposal and dealing objectively and factually with complexities which would otherwise be beyond human capacity We are woefully incapable of resolving in a rational way the human problems which have issued from automation, telecommunications and other advances of technology which have created highly complex sociological problems. The scale and pace of effort in the physical sciences are still increasing, and we need a more serious attempt to establish the human sciences in a saner relation to the physical ones

A recent survey by the British Academy has emphasized the wide disparity in support given in Britain to the human and physical sciences Referring to this report, Sir Willis Jackson contrasts the £40,000 awarded by the Department of Scientific and Industrial Research, through its Human Sciences Committee in 1959–60, to the investigation of human problems in industry with the £5·775 million awarded by the Department for the support of research Sir Willis does not press the comparison, which may stand, for the supply of competent investigators is also too limited and one problem is to attract a greatly increased number of able investigators and provide them with experience Meanwhile, however, it is equally important that the minds of the increasing proportion of able recruits attracted to the physical sciences and technology should at least be open to the sociological consequences of their work and that they should be equipped in some measure to discharge the widening human, as well as the scientific and technical, responsibilities which lie ahead of them, and it is refreshing to note that, in concluding his lecture on this note, Sir Willis does not omit to look at the place of our scientists and technologists as agents of a new diplomacy nor hesitate to assert that they will have an increasing part to play as advisers and as teachers in the progress of the under-developed countries, not only carrying conviction in regard to particular scientific and technical expertise but commanding also respect as ambassadors of broad, sympathetic vision, sound judgment and unassailable integrity

This inaugural lecture is one that the Minister for Science will no doubt be prompt to note, and this is as it should be for it argues on lines which Lord Hailsham has often been disposed to argue, and on him must fall some of the responsibility for ensuring that the necessary resources are made available The theme, however, is one that no less concerns the universities, the professional institutions and industry Even on the level of finance all the responsibility cannot be attributed to the Government industry itself must bear a major share, and in regard to research into the human sciences, the universities have some responsibility for finance as well as organization if they expect to avoid the earmarked grant being brought into use Especially perhaps, however, the responsibility rests on the professional institutions, for much must depend not only on their readiness to co-operate with Government departments and institutions, with the universities and the colleges of technology and also with industry, but equally on the outlook and the professional ideal which they set before their members In the end the character of the partnership between science and electrical engineering, or between science and other technology, will depend on the vision and judgment and public spirit which the technologist brings to the service of his profession.

ADHESION AND ADHESIVES

Adhesion

Edited by Prof D D Eley Pp x+290+11 plates (London Oxford University Press, 1961) 55*s* net

THE subject of adhesion, like so many subjects of technical importance, does not fall within the boundaries of any one branch of science, but draws on the activities of chemists, physicists, engineers and others This renders the need for a good systematic text-book both more desirable and more difficult to satisfy If the book is to be anything like comprehensive, it is almost inevitable that it should be a co-operative venture

The book under review, of which Prof Eley is the editor and part author, is the outcome of the discussions of a committee on the subject of adhesion Its outlook is fundamental in the sense that it is concerned primarily with scientific principles rather than with detailed applications The subjects included have been chosen with care so as to cover all the principal lines of development

The introductory chapter, on the fundamentals of adhesive joints (D D Eley and D Tabor), sets out the main phenomena to be examined Chapter 2, 'Molecular Theories of Physical and Chemical Adsorption" (C Kemball), gives an excellent account of the types of molecular forces operating between surfaces of dissimilar materials Chapter 3, "Thermodynamic Adhesion" (D D Eley), and Chapter 4, "Thermodynamics and Imperfections of Solid Surfaces" (W J Dunning), deal with the fundamentals of adsorption phenomena, with particular emphasis on the adsorption of liquids on solids Experimental data on surface free energy, enthalpy and entropy, and their interpretation, are discussed Chapter 5, 'Friction and Adhesion between Metals and Other Solids" (D Tabor), discusses in a very illuminating manner the information which has been obtained on the nature of the actual surfaces of contact in the case of clean metals and metals covered with surface films

These five chapters are concerned with 'adhesion' in general rather than with 'adhesives', and it is only in later chapters that the chemical and physical properties of adhesive materials, which are usually high polymers, are considered Thus in Chapter 6 (by A Baker) the principal types of adhesive materials are classified in relation to the chemical reactions involved in the setting process, and their general fields of application are indicated Chapter 7 (by H Warburton Hall) gives a general account of the mechanical properties of thermosetting polymers, which emphasizes particularly the phenomena of visco-elasticity and fracture, while Chapter 8 (by W C Wake) discusses the closely related subject of the rheology of adhesives, but with more particular emphasis on soft or rubbery materials in which the ability to flow and wet a surface is a primary consideration This leads on to "The Distribution of Stress in Adhesive Joints" (I N Sneddon), which to the engineer will appear as the central and most informative chapter in the book This chapter gives an extremely clear account of the remarkable theoretical and experimental work which has been carried out on the analysis of stresses in joints, and has perhaps a more direct bearing on practical design than any of the earlier chapters After this, a short chapter on the physical testing of adhesives and adhesive joints, which is not free from technical jargon, and the editor's "Concluding Remarks" come rather as an anti-climax

While the presentation is generally clear, there are a few places in which difficulty may be encountered Thus, for example, Plate 1, which shows nothing at all clearly, is without value—at least the position (and form ?) of the solid surface, and the specific atoms in the molecules, should have been indicated On p 123 it is not the 'softness' of the metal (which to me means "low modulus of elasticity") but its low yield stress which is relevant to the argument The formula for the elastic energy of a rubber (p 173) is incorrectly given, the 'strains' should be replaced by 'extension ratios' The argument (p 202) concerning the effect of strain energy on the force required to rupture an adhesive bond fails to define the problem clearly In the same chapter 'Griffiths' is used repeatedly for 'Griffith' On p 207 it is difficult to see how a 'stress concentration' factor can have a value less than unity

These, however, are minor defects Bearing in mind the inherent difficulties of the subject, already referred to, and the inevitable additional difficulties arising from multiple authorship, the book as a whole is successful in achieving its purpose, which is to bring together the most important basic facts and theories of adhesion and related phenomena It is a notable addition to the not too extensive scientific literature on the subject, and it can be confidently recommended The comparatively low price should encourage its purchase by individuals as well as by libraries.

L R G. TRELOAR

BIOLOGY OF MAMMALS

Systematic Dictionary of Mammals of the World

By Dr Maurice Burton Pp 307 (London Museum Press, Ltd , 1962) 35*s* net

FACTUAL information concerning the many genera and species of mammals is scattered through a wide and diverse literature often to be found only in specialized libraries The author of this work, perhaps better known in other fields of natural history, has attempted to remedy this situation and has largely fulfilled his aim of providing a source of easy reference to facts not readily available elsewhere in one book The book, not a dictionary in the alphabetical sense of the word, is a compilation of basic biological information for many of the more important species of mammals, and follows the usual systematic arrangement, with species listed in their appropriate orders, suborders and families Any disadvantage to those unfamiliar with detailed mammalian classification is overcome by the provision of an excellent index The author supplies brief but useful notes on the more important features of the higher systematic categories while presenting the distinguishing characteristics, ecology and life-history of individual species in considerable detail, with statistical and other information wherever possible The text is enlivened throughout with small but attractive scraper-board drawings

It is facile to criticize such a book as this, the more so since varying levels of knowledge of its raw material render inevitable some degree of uneven treatment and for reasons of space much has had to be omitted These features are emphasized by the arrangement of the book at the lower levels of classification with related genera and species or those on which information is lacking listed in conjunction with the more detailed notes on one particular species This is confusing at first sight for example, the entry for the European wild cat is followed by brief details of six other genera of cat, and that for the snowshoe rabbit (*Lepus americanus*) of North America by notes on thirteen other species of African and Asiatic hare Scientific names are occasionally antiquated, such as *Rhachianectes* for *Eschrichtius*, the Californian grey whale, or incorrect, such as *Enhydris* for the more usual *Enhydra*, the sea otter There is no bibliography, the author instead listing some of the more important sources in the preface A few of the drawings lack a caption or other indication of the animal portrayed Small errors which have been overlooked include a cross-reference to the New Guinea water rat, *Parahydromys*, which receives no further mention, *Crossomys* being correctly included, *Talpa europaea* is said to extend to Japan the Japanese mole is *T micrura*, the British water vole (*Arvicola*) does occur in the Scottish Highlands, perhaps through summarizing too zealously, the author implies that the world record African elephant tusk is 11 ft 5½ in in length and weighs 226½ lb In fact, he has combined the length of the longest tusk with the weight of the heaviest

Nevertheless, this book will be invaluable to amateur and professional student alike Its detailed treatment of representatives of the entire class sets it apart from others of its kind, and the author is to be congratulated on his useful and comprehensive summary of the literature J H HILL

DRUGS AND THEIR ACTIONS

Drugs, Medicines and Man

By Prof Harold Burn Pp 232 (London George Allen and Unwin, Ltd , 1962) 25*s* net

THE readers of this useful and clearly written work will be grateful to Prof H Burn for having used his great gifts of exposition in presenting them with an easily understandable account of drugs and their actions This is a subject that deserves such a treatment, not only because of its scientific interest, but also because drugs are of importance to all, patients and users of the National Health Service, and the tax-payers For all these reasons it would probably have been more appropriate to have this book reviewed by one who is not in his daily work concerned with the action of drugs and who is not familiar with many of Prof Burn's ideas and with his contributions to the analysis of drug action However, I myself experience an additional pleasure in reading this book that of meeting old acquaintances in a new setting I realize, also, that there is much for me to learn in this volume One is glad to be reminded of the fact that the attempts to fight disease with the help of the synthetic chemist are of surprisingly recent date In the field of the hypnotics, of the general and local anæsthetics and of the analgesics, the efforts of the chemists can be traced back to the nineteenth century, but many other types of therapy by synthetic drugs, some of the most successful ones, such as the treatment of parasitic and infectious diseases, of hormonal dysfunction of the disorders of the cardiovascular system and of mental illness, are achievements of the twentieth century

Prof Burn describes many instances where the pharmacologist's efforts in the analysis of the effects of drugs on biological processes have guided the work of the synthetic chemist and led to valuable new therapeutic agents However, the book also gives many examples of advances that were the outcome of fortuitous findings The account of some of these accidental discoveries, or the chapter on the doctor–patient–drug relationship, will be readily appreciated and enjoyed by the non-scientific reader Prof Burn also deals at some length with the habit-forming drugs such as alcohol and nicotine One of the attractive features of the book is that he discusses the mode of action of these substances (and of other drugs) but that he does not pass many judgments of value, still, we do learn from him that we should neither eat too much nor take too little exercise, nor disregard the danger of excessive mental strain during our daily work

Anyone who really wants to learn something about the usefulness of drug therapy should read this book, and particularly the last chapter, which gives a brief summary of the achievements of drug therapy For having given the lay reader a clear account of these successes Prof Burn has earned the gratitude of all pharmacologists H BLASCHKO

COMPENDIUMS OF MINERALOGY

Rock-Forming Minerals

By Prof W A Deer, Dr R A Howie and Dr J Zussman Vol 1 Ortho- and Ring-Silicates Pp viii+333 (London Longmans, Green and Co, Ltd , 1962) 95*s* net

WHEN J D Dana, in his early twenties, put together the first edition of his *System of Mineralogy* (published in 1837) he followed the then current fashion and used external characters almost exclusively as a basis of classification, but in the third edition (1850) he rejected the whole of the so-called 'natural history system' and accepted chemical composition as fundamental This feature was retained through the sixth edition (by E S Dana, 1892) which, supplemented by a series of appendixes, remained the standard mineralogy for half a century A chemical classification was followed, too, in the *Handbuch der Mineralogie* by C Hintze In the seventh edition of 'Dana', now in course of publication, a change is being made where feasible to a classification based on crystal structure and chemistry as elucidated by X-rays, a scheme already adopted by A N Winchell in 1933 for the section on silicates in his *Optical Mineralogy*

The volume under review is the first of a series of five which is to treat the restricted field of the rock-forming minerals The first four, on silicates, follow the now customary structural classification, the fifth will describe non-silicates Volume 1 deals with twenty-two mineral groups or species classified as ortho- and ring-silicates The description of a group is prefaced with a brief general account, for each species the ideal chemical formula, the crystallographic and optical data, and the unit cell are listed No data of morphological crystallography are given, drawings of crystals being used only to show the optical orientation of biaxial minerals (in the single case of axinite recourse is had to a stereogram) A description of the atomic structure leads naturally to a discussion of the chemistry in terms of a selection of analyses, to each of which is appended a recalculation showing the structural balance, information from laboratory syntheses is also taken into account A correlation of the optical properties with structure and composition, and a brief mention of diagnostic criteria, are followed by a section on paragenesis This, and the discussions of crystal chemistry, are the specially valuable features of the book Each description is followed by a critical selection of references

The general nature of the production is pleasing Misprints are mainly trivial, in names of localities or titles of papers, but there are several incorrect simple indices in the crystal figures and 'fast' and 'slow' have been interchanged on p ix The distinction of line in the legend to Fig 29 is obscure, and Fig 68 shows decreasing birefringence with an increase in the alkali content of beryl, contrary to the correct statement in the text The minima for refractive indices and birefringence for beryl are inconsistent These, however, are small points of criticism of an important work, the remaining volumes of which will be awaited with interest by all petrologists The great disappointment is the high price, which will clearly place the whole series out of reach of most individuals

F C PHILLIPS

MEN OF PHYSICS

Biography of Physics

By Prof George Gamow (Hutchinson Science Library) Pp x+338+8 plates (London Hutchinson and Co (Publishers), Ltd, 1962) 21*s* net

BOTH as a physicist and as a popularizer of science, Prof Gamow enjoys world-wide esteem His larger works are soundly instructive, and the "Tompkins" books and other lighter contributions are both original and gay It is difficult to assess a book that falls midway between the two categories—and does so intentionally—when one feels that this design is misguided I enjoyed it, of course, as anyone who has lived with physics for many years must, and I can see that in the earlier chapters (where the pace is slower), and in the chapter on relativity (which has appeared before), a layman could learn a great deal, if he got down to close and careful reading My criticism is that the leavening that I find entertaining would distract the uninitiated, particularly in the later chapters There is, for example, a great deal said, in 'much-translated' verse, about an incident involving Niels Bohr and a motor-cycle in Cambridge The version I was brought up on took place in Manchester at the corner of Dickenson Road, involved a No 41 tram and a Dursley-Pedersen push-bike borrowed from an undergraduate named Etchells, a crocodile, and a trolley-boy, but the crocodile (of young ladies from a well-known school in Withington) vouchsafed no comment, and the accurately recorded observations all came from the trolley-boy Legends do get distorted in the telling, and it may be that the author's is the more precise, but will it really mean much to the person who takes the book up in the hope of being introduced to physics ?

There are many excellent features, which can be praised whole-heartedly in any frame of reference The work of Archimedes, of Kepler, and of Galileo is clearly set in perspective in relation to later developments, there is a long discussion of the riches of Newton's *Principia*, which brings out some points that illustrate further the vast range of problems which Newton himself considered The chapters on heat, light, and electricity (with tribute to Faraday and Maxwell in particular, and concise and clear accounts of the contributions of Ampère Oersted and Ohm) are sound, though naturally the pace accelerates Relativity is very well treated, and in the later chapters on modern physics, one is taken behind the scenes to the 'thought experiments' in which Einstein, Heisenberg, and others wrestled with the new concepts that were to revolutionize the world of physics

From the point of view of the scientist who already has some acquaintance with the subject of this biography, indeed, this is a delightful story of the way it has grown up and flourished But, with all respect, the presentation of the lighter side of individual physicists does not seem likely to persuade strangers that physics itself is rewarding to know or to understand, and this is what the author says in the preface he really wants to do

G R NOAKES

LINEAR DIFFERENTIAL OPERATORS

Linear Differential Operators

By Prof Cornelius Lanczos Pp xvi+564 (London D Van Nostrand Co, Ltd, New York D Van Nostrand Company, Inc, 1961) 80*s*

THIS unusual book has many distinctive features, and will be of interest particularly to those mathematicians whose research fields are on the frontier between pure and applied mathematics It emphasizes the close link between analysis, algebra and the theory of numerical processes, and the applications discussed extend into physics and electrical engineering

The first five chapters are concerned with purely mathematical developments, although it is clear throughout that the motivation is the application They cover the theory of interpolation, harmonic analysis, the matrix calculus, function spaces, and the Green's function A chapter on communication problems is given to the theory of the galvanometer the steady-state analysis of music and speech and the transient analysis of noise In another chapter, on Sturm–Liouville problems, the special functions play a large part A chapter on boundary-value problems covers potential theory, vibration and heat flow, and there is a very interesting and unusual discussion of the parasitic spectrum The last chapter deals with the numerical solution of trajectory problems

A notable omission occurs in the lists of references It is surprising that while there are many references to books there is none at all to original papers It would certainly have been proper to refer to some of the many papers on aspects of the book's contents, for example (in §3, 12), on error analysis, ill-conditioned systems of linear equations, and the problem of defining an inverse for a singular matrix

A reference to the author's style may not be out of place In an age where it is fashionable to present mathematics in a condensed form it is a tonic to come across a book written in a style which, while somewhat discursive, is notable for its clarity In places the author would seem to assume scant mathematical knowledge on the part of the reader, but on the whole the book is for the mathematically mature, and the style is such that the message gets across very well, and in a unified fashion The graduate student, about to start reading for research, will find the book particularly attractive

L S GODDARD

Finite Markov Chains

By Prof John G Kemeny and Prof J Laurie Snell (The University Series in Undergraduate Mathematics) Pp viii+210 (Princeton, N J D Van Nostrand Company, Inc , London D Van Nostrand Company, Ltd , 1960) 37*s* 6*d*

THIS book presents an undergraduate course on finite Markov chains by using mathematics no more complicated than elementary matrix algebra. The authors provide a lucid summary in Chapter 1 of the mathematical prerequisites needed so that a conscientious non-mathematician may also make use of the book The theory is developed in Chapters 2–6 and applications are discussed in the final (seventh) chapter Throughout there is adequate use of numerical examples to illustrate the line of the argument The gain in conciseness which results from the ruthless use of matrices is so great that it seems scarcely just to protest that it has not been put to better use However, the following omissions cannot be left unremarked no solutions are given to the exercises given at the end of Chapters 2–6, no comparison is made between the matrix methods used here and other methods of investigating Markov chains, the references given to other work are inadequate This last fault is the most serious, particularly in view of the two audiences to whom the book is addressed Some of the virtues of the book have already been mentioned To these, I would add style and presentation, coherence and, finally, a special commendation for the last chapter which gives a much broader picture of the application of finite Markov chains than the usual questions about queues and cosmic rays ALISON DOIG

Introduction to Space Dynamics

By Prof William Tyrrell Thomson Pp xiv+317 (New York and London John Wiley and Sons, Inc , 1961) 87*s*

ALTHOUGH several specialized books on the dynamics of space vehicles have appeared in recent years, there has been no suitable introductory text-book for university students This deficiency Prof Thomson has now made good He begins with 40 pages on the fundamentals—vectors, kinematics, transformations of axes Then, after a long chapter on particle dynamics and satellite orbits, come discussions of the rotational movements of rigid bodies and the dynamics of gyroscopic instruments Chapter 7 deals with the motions of space vehicles, particularly in rotation, including such topics as the 'de-spinning' of satellites by means of weights flung out on unwinding strings Chapter 8 outlines the methods for optimizing the performance of multi-stage rockets, and the subject of the last chapter is generalized theories of mechanics, as typified by Lagrange's equations The text is generously interspersed with worked examples and problems, which have the virtue of being more realistic than those found in most text-books

The book is sound, well written and easy to follow The topics are, on the whole, well chosen, but, as is inevitable in a book in which the explanations are so full and the examples so numerous, some subjects are dismissed too cursorily (for example, Earth-satellite orbits) and others go unmentioned The omitted topics could with advantage have been outlined, so that students would realize that the book, although an excellent introduction, is an incomplete one

D G KING-HELE

General Microbiology

By Prof William G Walter and Prof Richard H McBee Second edition Pp ix+405 (Princeton, N J D Van Nostrand Company, Inc , London D Van Nostrand Company, Ltd , 1962) 54*s* 6*d*

DESIGNED for use with elementary students, the second edition of this introduction to microbiology needs little commendation The launching seven years ago was quickly followed by three reprintings and now the authors have grasped the opportunity presented by a second issue to make a thorough revision of the whole work

The historical chapter has been omitted, and relevant historical details have been included with the topic under discussion Additional sections deal with lyophilization, the preservation of cultures by freeze-drying techniques, etc In the chapter on the microbiology of foods, the authors discuss preservation by irradiation and the use of food additives The overall production is such that Profs Walter and McBee can fairly claim that they have succeeded not only in providing a well-balanced and scientific introduction to microbiology but also in relating this study of micro-organisms to the everyday life of the student The detailed excellence of the line-drawings shows up the fuzzy inadequacies of many of the photographs T H HAWKINS

Systematic Qualitative Analysis

An Introduction. By Dr. G A Morrison Pp. ix+198 (London Butterworth and Co (Publishers), Ltd , 1961) 25*s*

ALTHOUGH qualitative analysis has a well-established place in school and university courses, there is some difference of opinion as to its relative merits in chemical education It can serve to develop powers of observation and deduction, skill in certain laboratory techniques and practice, and an appreciation of certain physico-chemical principles because it is the study at first-hand of numerous chemical reactions On the other hand, as Dr. Morrison is well aware, analysis can be performed almost entirely "in an automatic way without mental effort"

Systematic Qualitative Analysis is based on the experience of teaching inorganic analysis to first-year university students, and is clearly designed for their use The opening sections offer an outline of the scope of the book, a rather unsatisfactory treatment of dissociation constants, buffered solutions and solubility constants, some descriptions of the usual semi-micro techniques, and details of preliminary tests A section then deals with the semi-quantitative application of solubility product theory to group separations The next section gives details for effecting the separation of cations into groups, and then the analysis of each group is treated fully in separate sections Finally, there are two sections elaborating the analysis and confirmation of anions, an appendix of useful reagents, and a good index

Using Dr Morrison's book it may still be possible to perform analyses in an automatic way However, some mental effort will be needed if only to interpret his 'running nomenclature' This labels each stage of the analysis systematically and quite unambiguously, but its extensive use in the text results in such statements as "If IIBSL^{4}S is Se it should be red, and if IIBSL^{5}S is Te it should be black" (p 113) The book is compact, well bound and presented, but it may seem somewhat expensive at 25*s*

MICHAEL C COX

THE PARTNERSHIP BETWEEN SCIENCE AND ELECTRICAL ENGINEERING*

By Sir WILLIS JACKSON, F R S.

Professor of Electrical Engineering, Imperial College of Science and Technology, London

I DO not think my civil and mechanical engineering colleagues will disagree when I say that electrical engineering differs from their subjects in that it is much younger and had its origins in scientific speculation and experiment rather than in practical achievement Granted that the first practicable electric telegraph was in operation in 1797, the subject of electrical engineering is nevertheless normally, and rightly, regarded as starting from Faraday's discovery of the principle of electromagnetic induction in 1831 Since that date its progress has been continually stimulated, and periodically revitalized, by scientific discovery, notably by discovery in physics But it has at intervals reciprocated by providing science with new techniques and new tools for further scientific investigation, and this interchange and interdependence—this partnership—has been of profound benefit to both

There are, no doubt, many permissible definitions of engineering One, which I myself like, is that it is the economic exploitation by one technique or another of the properties of materials Indeed, when I chose the title of my inaugural address I had in mind devoting the greater part of it to this subject, for it illustrates perhaps best of all the ways in which the work of physicists, chemists, metallurgists and mathematicians has affected, and to a large extent determined, the trends of progress in electrical engineering—how the refinement and utilization of special properties of particular materials has opened up quite new facets of electrical development.

The materials of interest to the electrical engineer are classified as conducting, semi-conducting, insulating, magnetic, structural and nuclear In the full, written version of my address I have summarized some of the most significant recent developments in semi-conducting, insulating and magnetic materials and the engineering consequences of these developments.

I have outlined, for example, how the science of semi-conductors has been transformed during the past 15–20 years from an interesting commentary on an empirical practice in the rectification of alternating current—a commentary which permitted no very useful deductions to be made as to how improved solid-state rectifiers might be manufactured—to a framework of knowledge and understanding providing for the production of devices—notably the transistor—which are predeterminate, more efficient and reliable, and vastly more extensive in scope, than could possibly have resulted merely from practical observation and common-sense improvements With these devices have come new techniques of repetitive manufacture, under conditions of laboratory cleanliness and precision, and a new form of electric circuit engineering

I have given similar illustrations, in respect of insulating and magnetic materials, of the contributions which the scientific disciplines I mentioned have made to the progress of my subject

The story of the impact on electrical engineering of the science and technology of materials is at a fascinating stage New solid-state devices of great scientific ingenuity and carrying baffling names—the Hall effect multiplier, the tunnel diode, the maser, the laser, the cryotron—are appearing with amazing rapidity Their engineering significance is necessarily still largely unestablished, but they herald important further changes in electrical engineering technique Among other prospects, low-temperature physics may soon penetrate not only the domain of computers, but also that of power engineering Scarcely have the manifold materials problems of what is already called the conventional nuclear power station been reasonably resolved, and the prospect of early economic power generation by this means established, but the race is on for other new means of power production—the thermoelectric generator, the thermionic generator, the magnetohydrodynamic generator, the fuel cell and the thermonuclear fusion type reactor—and in each case the production and use of suitable materials in appropriate context are likely to provide the key to success

There is also the parallel story of how, by playing his part in a continuous game of leapfrog, the electrical engineer has helped the scientist through his commercial production of scientific equipment such as the electron microscope, the mass spectrometer, various forms of particle accelerator, and electronic components of increasing reliability, decreasing cost and vanishing size until, for use in satellites, they can be compressed to a density of some 10^8 components per cubic foot

Perhaps I should have been wise to adhere to these comparatively uncontroversial themes In the event, however, my original intentions were overridden by a stronger urge, the desire to discuss a number of factors bearing on the translation of the results of scientific investigation into engineering achievement—factors which I believe give cause for concern and to be in need of more attention, and of action, in Britain than they are perhaps receiving

The factors to which I shall refer do not apply only to electrical engineering, but it is there, of course, that I have mainly observed them Nor do I pretend that they are the only factors In fact, some people may consider that they are not the most important ones I also recognize that they have widely varying relevance in respect of individual organizations and of persons Some may rightly feel, therefore, that what I say has no relationship to themselves, in which case I beg them to bring their influence to bear on situations to which my remarks may be thought more applicable

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* Substance of Sir Willis Jackson's inaugural address delivered on May 8

First, I want to refer to the pure scientist and his attitude on occasions to engineering and to engineers In a recent lecture Dame Kathleen Lonsdale said "Science is knowledge obtained by observation, by experiment and by subsequent deduction Natural science is the investigation of the nature and properties of natural bodies and of natural phenomena The object of the scientist is the determination of general laws and the deduction of principles from the effects which are observed"

It follows that outstanding scientists, and those who show promise of becoming so, must be free to follow the directions which their scientific curiosity dictates, without concern for the possible engineering significance of the results of their work This freedom has been a well-safeguarded characteristic of university science in Great Britain and I hope it will remain so It can be tampered with only at grave risk to the seed-bed from which important contributions to future progress in engineering will spring It follows also that these scientists must, so far as is practicable, be provided with the most up-to-date facilities and tools of investigation, if they individually—and through them the country—are to remain in the forefront of scientific endeavour and achievement

It is understandable, therefore, that they should be critical of Government—since funds come in increasing proportions from this source—when they consider that the resources being made available to them are inadequate and restricting, and critical of our industry when they consider that it is failing, relative to that of other countries, to translate the results of earlier scientific work speedily enough into the kinds of equipment necessary for their further pursuits

But what I myself do not find understandable is a tendency I have observed among some of these scientists to regard the process of translation of the results of scientific work into engineering achievement as a markedly lower-grade activity than their own, to be unsympathetic to the problems and difficulties which can be associated with it, and to generate in their better students an attitude of mind which is antipathetic to their later participation in it The outcome can only be to retard an improvement in the situation about which they may rightly be constructively critical

Whatever may be the individual view as to how much of available moneys should be spent on research by Government, or by industry within itself, and for what purposes, the fact is that the amount of these available moneys is dependent on the productivity, and in our kind of society on the profitability, of our manufacturing industry in the face of an increasingly competitive situation If this is a case of the hen and the egg, and if the pure scientist and his industrial compatriots appear sometimes to have different views as to which is the egg, I would beg the pure scientist to show a greater willingness than is sometimes apparent to recognize that the conduct of applied research and development in industry, and the speedy translation of its results through design and manufacture into efficient, reliable and economic pieces of engineering equipment, are activities which can make intellectual demands no less severe than, though different from, his own and involve problems of human relationships from which he is largely protected, and that it still lacks in Britain a public prestige at all commensurate with what he himself enjoys

Those who are rightly concerned to increase the research activity of the engineering schools should also be cautious lest their student participants fail to appreciate that research applied to engineering is not a thing apart, but something which to be effective must be woven into the whole fabric of productive activity, and that it demands of the research worker experiences which cannot be gained in divorce from the environment in which this activity is conducted

The training of selected young people in the processes of research is the highest level of teaching—in the engineering no less than in the science schools—and is an essential part of the activity of these schools Its basic aim should be to stimulate inquisitiveness, and develop the powers of satisfying it, in relation to the problems of engineering, and care must be taken that its pursuit does not obscure the fact that the aims of engineering are different from those of science—though the two activities are inextricably interwoven—and that the scope for inquisitiveness in engineering is not restricted to its research domain

If our national weakness lies not in the quality of our research but in our relative inability to translate its results into engineering achievement, it is of limited value for the research worker to criticize from the touch-line an unwillingness—whether this be real or apparent—of the practising engineer to take advantage of his work, and detrimental to give the impression that he regards the work of these engineers as a comparatively straightforward matter with which he himself does not need, or wish, to be associated Where the situation is weak, he adds little strength merely by writing papers and reports What is often needed is a willingness of the research worker to go into the engineering domain for the express purpose of helping to ensure that his results are used effectively To do this he will need more than research ability, and if he succeeds, and in succeeding attains a position of senior responsibility and authority in this, for him, new domain, he will have done much more than correct a previous weakness in it—he will have established for the research laboratory from which he came an enhanced importance and respect, and an assurance of support for it which might otherwise be lacking

In my opinion the problems of staff mobility lie at the root of much of our difficulty in gaining full benefit from our national research effort The most objective discussion of these problems of which I am aware is contained in the report on the *Management and Control of Research and Development*, published in September last In the commentary on the structure of the Scientific Civil Service, the report says

"The principal defects which it reflects are that too many young scientists are committed prematurely to a permanent career in Government research and, second, that those in their forties and fifties have no option but to stay on as research workers (usually at Principal Scientific Officer level) long after they have made their main contribution to research, and in a period of their careers when their experience and ability would be of value if directed to other activities

"Another major criticism levelled at the administration of, and conditions of service in, the Scientific Officer Class by many of those with whom we discussed the staffing arrangements was lack of mobility According to our evidence, this reveals itself in two ways First, there is insufficient movement of research staff between establishments or, indeed, from one line of research to another As a result, Government scientists are deprived of the stimulus of new work, at the same time as they are denied the wider experience necessary for those who ultimately rise to higher positions of responsibility Secondly, there appears to be an absence of any planned arrangements for exchange or secondment of research staff between Government research establishments on one hand, and the universities or industry on the other, or even indeed, with other branches of the Civil Service Because of this, members of the Scientific Civil Service enjoy fewer opportunities than they should of broadening their experience in a way which would be beneficial both to themselves and to the Government"

It is to be hoped that, arising out of the Committee's suggestions, the situation within the Scientific Civil Service will gradually be improved In varying degrees, however, the same sort of criticisms apply to industry and to our educational institutions None has yet given much evidence of a belief that systematically encouraged and deliberately arranged movement of staff between their respective domains would bring benefit to all

There is one aspect of this in which I have a special interest—namely, the further development of postgraduate courses in science and technology It becomes increasingly evident that a substantial proportion of engineering graduates must supplement their undergraduate courses by a fourth year of more advanced specialized study I am one of those who regard it as highly desirable that participation in the latter should be preceded by a period of training and experience in engineering practice partly as a foundation for a reliable choice of specialist field, and also so that the graduate may gain an appreciation of the grounds on which existing practices in his chosen field have emerged and of the directions in which improvement is required I know that many of the university staff members concerned in the development of postgraduate courses hold this view and have been disappointed with the limited response of industry to the steps they have taken, and have concluded that the only means of ensuring participation in these advanced courses is to accept as students men who have just graduated

There are, no doubt, several reasons for this limited response One has been the shortage of scientists and technologists at a time of rapid industrial expansion Another, in respect of which Imperial College has set a better example than most, though, as I see it, there is still scope for improvement, is that many of the courses provided have been essentially departmental in character, have not drawn on the wider resources of the university and have therefore failed to reveal deliberately and adequately enough the interdependences between the sciences and technology to which I referred initially, and to illustrate the kind of teamwork between representatives of the various disciplines now essential to new industrial development A third, and perhaps the fundamental, one is that of lack at sufficiently senior levels of industry of adequate contact with, and knowledge about, what is being attempted There is as yet too great a separation between the two sides

In this respect I believe we have something to learn from other countries in which for limited periods, carefully selected men, whose primary employment is in industry or Government establishments, are given academic status and contribute to advanced teaching work It is to me quite unrealistic to suppose that the teaching of technology at its highest level can be carried out wholly by men who occupy full-time teaching posts, unless these men return to industry—to a responsible position in industry—or a similar environment at appropriate intervals In my opinion the raising of technological teaching in Great Britain to the level required demands a much greater mobility of staff, between educational institutions on one hand, and industry, Government establishments and the public services on the other, than has yet been achieved, and, in supplementation, that carefully selected men should be fully recognized members of both sides, as has long been the case in medicine

It may be that the initiative in this matter will be taken by the colleges of advanced technology, though I hope that the initiative will not be limited to them

In two respects this kind of help is particularly needed—one, to contribute to the formulation and the teaching of the principles of design, an aspect of engineering on which we are sometimes much criticized and which receives little systematic treatment in present postgraduate courses, the other, to portray the interplay of scientific, technical, economic and human considerations in the pursuit of new developments and in the handling of major national and international projects

I would like to see a much more deliberate and extended movement of selected men between the interrelated activities I have mentioned used as a vehicle of staff development Our arrangements are much too compartmented in all sorts of ways, leading to lacks in mutual understanding, sympathy and respect, to delays in implementing in one domain what is emerging from another, and to the inadequate production of men who can lift themselves above particular loyalties to help resolve the increasingly complex problems with which our community is faced Long tradition has built into the various aspects of our national life a great diversity of restraints and inhibitions, the relaxing of which is, of course, full of difficulties and is not free from dangers But the greatest danger is that at critical points of policy decision we shall delay too long, and that the price we shall have paid is higher than we can sustain The most expensive decisions can be those which are not taken

In one such respect the industry of Great Britain seems to me to give cause for considerable anxiety In his presidential address to the Institution of Electrical Engineers in October last, Mr G S C Lucas gave the following figures, taken from the return of Trade Associations in 1958, for the numbers of separate companies designing and manufacturing particular lines of electrical equipment and prime movers power transformers, 19, steam turbines, 11; domestic appliances, 67, switchgear, 50, control gear, 34, small switch and fuse gear, 32, domestic radio and television sets, 60, semi-conductor devices, 26

This subdivision of our industrial effort—and it occurs on a larger scale in some other industries—produces among the larger organizations a considerable multiplication of research, development and design effort directed to the same, or substantially the same, technical objectives, and the possibility that no one of these concerns has the resources either of manpower or finance to launch a really major attack on a new field Among the smaller ones, it means all too often not only an absence of research and development effort within themselves, but also, in varying degrees, an inability to take advantage of the results of work of this kind being done elsewhere—in particular in the research associations specifically on their behalf

Following my participation in a visit to the U S S R in 1955, I included in an article published in the Institution of Electrical Engineers' *Journal* the question "How can we develop a more effective co-ordination of our own national resources without loss of the benefits associated with a free society?"

Since that date there has certainly been a trend towards the creation of larger industrial units, but there has been little self-initiated movement towards the organization of co-operative research and development arrangements within industry itself on subjects of basic and common interest and importance, such as appear to have occurred between otherwise competing continental companies within the European Common Market In consequence, the scientific and technological manpower of the electrical industry is still dispersed to a degree which throws doubt on its ability to compete in large-scale new development with these continental arrangements, let alone with those of the United States and the U S S R—and this notwithstanding that these countries seem unlikely to be any shorter, if as short, of scientists and technologists per unit of population as Britain is The position appears to me to be worse in some, if not most, of our other industries

Different people lay different emphasis on the responsibility which falls to Government, to the Department of Scientific and Industrial Research and the research associations, to the defence and public services and to industry itself, for finding a satisfactory solution to this situation In my opinion there can be no satisfactory solution without considerable initiative by industry, but if it cannot, or will not, do within itself the research and development work necessitated by the national interest, if not indeed for its own survival, this work must necessarily be carried out in national laboratories There is, of course, much work which can best, and some which can only, be done in this way, and this is no doubt likely to increase But the more research and development are separated organizationally and in locality from design and production, the greater are the difficulties, in our society, of effective transfer, and these will be the more serious if the research and development is carried out by staff lacking substantial experience of the manufacturing domain, and if, having been recruited, they are locked up in permanent separation from it An important, if not essential, component in the answer may be the emergence on an extensive scale of civil research and development contracts in industry financed by Government and the public services

There is a further aspect of our utilization of scientific and technological manpower which requires much more attention than it has yet received, namely, the extent to which some of this manpower is performing duties which, with appropriate replanning of its work, might well be undertaken by men or women of lower academic attainment This process of devolution is, of course, a continuous one in any progressive situation, and it is characteristic that the highly qualified persons so relieved move into other fields of creative effort with similar later consequences It demands for its progression, however, the availability of people, themselves well educated and trained but to a different level, on whom the work can be devolved In this connexion I believe that in stressing our shortage of scientists and technologists we have given inadequate recognition to the importance of the supporting contribution, and too little attention to the needs in education and training, of our technicians, men who in the electrical industry perform such duties as supervisors of the manufacture, installation and maintenance of electrical equipment, as inspectors and testers, as draughtsmen and as assistants to professional people in research, development and design departments It may well be that our greater present national shortage is at this level

So far the technician has been provided mainly by the promotion of craftsmen—whose scientific and technical knowledge and understanding have too often been inadequate for the needs of the rapidly changing situation—or from unsuccessful aspirants to professional status Fortunately, it is now widely recognized that he must be prepared much more deliberately for the duties to be performed, and the recent Ministry of Education White Paper entitled *Better Opportunities in Technical Education* did a great service in defining an improved structure of technical college courses for this purpose It is to be hoped that employers, and the trade unions, will take steps to ensure, for it is a joint responsibility that the improved technical college facilities now being provided will be matched by commensurate facilities for practical training I wish I were confident that this will soon occur, but fear that nothing short of radical changes in our systems of apprenticeship, with inevitable legislation, will achieve the desired results

It is gratifying that the Scientific Manpower Committee is now devoting itself to an assessment of the technician situation and to a study of the problems involved in it

The factors, affecting the translation of the results of scientific investigation into engineering achievement, which I have mentioned bear with others on what is referred to as economic growth—nowadays and everywhere a paramount aim of national and international policies

This aim found expression in the recent decision of the Ministerial Council of O.E C D to set as a collective target for the twenty member countries the attainment, during the 1960–70 decade, of a growth in real Gross National Product of 50 per cent

It was also reflected in the following statement taken from the report of a NATO Study Group entitled *Increasing the Effectiveness of Western Science* "A goal of Western nations should be to devote as much of their national income as possible to encourage research and development appropriate to their needs and their interests For basic scientific work we recommend a level of the order of 0 2 per cent of the Gross National Product as a target for all countries For applied research and development the experience of the technically advanced countries indicates that a goal of 2 per cent of Gross National Product is both desirable and achievable"

I do not think there is much risk of giving offence to the authors of such statements in saying that there is nothing very scientific about them The fact is that we have not yet seriously begun the attempt to relate the advance of scientific knowledge and of its technological application to the growth-rate of Gross National Product Some hard thinking needs to be done on the economic aspects of research and development in this sense, and it seems to me that we would be wise to devote a greater proportion of our increasing scientific potential to the doing of it, lest we increase less effectively, and less profitably in the national interest, than we should the great momentum which the pursuit of physical science and its applications has acquired

The economic aspects of this pursuit are not the only ones which call for increasing attention It is a commonplace to say that the achievements of scientists and engineers are producing changes in man's mode of life over an extending range and with increasing speed and directness, and are raising sociological problems of great complexity In my own field I need refer to no more than the consequences of automation and of world-wide telecommunications These, combined with other recent innovations not restricted of course to electrical engineering, have found us woefully incapable of resolving in a rational way the human problems which have issued from them Yet the scale of effort in the physical sciences and in technology is increasing rapidly, and the pace of its impact will do likewise, though its character may change It will bring further material benefit, and indeed this is greatly needed in many places and by many people, and no doubt the human race will devise means of dealing with its other consequences, however uncomfortably, as it has done throughout history

But is it not time a more serious attempt was made to establish the human sciences in a saner relationship to the physical ones? A recent survey by the British Academy has revealed the enormous discrepancy between the support given to the two sides in Britain In the field which concerns me most directly as an engineer, of the £5 775 million allocated by the Department of Scientific and Industrial Research in 1959–60 to the support of research, only £40,000 was awarded through its Human Sciences Committee for the investigation of problems of human relations in industry It may be that this sum was not out of scale with the worth-while research effort available The point is that the subject needs to attract a much increased number of good people, and I believe it would be a good investment for the physical sciences to lose some of its best for this purpose—not in any expectation of early benefits, but because longer-term ones will not come otherwise

Meanwhile, in so far as our plans draw to the physical sciences and to engineering an increasing proportion of the best young people available in our schools, we must at least open their minds to the sociological consequences of their work and ensure that they are properly prepared to fulfil the widening human as well as scientific and technical, responsibilities which lie ahead of them

As matters now stand Great Britain is engaged in a scientific and technological battle with countries that can draw on richer natural resources and can bring larger numbers of trained men to bear on defined objectives It is a battle in which, whether we like and agree with it or not, national prestige is linked with priority of scientific and technological achievement, and where what is achieved in one part of the world—or well outside it—is known everywhere within a few hours Our scientists and technologists have become not only the guardians and guarantors of our national economy, but also the agents of a new diplomacy in which more than an expanding export trade is at stake They will have an increasing part to play as advisors and as teachers in the progress of the under-developed countries, and must not only carry conviction as experts in their particular scientific and technical specialities, but also gain respect as ambassadors of broad, sympathetic, vision, sound judgment and unassailable integrity

[See also page 1 of this issue]

THE SNOWY MOUNTAINS SCHEME, AUSTRALIA*

By Sir WILLIAM HUDSON, K.B.E.

Commissioner, Snowy Mountains Hydro-Electric Authority

AUSTRALIA, a continent of about three million square miles and approximately equal in area to the United States of America, carries a population of only some ten million people Nevertheless, owing to its active immigration programme and progressive national development policy, expansion in all fields of industry is proceeding apace This calls for a corresponding augmentation of public services such as roads, transport, water supplies and, last but not least, power

In the field of power production, the growth in demand during recent years has been most marked Superimposed on the load growth brought about by the increase in population there has been an average annual increase of approximately 9 per cent in total energy consumption and approximately 7 per cent in energy consumption per capita

Owing to its geographical position astride the dry latitude band of the southern hemisphere, the limited extent of high mountain ranges and the great distance of its interior from the sea, Australia is the world's driest continent Except in Tasmania and at a few localities along the eastern fringe of the mainland, the scope for hydro-electric development on any large scale is limited On the other hand, New South Wales, Victoria and Queensland, the three States which support 80 per cent of the country's population, have extensive reserves of black and brown coal deposits up to 400 ft in thickness in the case of brown coal and more than 100 ft in the case of black Consequently, coal-burning stations account for about 73 per cent of Australia's total power output Excluding Tasmania, which depends almost entirely on its adequate hydro-electric resources for power, the proportion becomes about 83 per cent

There is no commercial production of nuclear power at present, but developments in Britain and North America are being watched with great interest In Tasmania, hydro-electric resources are likely to provide the most economic form of power develop-

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* Substance of the Citrine Lecture delivered at the British Electrical Power Convention meeting at Brighton during June 4–7

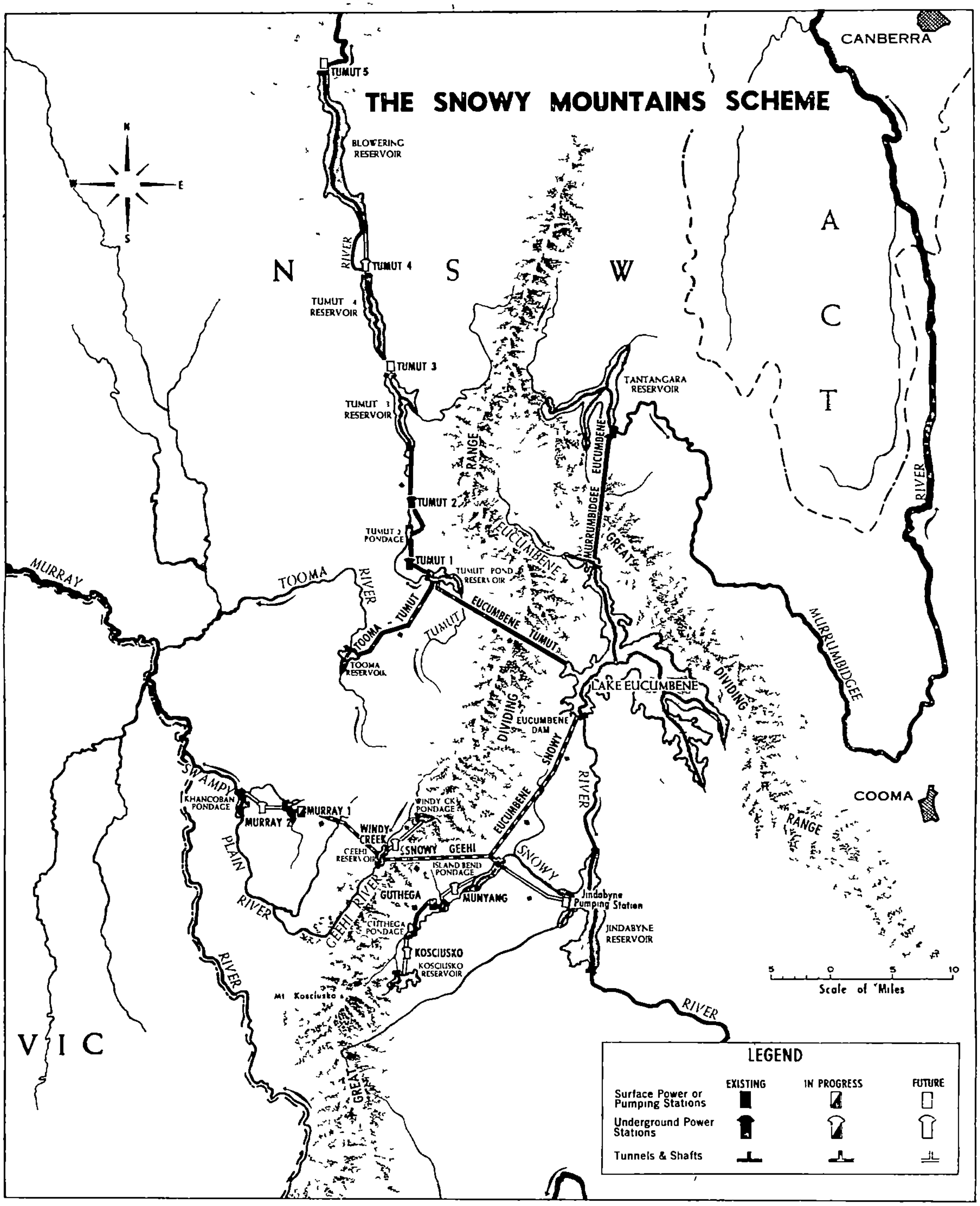
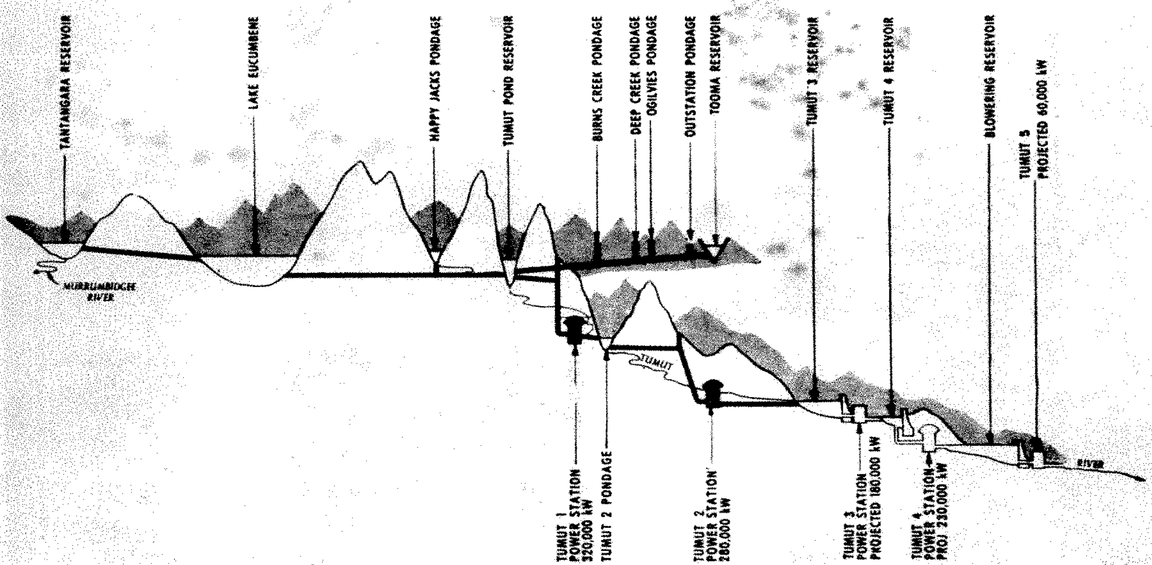


Fig 1

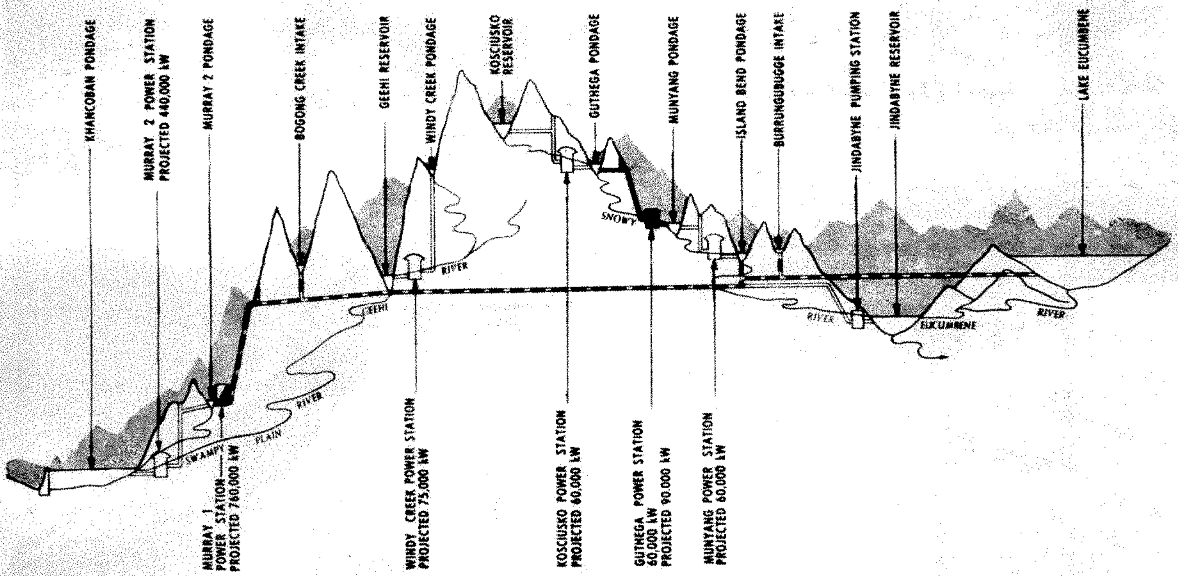
ment for many years to come, and the more highly populated eastern States will continue to utilize their abundant coal reserves and water-power potential, but South Australia and Western Australia are likely to turn to nuclear power stations in the 1970's, dependent on the development of economic plants of the order of 100 MW

Taking advantage of modern improvements in power generation and transmission, New South Wales will be installing 275 MW units and Victoria 200 MW units at new thermal stations now under construction on their coal fields, future plans provide for generators of 350 MW capacity The two States have established a 330-kV transmission system which, when completed, will extend from near the Queensland–New South Wales border in the north to Melbourne at the southern extremity of Victoria, an overall distance of 800 miles

It is perhaps paradoxical that Australia, the world's driest continent, has under construction one of the world's largest hydro-electric projects, the Snowy Mountains Scheme (Fig 1) This Scheme makes a



SNOWY - TUMUT DEVELOPMENT


KHANCOBAN PONDAGE
MURRAY 2 POWER STATION PROJECTED 440,000 kW
MURRAY 2 PONDAGE
BOGONG CREEK INTAKE
GEEHI RESERVOIR
WINDY CREEK PONDAGE
KOSCIUSKO RESERVOIR
GUTHEGA PONDAGE
MUNYANG PONDAGE
ISLAND BEND PONDAGE
BURRUNGUBUGGE INTAKE
JINDABYNE PUMPING STATION
JINDABYNE RESERVOIR
LAKE EUCUMBENE
SWAMPY PLAIN RIVER
GEEHI
RIVER
SNOWY
RIVER
EUCUMBENE
RIVER
MURRAY 1 POWER STATION PROJECTED 760,000 kW
WINDY CREEK POWER STATION PROJECTED 75,000 kW
KOSCIUSKO POWER STATION PROJECTED 60,000 kW
GUTHEGA POWER STATION 60,000 kW PROJECTED 90,000 kW
MUNYANG POWER STATION PROJECTED 60,000 kW

SNOWY - MURRAY DEVELOPMENT

Fig. 2

major contribution to both irrigation and power development, its principal feature being the diversion to inland irrigation areas of the Snowy River waters which previously traversed already well-watered areas and ran to waste in the Tasman Sea.

The Scheme embraces an area of nearly 3,000 square miles of mountainous country and ranks among the largest individual engineering undertakings ever attempted. It involves the construction of nine major dams and several smaller ones, approximately 100 miles of tunnelling, ten power stations and more than 80 miles of aqueducts high up on the mountain ranges to collect the mountain streams which would otherwise miss the reservoirs and tunnels. On completion the Scheme will provide $2\frac{1}{2}$ million kW. of peak load power and 2 million acre-ft. of water per annum for irrigation. Capital cost will be £320 million sterling. By the sale of electricity at competitive rates the Scheme can meet all costs of power production plus those attributable to the supply of water for irrigation without receiving any revenue for the latter.

Earlier investigations and negotiations eventually led to the agreement between the Commonwealth Government and the Governments of the States of Victoria and New South Wales on the distribution of power and water between the two States. The Scheme's main storage, Lake Eucumbene, has a capacity of 3·8 million acre-ft. (Fig. 3), and this, together with the hydraulic control provided by the complex trans-mountain diversion system, guarantees a high degree of regulation and also flexibility in the operation of hydro plants. Underground construction in the form of long trans-mountain diversion tunnels

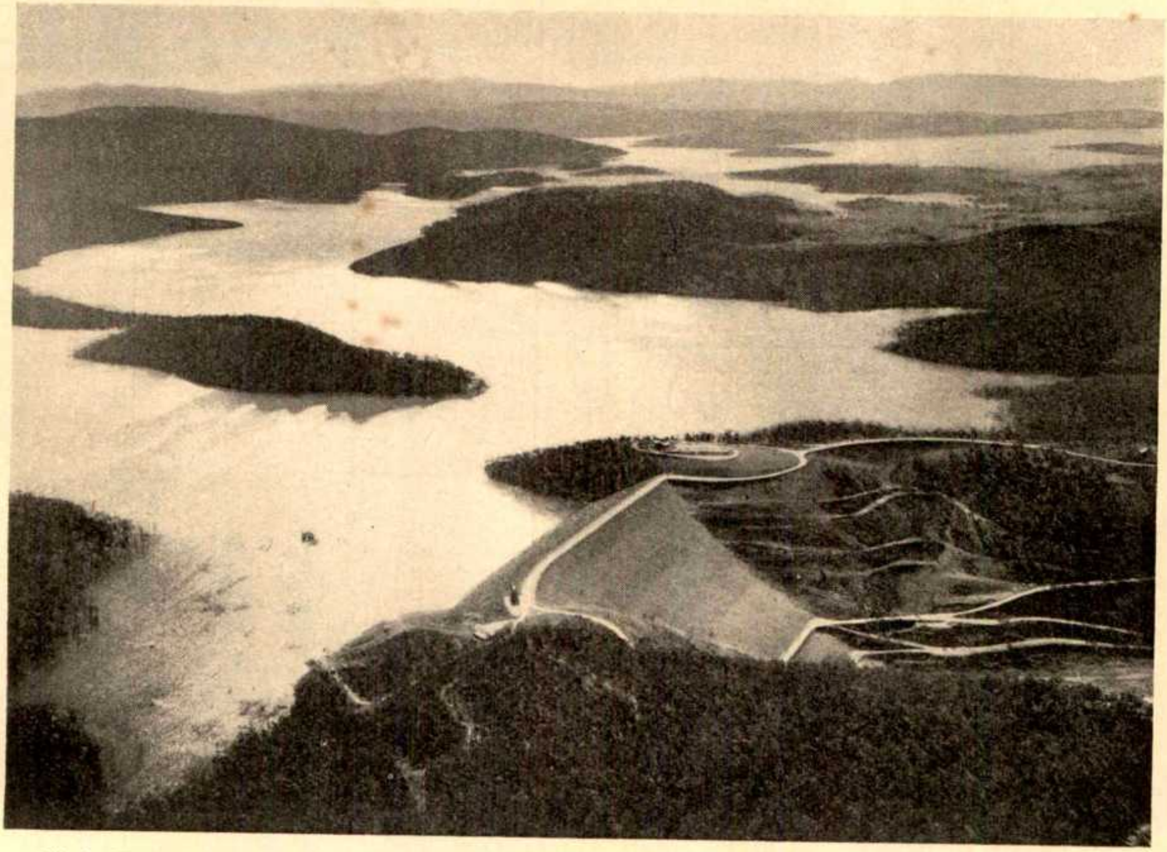


Fig. 3. The 380-ft.-high earth and rock fill Eucumbene Dam forms the Snowy Scheme's main storage, Lake Eucumbene. Part of the vast 3,800,000 acre-ft. storage capacity Lake can be seen in this aerial view

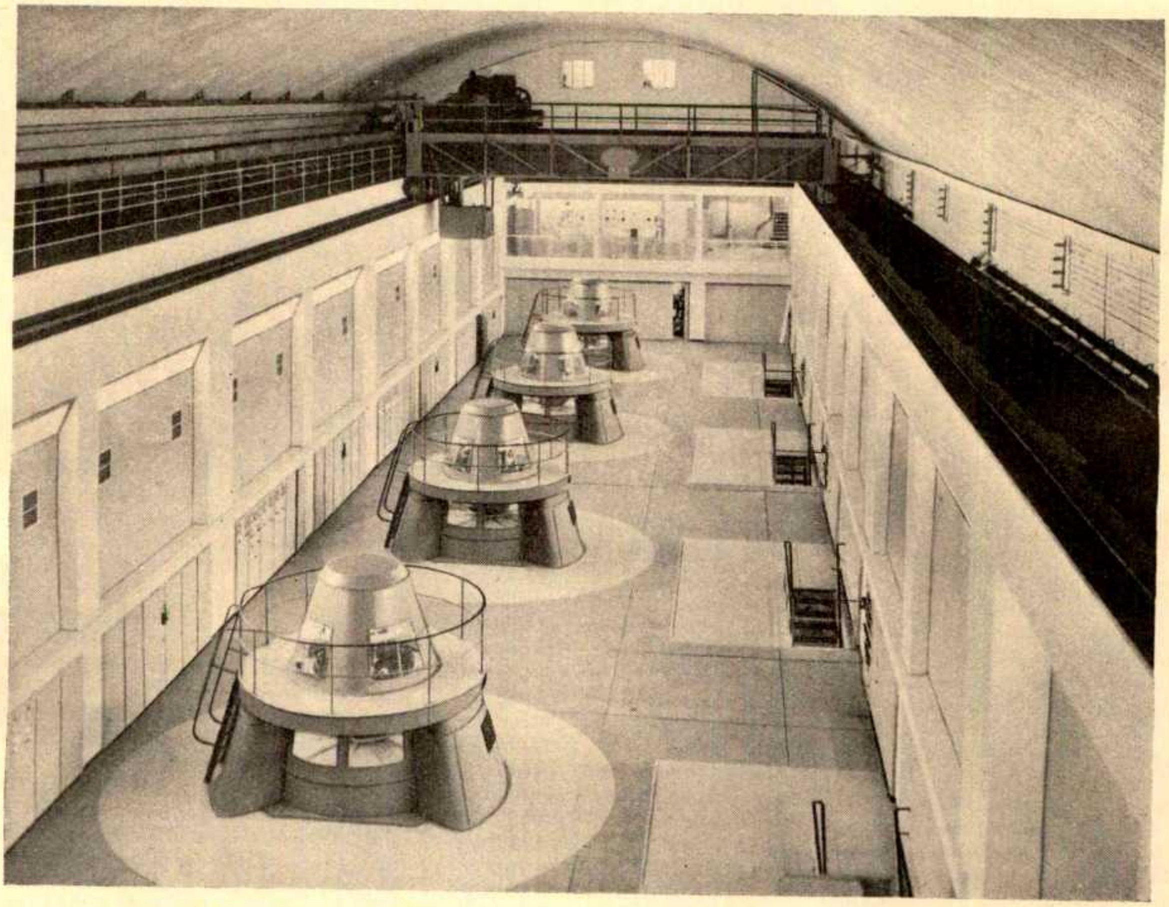


Fig. 4. Tumut I Power Station has an installed capacity of 320,000 kW., and is situated more than 1,000 ft. underground. It has been in service since October 1959

and underground power stations is a characteristic of the Scheme.

The layout of the Scheme falls under two geographical groupings, the Snowy–Tumut Development and the Snowy–Murray Development (Fig. 2).

The Snowy–Tumut Development centres on the diversion of the Eucumbene River, a tributary of the Snowy River, from Lake Eucumbene through a trans-mountain tunnel to Tumut Pond Reservoir on the Tumut River, a tributary of the Murrumbidgee. Storages are also provided on the Upper Murrumbidgee and Tooma Rivers and diversion tunnels bring the waters of these rivers into the Tumut Pond Reservoir. From there, the combined flows of all four rivers will pass through four power stations spaced along the Tumut Gorge, to Blowering Reservoir, where the water discharged from the power projects during the winter months will be held for release during the summer, as and when required, for irrigation in the Murrumbidgee Valley. By utilizing the 2,700-ft. fall from Tumut Pond Reservoir to Blowering Reservoir, the four power stations in the Tumut Gorge will have a total generating capacity of more than 1,000,000 kW. On completion of Blowering Reservoir, the total gain of water for irrigation to the Murrumbidgee River will be approximately 1,120,000 acre-ft. per annum.

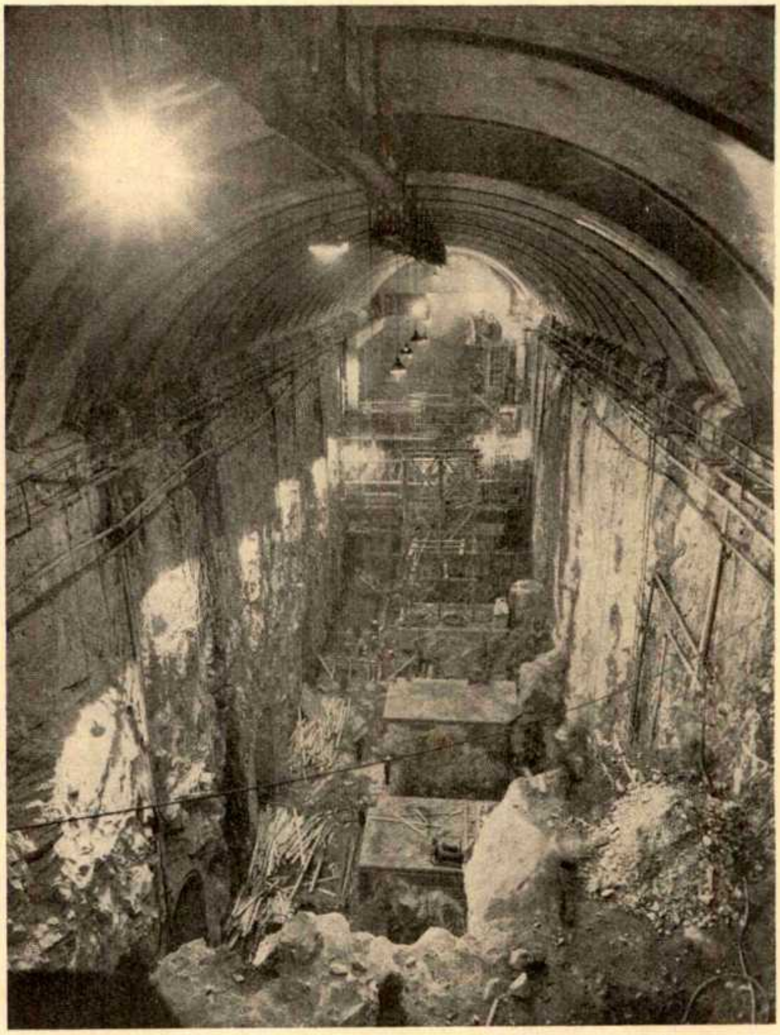


Fig. 5. The 280,000-kW. Tumut II Power Station during construction. The Station is situated more than 800 ft. underground, and this view shows the excavation for the main chamber, which measures 320 ft. long, 51 ft. wide and 105 ft. deep

Of the three completed power stations, Tumut I (Fig. 4), Tumut II (Fig. 5) and Guthega, two are located about 1,000 ft. underground and much use was made of rock bolts which the Authority adopt extensively for rock support in underground workings, eliminating the need in many situations of steel supports and concrete lining.

The Snowy–Murray Development involves the diversion of the Snowy River on the eastern side of the mountains by a dam at Island Bend and a trans-mountain tunnel system, more than 30 miles in length, to the Murray Valley on the western side of the mountains. This tunnel system includes a two-way flow connexion 15 miles long between Island Bend Reservoir on the Snowy River and Lake Eucumbene. Flowing through the tunnels, shafts and pipeline, the diverted waters will fall 2,600-ft. to generate 1·2 million kW. of power in two large power stations. The total gain to the Murray River of water for irrigation will be approximately 800,000 acre-ft. per annum.

The run-off of the portion of the Snowy catchment downstream of Island Bend will be impounded in Jindabyne Reservoir at the point where the Snowy leaves the mountains. A pumping station using off-peak energy will pump water from this reservoir through a tunnel and pipeline 6 miles in length to the trans-mountain tunnel system. The amount of energy required for pumping against a head of 800 ft. to lift the water to the level of the Geehi Reservoir will be about two-fifths of the more valuable 'peak load' energy generated in the turbines of Murray I and Murray II Power Stations by the same water falling 2,600 ft.

Construction of the various components of the development up to and including Murray I Power Station is now proceeding, and plans for the Murray II Project are well advanced. The 760,000-kW. Murray I Power Station will be the largest in the Scheme and, at the time of ordering, the Francis type turbines (160,000 b.h.p. at maximum net head of 1,710 ft.) represented a unique combination of high head and large output for this type of machine. Twin pipelines, a mile long and approximately 13 ft. in diameter, supplying this station will involve 10,000 tons of steel, of which a large part will be high-tensile, notch-ductile grade.

Many problems have been met in establishing in a remote and mountainous area an organization to carry out the work; for example, the need to provide several townships, including housing, schools and modern amenities for the works forces, and measures to encourage speed of construction. A feature of the work so far carried out has been the increasing rate of progress achieved, particularly in hard-rock tunnelling where maximum advancement per face represented substantial increases on the world record tunnelling speeds current at the commencement of the Scheme. Also notable is the almost complete absence of industrial trouble during the past twelve years.

Two out of every three persons comprising the working forces are 'New Australians', recent arrivals from Europe who have chosen Australia as their future homeland. More than 30 countries are represented. The integration of these men and their families, many of whom on arrival could not speak English, into an Australian community proved to be an interesting and successful experience.

[After describing the Authority's Engineering Laboratories and the work of survey, diamond drilling and hydrology teams as examples of the thorough investigations carried out before and during construction, Sir William closed his address with a reference to the indirect but nevertheless substantial benefits arising from the Scheme.] The construction of an extensive road system has greatly facilitated public access to one of the most attractive winter and summer recreation areas in the country, and the Authority's policy is to encourage and assist people wishing to visit the Scheme, 60,000 visitors having been accommodated, fed and transported on two-, three- and four-day conducted tours over the projects in 1961.

The Scheme has now reached the half-way mark. Approximately 300 miles of mountain roads, 50 miles of tunnels, 6 dams, 30 miles of aqueducts have been constructed, and three power projects are in operation supplying water to irrigate dry inland areas and peak load power to the interconnected power systems of south-eastern Australia.

CONTINUOUS PROCESSES BEING INVESTIGATED IN AUSTRALIAN STEEL INDUSTRY RESEARCH

By Dr. HOWARD K. WORNER

Director of Research, The Broken Hill Proprietary Co., Ltd., New South Wales

PRACTICALLY every steel-making plant in the world has greatly increased its output in recent years. Some of this increase has resulted from improvements in technique and efficiency of operation, but in other cases it has been mainly due to the building of more or bigger ovens, furnaces and rolling mills. The high cost of major steelworks units is a matter of serious concern and is leading to re-appraisals of long-established practices like coke-making in by-product ovens, blast furnace iron production, and open hearth steel-making.

It has seemed to me that one way to arrest the trend to bigger and bigger furnaces, ladles, cranes and locomotives in steelworks is to follow the lead of petroleum refiners and chemical manufacturers generally, in replacing batchwise processes, often widely separated, with closely integrated continuous unit operations, and to use, wherever possible, launders pipes and belts to transport materials.

It is appreciated that the handling of molten steel presents difficulties which are not encountered in the transport of oil or plastics, but it is felt that advances in knowledge, the advent of new materials and improved systems of measurement and control have brought the goal within the realms of practical realization.

There are those, of course, who hold that the best coke and steel can only be made batchwise, and with ample time for 'conditioning'. I contend that this is a mistaken view and one which, if maintained, will retard the advancement of the iron and steel industry into the era of full and effective utilization of computer controls.

It is of interest that the trend in this direction has begun at the two ends of steelworks operations; blast furnace ferruginous burdens are now frequently prepared in continuous sintering or pelletizing units, and at the steel finishing end, strip rolling, electro-galvanizing and electro-tinning have become highly automated continuous operations.

An announcement[1] was made in May 1961 at the annual conference of the Australian Institute of Metals that work had started on continuous steel-making[2] in the research laboratories of the Broken Hill Proprietary Co., Ltd. Further information on this and other projects was given[3] at the symposium held at the BHP Central Research Laboratories in March 1962 to celebrate the fifth anniversary of their opening (Fig. 1).

Research on continuous steelmaking has so far been concerned with an appraisal of metallurgical features and with the obtaining of data for the design of a small production unit. It has already been demonstrated in furnaces up to 5 tons per hr. capacity that by the method of continuous 'staged' lancing with oxygen it is possible to produce a wide range of grades of steel; all that is necessary to change grade is to alter the oxygen metered through the last lance, or alternatively to modify the metal flow-rate. Adequate heat is generated in the refining process; increases in temperature of up to 500 deg. C. have been measured along the bath and low carbon steel has been tapped from the experimental units with a superheat of 200 deg. C. The indications are that in full-scale production units there should be appreciable potential for scrap consumption and/or for incorporation of ore, which incidentally need not be in the form of hard lumps as required by the open-hearth process. High magnesium oxide refractories have stood up well to the conditions in the small experimental furnaces; they do not suffer the temperature cycling which is so prone to cause spalling in furnaces operated batchwise.

In certain experiments designed to test the refining potential, remarkable lowering of phosphorus and sulphur contents has been achieved. An intriguing new field of slag-metal chemistry has been opened up, particularly with a practice in which slag can flow countercurrent to metal. The gases, which, of course, are emitted continuously, are both very hot and rich

Fig. 1. Broken Hill Proprietary Co., Ltd., Central Research Laboratories, set among tall eucalyptus trees at Shortland, New South Wales

in carbon monoxide. They would therefore be suitable for use in scrap pre-heating even pre-melting. They could also be used for ore pre-reduction or limestone calcination or for steam raising.

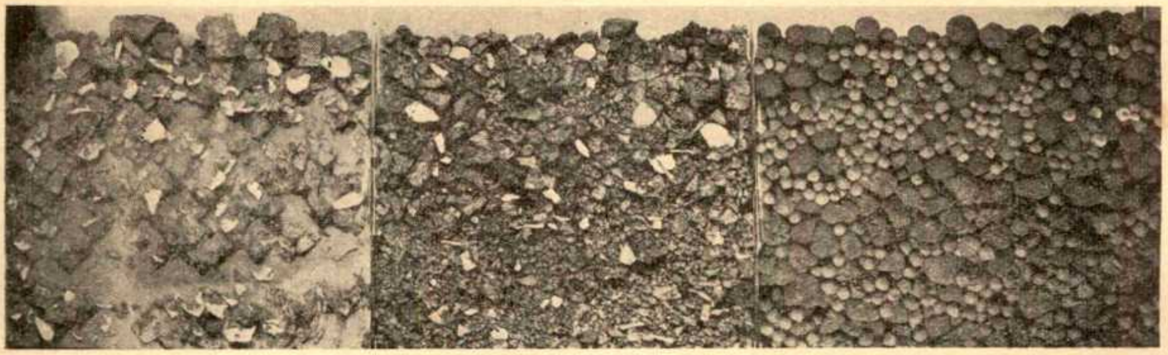
The demonstration of the metallurgical feasibility of continuous steel-making presents a challenge not only to push on with the engineering of full-scale plant but also with: (*a*) the evolution of means of continuous analysis of steel in a slowly flowing stream; and (*b*) continuous or near continuous tapping of blast furnaces. For the former, both physical and chemical methods are being investigated. The solution to the latter will probably lie in two-tap hole practice, hot metal flowing in turn from each tap-hole.

In a production plant it would be necessary to interpose a form of tilting 'mixer' between the blast furnace and the continuous steel-maker and a somewhat similar holding furnace for the steel ahead of casting. The indications are that continuous oxygen steel-making can achieve a steady flow of metal at practically constant temperature, and this is, of course, greatly to be desired in feed to a continuous casting plant. Many troubles arise in present continuous casting plants from changes in temperature in the steel delivered from individual ladles. Finishing additions, preferably in a finely divided form, are appropriately made to the steel as it flows into the tilting holding vessel.

As already implied, future work will seek to achieve not only high metallurgical efficiency in steel-making but also economy in materials handling and site utilization by making the maximum use of gravity flow of liquid metals in hooded refractory launders.

The high productivity of oxygen steel-making processes, whether batchwise or continuous, is possible because of the extremely high rates at which the metalloids, silicon, carbon and phosphorus, burn out of the molten iron when it is jetted with elemental oxygen. This fact has helped the blast furnace to stave off challenges by direct reduction processes. Most of the latter are not able, as is the blast furnace, to produce hot metal with 'internal fuels' ready for oxygen refining.

Contributing materially to the recent great increases in the productivity of iron blast furnaces has been the replacement of ore burdens with either sinter or pellets. The latter, being uniform in size and shape, are showing up to particular advantage



a *b* *c*

Fig. 2. Simulated blast furnace burdens: *a*, unscreened ore, coke and limestone; *b*, screened coke, limestone and fine sinter; *c*, fluxed ore pellets and coke pellets which have by far the greatest permeability to gases

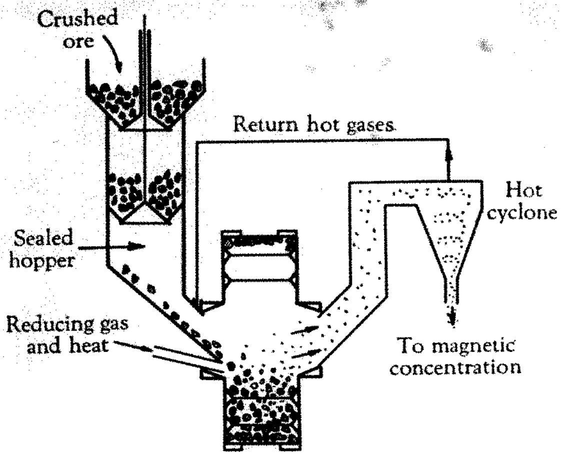


Fig. 3. Diagrammatic section of the pilot plant being used to evaluate simultaneous autogenous grinding and reduction of siliceous ores such as jaspilite

in the relatively few furnaces to which they have so far been charged. It has seemed to the research workers here that if ore pellets can confer such benefits to furnace 'driving-rate', primarily because of their contribution to uniform stack permeability, there should be a further gain if the coke also be added in rounded forms. As yet the researches in this field have not advanced beyond the point of small-scale melting trials in cupolas, but the technical feasibility of producing strong, fissure-free coke pellets or briquettes and of burning them in shaft furnaces has been demonstrated. Significantly, this development has evolved from a systematic study of different methods of continuous coking, and on inferior coking coals at that[4,5]. It has been found that the efficiency of continuous coking can be increased by feeding the coal to the coking unit (strands, columns and kilns are being evaluated) either in the form of pellets or as rounded briquettes.

Not content with the making of strong coke pellets, current research here is concerned with the production of composite pellets in which fine ore, and possibly some flux, are also incorporated. By controlling the heating-rates and with appropriate gas circulation, a substantial degree of ore reduction can be achieved simultaneously with coking. It is felt that the perfection of this practice could contribute to even greater productivity of blast furnaces which are already very efficient 'reactors'.

Investigations of a possible new method of treating siliceous ores have stemmed from a fundamental study[6,7] of the phenomena which occur in a fine-grained hæmatite–quartzite (jaspilite) on heating to about 600° C. in lean combustible gases. Severe intercrystalline cracking occurs due to: (1) the thermal gradients built up in the ore lumps; (2) marked changes in volume in the hæmatite grains as they are reduced to magnetite; (3) the considerable dilation of quartz as it undergoes the $\alpha \rightarrow \beta$ inversion at about 570° C.

The process[8] has been tentatively designated 'CRAG' because it achieves in one unit Combined Reduction and Autogenous Grinding. Experiments in a small pilot plant with 4-ft. diameter grinding drum have yielded very promising results and data are being collected for the design of a large pilot plant.

A new type of dry magnetic separator[9] suitable for recovering the fine magnetite (minus 200 mesh) from the product blown out of the mill is also being developed in the Broken Hill Proprietary Central Research Laboratories.

Thanks are due to the Directors of the Broken Hill Proprietary Co., Ltd., for permission to publish this article and for their continued support of the researches described. Responsibility for the opinions expressed is, of course, accepted by me.

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THE INDIAN BOTANIC GARDEN, SIBPUR, CALCUTTA

By Dr. J. SEN

Superintendent

THE Indian Botanic Garden celebrated its 175th anniversary on July 6. The Garden, with its famous great banyan tree, is perhaps the largest of its kind in Asia and occupies an area of 273 acres on the west bank of the River Hooghly. It is situated in Sibpur only a few miles from Calcutta, and, in the words of the late Sir Arthur Hill, a director of the Royal Botanic Gardens, Kew, "offer[s] an interesting and remarkable parallel to those other Royal gardens . . . at Kew, since Kew is only a few miles from London; while . . . Sibpur gardens are within easy distance of the second city of the Empire and are situated on the banks of the Hooghly which washes the shores of Calcutta. Both . . . gardens suffer somewhat from being on the flat alluvial soil of the riverside; the skill of the gardener, however, has transformed both sites into gardens of high scientific interest as well as of great aesthetic beauty, so that they serve as a source of inspiration, education and research for their many visitors".

For nearly two centuries now, Sibpur Garden has been a centre of botanical and horticultural research. Some of the pioneer works on the flora of India, Burma and Malaya, as also the introduction of plants like tea, cinchona, mahogany, etc., to India are credited to men who have worked here. The utilization of jute, improvement of Indian cotton and cultivation of flax, hemp, ramie, tobacco, vanilla, coffee (Arabian and Liberian), cocoa, ipecacuanha, india-rubber, tapioca, cardamoms, potato, henbane, aloes. sarsaparilla, jalap, Japanese mulberry, varieties of sugar-cane and plants of varied economic importance were also made possible in this Garden and through its agency. Since the 150th anniversary about seventy-five plants were successfully introduced in spite of the fact that the Plant Introduction Office in Delhi has already taken over this work. It is needless to discuss in detail the numerous experiments in the cultivation of economic plants which have been conducted in the Garden since its beginning. As regards horticulture, it may suffice to say that a large proportion of the kinds of exotic plants now found in different gardens in India was introduced into the country through the efforts of the Garden authorities, and that the present improved methods of cultivation were to a great extent initiated here. Thus this Garden has played an important part in the economic growth of India.

The present plant population of the Garden is more than 12,000, grouped on a geographical basis (that is, typical plants of a country are planted in a particular area). Among the interesting show-spots in the Garden are the large palm-house, the small palm-house, the orchid-house, the fernery, the cacti and succulents, the branching palms, the screw-pine collection, the bamboo collection, the giant water-lily of the Amazon, the picturesque vistas, and some of the pleasant lakes and delightful walks. The Garden is celebrated as the most useful and the most beautiful in the East, as in the words of the late Sir Joseph Hooker, another director of the Royal Botanic Gardens, Kew, it having "contributed more useful and ornamental tropical plants to the public and private gardens of the world than any other establishment before or since".

The herbarium and library have gradually developed as necessary adjuncts to the Garden. The herbarium was established by Dr. William Roxburgh in 1793, and now contains more than 2.5 million specimens. Since April 1957, the major part of the collection was transferred to the newly re-organized Botanical Survey of India. The library has a valuable collection of about 30,000 volumes on botany, forestry, agriculture, entomology, geology and related sciences. Some old manuscripts, scientific correspondences and albums containing original paintings of Indian plants, which have been and are still being prepared since the early nineteenth century, are part of its rich heritage.

The Lloyd Botanic Garden, Darjeeling, has been developed as a subsidiary garden to the Sibpur Garden. Established in 1878 when Dr. T. Anderson was the superintendent, the Garden in Darjeeling has been the centre of introduction and cultivation of temperate plants, which do not grow in Sibpur. The herbarium contains about 30,000 specimens covering nearly all the species of the Eastern Himalayas. There are a small library and a laboratory.

Thus the Indian Botanic Garden has become an important venue for the botanists in India. They, and often the Indian people, owe a great debt to its founder, Lieut.-Colonel Robert Kyd, and the long chain of his successors, who gave India important economic plants to increase her wealth, and laid the foundation of Indian botany. Dr. William Roxburgh (1793–1814), who was the immediate successor of Colonel Kyd, is rightly called the 'father of Indian botany' for his *Hortus Bengalensis*, *Flora Indica* and *Plantae Coromandelianae* in three large volumes. Some of his original specimens are still preserved in the herbarium. Dr. Roxburgh established jute in this Garden and exported the first 100 tons of this crop to the European market. He was succeeded by Dr. Francis Buchanan (afterwards Sir Buchanan Hamilton) (1814–16), a naturalist of great reputation.

In 1817, Dr. Nathaniel Wallich succeeded Dr. Buchanan. Dr. Wallich organized expeditions into the remote and then little-known regions of Kumaon, Nepal, Sylhet, Tenasserim, Penang and Singapore; in fact, undertook a botanical survey of a large part of the Indian sub-continent, and made extensive collections, which together with other collections were distributed among the herbaria of Europe. His *Plantae Asiaticae Rariores* in three superb volumes illustrated by coloured figures are of a high degree of excellence. He retired in 1846 and died in 1854. When Dr. William Griffith (1842–44) officiated during the lengthened absence of Dr. Wallich in Europe, he was upset to find the denuded condition of the herbarium. His extensive notes and drawings were published in nine volumes after his premature death. Dr. Hugh Falconer, well known for his researches on the Siwalik fossil mammals, succeeded Dr. Wallich and remained in office until 1855. Dr. Thomas Thomson came into office in the same year and retired in 1861. He is well known as the coadjutor of Sir Joseph Hooker in the collection and distribution of an extensive herbarium of East Indian plants, and the joint author of the first volume of a new *Flora Indica*.

Dr. Thomson was succeeded by Dr. Thomas Anderson, who died prematurely in 1870 of a disease contracted during his efforts to introduce the quinine-yielding cinchona plants into the Sikkim Himalayas. Dr. Anderson was also at the same time the first conservator of forests for Bengal and officer-in-charge of the introduction and cultivation of cinchona in India. Mr. C. B. Clarke officiated as superintendent for about two years (1869–71) and was one of the collaborators in Hooker's *Flora of British India*. When Dr. (afterwards Sir) George King became superintendent in 1871, the Garden was still suffering from the effects of devastation caused by the two great cyclones of 1864 and 1867. Besides reorganizing and reviving the Garden and the Herbarium, Dr. King initiated the publication of the *Annals of the Royal Botanic Garden* and himself contributed a number of important papers and monographs to the *Annals*. He was largely responsible for the establishment of the Botanical Survey of India in 1890 and became its *ex-officio* director, and this arrangement of superintendent holding the office of director of the Survey continued until the post of director was abolished about twenty-five years ago.

Surgeon-Major (afterwards Sir) David Prain succeeded Sir George King in 1897 and gained a world-wide reputation by his works on the genera *Pedicularis* and *Dioscorea*, and regional floras. He retired in 1906. Captain (afterwards Colonel) A. T. Gage succeeded him and continued in office for about seventeen years. His works on Euphorbiaceae and the Burmese flora have earned for him a good reputation. Mr. C. C. Calder, well known for his works on *Oxalis* and vegetation patterns of India, took office in 1923. He was succeeded by Dr. K. P. Biswas (1937–55), followed by Dr. D. Chatterjee (1955–60), both well known to Indian botanists. Among the important personalities who at one time officiated as superintendent are Dr. William Carey (1813–14), Sir William Wright Smith (1908), and Dr. J. M. Cowan (1926–27).

This is a brief record of the almost uninterrupted history of the Indian Botanic Garden, which has been thriving under the Government in Calcutta.

NEWS and VIEWS

Ipatieff Prize of the American Chemical Society
Prof. C. Kemball

The Ipatieff Prize for 1962 of the American Chemical Society has been awarded to Prof. C. Kemball, professor of chemistry in the Queen's University of Belfast. The prize, consisting of 3,000 dollars, is named after V. N. Ipatieff (1867–1952) and is awarded once every three years for outstanding chemical experimental work in the field of catalysis or high pressure to a person not over forty years of age. The rules of eligibility state that preference shall be given to American chemists, and the award for 1962, although it is the sixth in the series, is the first to have been made to a non-American. Prof. Kemball has developed a research school at Belfast in the field of heterogeneous catalysis which is acquiring an international reputation. He received a Meldola Medal for 1951 on the recommendation of the Royal Institute of Chemistry and the Corday-Morgan Medal of the Chemical Society for 1958.

Shewhart Medallist of the American Society for Quality Control:
Mr. L. H. C. Tippett

The Shewhart Medal of the American Society for Quality Control "for outstanding leadership in the field of modern quality control" has been awarded to Mr. L. H. C. Tippett, an assistant director of the Cotton, Silk and Man-made Fibres Research Association, Shirley Institute, Manchester. This is the first time that the award has been made to a non-American. Mr. Tippett, who has been at the Shirley Institute since 1925, is the author of many books and papers on applied statistics and quality control and is a leader in the field of statistical investigation of process operations in the textile industry. Earlier recognitions of his work include the Guy Medal in Silver of the Royal Statistical Society and the Warner Medal of the Textile Institute. He was the first chairman of the Committee on Statistics in Industry and Technology of the International Standards Organization during 1949–58, and the first chairman of the Textile Institute's Quality Control Group during 1959–61.

Physics at Minnesota
Prof. C. J. Waddington

Dr. C. J. Waddington has been appointed an associate professor of physics in the University of Minnesota. Dr. Waddington was an undergraduate in the University of Bristol, and afterwards became a research student there, taking his Ph.D. in 1955, after making investigations on the helium nuclei present in the primary cosmic radiation. These experiments showed that the conventional theories of geomagnetism were unable to predict precisely the behaviour of cosmic ray particles in the Earth's magnetic field, and they led to a greater appreciation of the importance of the non-dipole components of this field. During 1955–59, Dr. Waddington held a Royal Society McKinnon research studentship which enabled him to continue his work in Bristol on the composition of the primary cosmic radiation, and the eleven-year variation in the intensity of these particles. During this time, 1957–58, he spent one year in the University of Minnesota. In 1959 he was appointed lecturer in physics in the University of Bristol—a post which he has continued to occupy until the present time. He spent six months at the National Aeronautics and Space Administration in the United States in 1961, continuing researches into the various aspects of primary cosmic radiation, and contributing to the development of techniques whereby photographic emulsions are used to detect temporal changes in the intensity of the primary cosmic radiation. Dr. Waddington is the author or the co-author of more than forty papers on subjects related to cosmic radiation.

Biochemistry, Nutrition and Food Science in Ghana
Prof. F. Aylward

The new Department of Biochemistry, Nutrition and Food Science in the University of Ghana is the first of its kind in Africa. The appointment of Dr. Francis Aylward as professor and head of this Department will ensure that this important development gets off to an excellent start. The Department will be concerned chiefly with courses leading to the degree of B.Sc. in Food Science, and with development and research in foods and food processes. Dr. Aylward's initial training was as a chemist and a biochemist. He was awarded first-class honours in chemistry at the B.Sc. examination in the University of Liverpool in 1932, and a Ph.D. in biochemistry in 1934. He has done research at the Hannah Dairy Institute in Ayr, in the Universities of Liverpool, Manchester and Johns Hopkins in Baltimore, and with Imperial Chemical Industries, Ltd. Recently, he was head of the Department of Chemistry and Food Technology in the Borough Polytechnic, London.

Dr. Aylward's publications demonstrate the growth of his increasing interests in the problems of food and nutrition. His early work was on the lipids, more recently, it has extended to studies on chemical additives to food and to investigations of dietary patterns and food habits. During the past two years, Dr. Aylward served as scientific adviser on foods and nutrition to the University of Ghana, on behalf of the Food and Agriculture Organization of the United Nations. He has produced a short but comprehensive report on foods and nutrition to the Government of Ghana (see p. 27 of this issue). The initiation of the new Department, with Prof. Aylward as its enthusiastic and versatile head, can be expected to serve as a model for other African countries in their attack on hunger and malnutrition.

Psychology at Hull:
Dr. A. D. B. Clarke

Dr. Alan D. B. Clarke, at present consultant psychologist and head of the Department of Psychology at the Manor Hospital, Epsom, has been appointed to the chair of psychology in the University of Hull with effect from October 1, in succession to Mr. G. Westby, who has been appointed to the chair of psychology in the University College of South Wales and Monmouthshire (see *Nature*, 192, 918, 1961). Dr. Clarke was appointed senior psychologist and head of the then new Department of Psychology at the Manor Hospital at Epsom in 1951, and under his guidance the Department has become one of the leading psychology units in Britain. His pioneer work on the training of mental defectives has revised all previous ideas on this subject and has been of the greatest social and psychological value

Dr Clarke is an active member of various committees of the British Psychological Society's Division of Professional Psychologists, and during 1954 he acted as secretary of the Society's Working Party on Mental Deficiency which prepared the memorandum of evidence submitted to the Royal Commission. His book *Mental Deficiency: the Changing Outlook* is considered to be one of the best on mental deficiency by an English-speaking psychologist, and he has contributed many papers to learned journals on clinical problems relating to intelligence, personality, learning in imbeciles and their employability, on social and emotional deprivation and also on general areas of psychology. With his wife, Dr Ann Clarke, who is also a psychologist at The Manor, he has recently been associated with the production of a documentary film on *Learning in Slow Motion* relating to their research. This film has been taken over for distribution purposes by the Central Office of Information.

Nuclear Power Research and Development in Britain

In a written answer in the House of Commons on June 4, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that a design team had been established to consider nuclear ships by the Atomic Energy Authority in conjunction with the British Ship Research Association and other interested organizations. The principal systems being studied by the team were the steam-cooled and steam-generating heavy-water reactors and advanced designs of pressurized-water and boiling-water reactors. Contracts for design study work had been negotiated with the Nuclear Power Group, the United Power Company, Rolls Royce and Babcock and Wilcox. Work was also in progress on reactor physics, engineering development, fuel-element technology and reactor materials in support of the marine reactor systems. In a further answer, Mr. Freeth gave the estimated cost of the experimental advanced gas-cooled reactor which should come into operation within the next two months as about £9.7 million, and the annual outlay for the operation and maintenance of the Dounreay experimental fast reactor as at present being about £2 million. On June 5 he stated that organizations for which the Minister for Science was responsible supported research in seismology by twenty-four qualified scientists, of whom twenty were employed by the Atomic Energy Authority and four were supported by research grants from the Department of Scientific and Industrial Research. The Meteorological Office employed two further scientists and the Universities of Aberdeen, Cambridge, Durham, Edinburgh and Liverpool had departments participating in seismological research.

Petroleum in the United Kingdom, 1961

United Kingdom industry statistics relating to consumption and refinery production of petroleum and its products for 1960 and 1961 was published by the Petroleum Information Bureau in April 1962, on behalf of the U.K. Petroleum Industry Advisory Committee (*U.K. Petroleum Industry Statistics—Consumption and Refinery Production, 1960 and 1961*. Pp. 9. London: Petroleum Information Bureau, 1962). This reveals that the internal demand for petroleum products was 7.8 per cent higher in 1961 than in the previous year, total consumption (excluding bunkers for ships engaged in foreign trade) being 46,014,950 tons. The standard products as now classified include aviation fuels, motor spirit, industrial spirits (with industrial benzole), white spirit, kerosene (burning and vaporizing oils), derv fuel, gas, Diesel and fuel oils, lubricating oils and greases, paraffin wax and scale, propane and butane, bitumen, chemical feedstock, light distillate feedstock, and refinery gases. Aviation fuels show for 1961 a 22.3 per cent rise, motor spirit increased by 6.8 per cent, burning oil consumption was 4.9 per cent lower, notwithstanding a rise of 50 per cent for domestic heating boilers compared with the previous year, due to the greater demand in the number of small and medium-sized houses for oil-fired central heating. Derv fuel was 9.9 per cent higher, lubricating oils only 1.5 per cent up, bitumen 9.7 per cent increase, and deliveries of propane and butane rose by 34 per cent, on account of heavier use in industry as gas-making feedstock. There was a rise of 11.1 per cent in consumption of refinery gases, but deliveries of chemical feedstock, which in recent years have advanced extremely rapidly, remained almost static compared with 1960. An interesting note on tax effect on consumption of motor spirit is that the 3*d*. per gallon surcharge imposed by the 'little Budget' in July 1961 (consolidated in the 1962 Budget to produce a standard rate of duty of 2*s*. 9*d*. per gal.) caused a tendency for lower grades of petrol to be favoured at the expense of the super brands.

U.K. Attitudes towards Population Control

The question of population pressure in Asia was again raised in the House of Lords by Lord Casey on June 6, who specifically asked the Government what it proposed doing to stimulate scientific research in the United Kingdom towards a socially acceptable solution of the problem applicable to Asian conditions. Referring particularly to population growth in India and Pakistan and the introduction of family planning, Lord Casey urged the need for research into biological methods of contraception. However, other speakers in the debate, including Lord Adrian, were more cautious regarding the value of recent introductions mentioned by Lord Casey and stressed the need to consider the subject in the widest possible context, Lord Milverton expressing the view that no scientific solution of the problem could be other than an adjunct to long-term educational, economic and cultural influences. Lord Brain emphasized the same point of view, urging that the population question was not solely a matter of food for the body, and besides stressing education pointed out the risk of epidemics spreading through air travel. He suggested that careful watch should be kept for any short-term reactions in introducing new oral contraceptives and that the Government should hold a watching brief on clinical tests of all contraceptives, if they did not sponsor such tests themselves and give a lead to the World Health Organization.

In replying for the Government on the debate, the Parliamentary Under-Secretary of State for Commonwealth Relations, the Duke of Devonshire, said that the Government was well aware of the problem and he agreed as to the need to increase food supply. On scientific research, he said that research into methods of contraception was linked with research into the whole field of infertility and that both fields were closely connected with some lines of cancer research. There was no indication of a shortage of funds for any promising lines of work and there was close co-operation between scientists in Britain and in India and elsewhere. Much work on the physiology of reproduction was being undertaken in university

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departments and by the Medical and the Agricultural Research Councils, in addition to a certain amount directly aimed at the control of conception, carried out by industrial enterprises. The Government would always be ready to accede to requests for help from Governments under the Colombo Plan. However, until such requests for technical assistance were received, Britain's main contribution to the problem of over-population should be to continue to give maximum support to the existing programme of capital aid and technical assistance. Such programmes would leave the Governments concerned free to judge how the help given could be best applied to overcome poverty and ignorance.

Forestry in Britain

IN his address to the Royal Society of Arts on the subject of forestry (the second of three Cantor Lectures on the Development of Natural Resources, May 14, 1962), Mr James Macdonald, deputy director-general of the Forestry Commission, dealt first of all with the productive side of forestry. Only some 7 per cent of Britain's land surface is covered with forests, which is a low figure compared with many European countries. The tree line in Britain is relatively low and exposure may be severe, thus preventing the use for forestry purposes of localities which would otherwise be suitable. On the other hand, tree growth in some localities is faster than on the Continent. As a result of plantings during the past forty years, the acreage under conifers (all exotic except the Scots pine) has increased and that under hardwoods has diminished. The distribution of age-classes is not ideal, there being an over-abundance of old hardwoods and a deficiency of old conifers. However, this state of affairs will be adjusted before very long, particularly with the conifers which are grown on shorter rotations than the hardwoods. It is considered that in the next seventy years production will be doubled from private forests and trebled from Forestry Commission forests. This increased production will be required in Britain, which in 1960 imported £456 million of timber. According to investigations by the Food and Agriculture Organization, the increasing deficit in the supply of timber will continue for the next few decades and there are some North American authorities who consider it probable that there will be no exportable surplus of timber from that continent by the end of the century. Forestry has an important part to play in upland areas in Britain, where it can increase production from the land and also help other land users with better communications and better grazing conditions without any diminution of livestock. The National Forest Parks provide facilities for recreation without interfering with the continued practice of forestry. Forests are also a haven for wild-life and thus help in its preservation. Mr Macdonald ended his talk by referring to the multiple uses of forestry in Britain, some of which are assessable in cash while others are not, but both private and State forests "represent a substantial contribution to national resources and a contribution which is growing greater year by year".

Materials Research

THE first number of a new international quarterly, *Materials Research*, was published in April (1, No. 1, April 1962. Published quarterly. Annual subscription £5. London: Heywood and Co., Ltd., 1962). The editors are Dr. E. G. Stanford (Banbury), Prof. T. J. Dolan (Illinois) and Prof. P. G. Bastien (Paris). Papers are in English, but abstracts of all the papers are printed in English, French, German, Italian and Russian. The sub-title of the periodical is "An International Journal of the Properties and Testing of Engineering Materials", and on the evidence of the seven papers included in the first issue, the emphasis is to be evenly distributed between these two fields. One group of papers is concerned with the use of ultrasonic flaw-testing equipment and with a rapid induction method for measuring electrical conductivity, while the other research papers deal with creep, notch-brittleness and an engineering aspect of fatigue. One of the declared aims of the periodical is to analyse the reasons for the particular properties of various materials. This aim has been pursued in an introductory review paper, which attempts a survey of the properties of metals in terms of crystal structure and two-phase distributions. In the light of this first issue, it appears likely that the journal will concern itself with comparatively empirical studies of engineering materials, together with an intensive examination of modern methods of materials testing and inspection. Since there is a shortage of journals dealing with testing methods, it is to be hoped that the editors will continue to give considerable space to good papers in this important field.

Topology

IN the course of this century, topology has grown from some isolated and special problems into one of the major disciplines of modern mathematics, with a considerable terminology and an array of methods which are influencing almost every other branch of the subject. The untimely death of that great topologist, Henry Whitehead, has fortunately not prevented his project of an international journal for topology from coming to fruition, thanks to the scientific enterprise of the Pergamon Press (*Topology*. Vol. 1, January–March, 1962. An International Journal of Mathematics founded by J. H. C. Whitehead, Oxford. Pp. 80. Published four times yearly. London and New York: Pergamon Press, 1962. Annual subscription rates: *A*, for libraries, government establishments, research laboratories, etc., £10 or 30 dollars; *B*, for individuals who place their orders directly with the publisher and certify that the journal is for their personal use, £3 10*s*. or 10 dollars). The new journal is a quarterly, and the first part appeared in the spring of this year. There is a strong editorial board consisting of M. F. Atiyah (Oxford), R. Bott (Harvard), F. Hirzebruch (Bonn), I. M. James (Oxford), R. Thom (Strasbourg), and an editorial advisory board of great distinction: H. Cartan (Paris), K. Kuratowski (Warsaw), M. H. A. Newman (Manchester), H. Seifert (Heidelberg), N. E. Steenrod (Princeton).

South African Museum, Cape Town

To an increasing extent museums are now seeking to advance knowledge by research and publication, and this is in addition to their previous functions of acquisition and conservation. In a recent issue of *Annals of the South African Museum* (46, Part 9: *On the Dentition and Tooth Replacement in Two Bauriamorph Reptiles*. Pp. 231–256+plates 12–14. Cape Town: South African Museum, 1962. 52 cents), Dr. A. W. Crompton gives the results of his researches on the dentition and tooth replacement in two Bauriamorph reptiles (*Ericiolacerta parva* and *Bauria cynops*). He finds that alternate tooth replacement was present in both genera *Ericiolacerta* represents an early stage in the transition from a sectorial to a pounding or crushing dentition, but it is doubted whether a true grinding action was present in *Bauria*

Biological Objects in Plastic

It has become increasingly the custom in Museums to embed various biological specimens in plastic and thus preserve colour and shape far better than by the older methods Messrs G Notini and Sigrid V Wettstein have just published in the *Bulletin of the Royal School of Forestry, Stockholm* (*Kungl Skogshogskolans Skrifter* Nr 36 "Embedding Biological Objects in Plastic" Pp 34 Stockholm Emil Kihlstroms Tryckeri AB, 1961) a very useful paper with full descriptive notes of the technical work involved The authors have carried out a prolonged investigation on this subject, and the paper includes details not only of the preparation of objects for embedding but also of the actual embedding procedure and the details as applied to the following types of objects Lepidoptera, Coleoptera, Hymenoptera, Diptera, spiders, fishes, crustaceans, centipedes reptiles, small mammals and anatomical preparations Also included is a useful list of references to previous work

Semiconductors and Transistors

The Special Subject List No 36 of the Library Association, entitled *Semiconductors and Transistors*, has been compiled by E H C Driver, librarian of the College of Advanced Technology Birmingham (Pp 19 6*s*, Library Association members, 4*s* 6*d* London The Library Association, 1961) It contains a preface by Prof R A Smith, University of Sheffield, and represents an adequate, but obviously not complete, bibliography of the large and rapidly growing literature on the subject Books published earlier than 1955 have been included only if especially useful and most of the entries refer to the years 1959–62 The 63 entries, apart from a separate list of periodicals, are grouped under the headings guides to the literature, abstracts, surveys and reviews, definitions, standards, and a select book list divided into five sections Popular paper-backs on transistor circuits for the home constructor have been excluded No prices of books are given and the entries have been limited to texts in English The List, which concludes with an excellent index, should be most helpful to those searching the literature or wishing to commence a small collection of books on the subject of semiconductors

The National Meteorological Library

Now that the Meteorological Office is fully established in its new building at Bracknell, Berkshire, its library is properly housed in suitable accommodation, and, except for its geographical location, is fully accessible Some of the material stored is on open shelves, and there are large stock rooms to which readers are not normally admitted The reading room is large and pleasant, with card index and loan facilities readily available The indexing is thorough, and in addition to the usual author-index there is a comprehensive subject-index which is based on the Universal Decimal Classification In this the author and title of every paper held on each subject are listed in chronological order A considerable number of translations of foreign literature are held, and this store is being continually augmented There is a room set aside for viewing micro-films and micro-cards These valuable facilities are available to the public for *bona fide* purposes

Testing Ferrous Metals for Corrosion Resistance

A useful booklet has been prepared by Mr K A Chandler for the Methods of Testing (Corrosion) Sub-Committee of the British Iron and Steel Research Association (*Testing Ferrous Metals for Corrosion Resistance* Pp 27 (MG/BF/19/61) London British Iron and Steel Research Association, 1962 3*s* 6*d*) In this he discusses a number of simple corrosion-testing procedures which require neither specialized experience nor complex equipment Designed, in the main, to meet the needs of small and medium-sized firms, it starts with a general introduction and then goes on to consider the choice of test and the preparation of specimens Laboratory tests are dealt with in the longest chapter and followed by field tests and service trials The final chapter is a very helpful account of the assessment of the results in terms of practical behaviour

Utilization of Activated Sludge

The activated sludge process is an aerobic and rapid method of sewage purification, which yields a sludge that settles down quickly, leaving a clear, well-oxidized effluent These changes are essentially brought about by micro-organisms which thrive in sewage under aerobic conditions Since the early days of the development of the process, attempts have been made to utilize activated sludge for agricultural purposes In recent years the possibility of utilizing sludge more directly as a feed supplement for animals has been investigated An article by S Sathyanarayana Rao and S C Pillai, of the Department of Biochemistry, Indian Institute of Science, Bangalore, gives a brief account of the studies, particularly on vitamin B_{12}, in activated sludge and on the utilization of the sludge as a feed supplement for farm animals, such as chicks and pigs (*J Sci Indust Res A* General 21*A* No 1, 1962) The work was started soon after the discovery of vitamin B_{12} and recognition of its importance in the nutrition of man and animals There is already some evidence to indicate the value of activated sludge as a feed supplement In addition to certain nutrients, activated sludge also may contain other substances as a result of intense microbial activity Activated sludge concentrates coenzyme $Q'2$, and it may be considered as a source of coenzyme Q in view of the recent attempt to produce it by bacterial fermentation of certain synthetic media

The Royal Commission for the Exhibition of 1851 Awards

The following awards for 1962 have been announced *Senior Studentships* K Bearpark (University of Oxford) for research in physics at Oxford, J F P Hudson (University of Cambridge) for research in mathematics at Cambridge, D I John (University College, Swansea, and Imperial College of Science and Technology) for research in organic chemistry at Imperial College, J R Waldram (University of Cambridge) for research in physics at Cambridge *Overseas Scholarships* (1) *Canada*, M C L Gerry (University of British Columbia) for research in physical chemistry at Cambridge, P Copper (University of Saskatchewan) for research in geology at Tubingen, J J Simpson (University of Toronto) for research in nuclear physics at Oxford, (2) *Australia*, J D Cotton (University of Melbourne) for research in inorganic chemistry at Cambridge, J L Black (University of Melbourne) for research in nuclear physics at Oxford, B D Jones (University of Sydney) for research in mathematics at Glasgow, (3) *New Zealand*, J F Young (University of Canterbury) for research in inorganic chemistry at Imperial College of Science and Technology, (4) *Pakistan*, E Ahmed (University of Dacca) for research in theoretical physics (place of study to be arranged), (5) *Singapore*, Miss P. H Yuen (University of Singapore) for research in zoology at the London School of Hygiene and Tropical Medicine; (6) *Republic of Ireland*, R E Burton (Trinity College, Dublin) for research in theoretical chemistry at Oxford.

Courses in Applied Mechanics

THE Postgraduate Department of Applied Mechanics of the University of Sheffield will hold the following introductory courses during the academic year 1962–63 electronics for engineers, stress determination, fine measurement in mechanical engineering, gear design, analysis of failures of machines and structures, corrosion and fatigue, non-destructive testing, hydraulic operation and control of machines, servomechanisms and automatic controls, gear loading, stress and life, friction wear and lubrication, analogue computing methods for engineers, noise, mechanical vibration, techniques in control engineering The courses are open to graduates in engineering or related subjects and to those with comparable technical training Further information can be obtained from Prof W A Tuplin, Department of Applied Mechanics, University of Sheffield, St George's Square, Sheffield

Physics Department at Stanford University

IN the note entitled "Nobel Prize for Physics Prof Robert Hofstadter" that appeared on p 799 of the December 2, 1961, issue of *Nature*, it was stated that Prof Hofstadter became head of the Physics Department at Stanford University Prof Hofstadter writes that this does not agree with the facts "Prof Leonard Schiff has been Executive Head of the Physics Department at Stanford University since July 1, 1948 Under his leadership the Physics Department has made many significant contributions to the field of physics Throughout this long period Prof Schiff has consistently been active in theoretical physics and he himself has made important progress in many different fields of physics" Prof Hofstadter is, in fact, a member of Prof Schiff's Department.

University and College News:

College of Aeronautics

THE following appointments have been announced J R Busing, to be senior lecturer in the Department of Aerodynamics, K H Griffin, to be senior lecturer in the Department of Aircraft Design, D Hyde, to be lecturer in the Department of Flight, D W McQue, to be instrumentation engineer in the Department of Electrical and Control Engineering, W G. Bradley, to be Research Fellow in the Department of Flight

Edinburgh

THE following appointments to lecturer have been made· Dr M M Harding (chemistry), Dr A L Haigh (veterinary physiology), Dr P Walker (zoology), Dr M Williamson (zoology)

Keele

THE following appointments and promotions have been made Dr I M L Hunter to be professor of psychology, R Shaw to be director of extra-mural studies, *Readerships*, Dr D E Davies (physics), Dr R McWeeny (chemistry), Dr R G Evans (biology), *Senior Lectureships*, Dr P Halmos (psychology), Dr C S Exley (geology), Dr S C Nyburg (chemistry). Dr P H Plesch (chemistry)

London

THE following titles have been conferred that of professor of zoology on Dr P M Butler in respect of his post at Royal Holloway College, that of professor of mathematics on Dr A Frohlich in respect of his post at King's College, that of professor of philosophy with special reference to sociology on Dr E A Gellner in respect of his post at the London School of Economics and Political Science, that of reader in comparative education on Dr E J King in respect of his post at King's College, that of reader in physics on Dr B Donovan in respect of his post at Westfield College The following appointments have been made Dr J Naylor Hunt, to the chair of physiology tenable at Guy's Hospital Medical School, Dr R W H Sargent, senior lecturer at the Imperial College of Science and Technology, to the chair of chemical engineering tenable at that College, Mr W G Spector, senior lecturer at University College Hospital Medical School to the chair of pathology tenable at St Bartholomew's Hospital Medical College, *Readerships*, Dr M E Fisher (physics), Dr A N Davison (biochemistry), Dr J A Elvidge (organic chemistry), Dr P J Higgins (mathematics) Dr D Robinson (biochemistry), J L Stafford (hæmatology), Dr M B Thorn (biochemistry)

University College of Swansea

THE following appointments to lectureships have been made for the session 1962–63 Mr F T M Smith (applied mathematics), Dr. C. A Wellington (chemistry), Mr M J Clarke (mechanical engineering), Mr D A Watts (geography)

Announcements

DR DENIS TAYLOR, who for the past five years has been a director and general manager of Plessey Nucleonics, Ltd , has been appointed co-ordinator of research and development for the Plessey Co , Ltd At one time Dr Taylor was head of the Electronics and Instrument Division of the Atomic Energy Research Establishment, Harwell

THE Joseph P Kennedy Jr Foundation is offering three international awards to recognize significant achievements in the past and to assist efforts of outstanding promise in research, service and leadership concerned with mental retardation Further information can be obtained from the Joseph P Kennedy Jr Foundation, 1413 K Street, N W Suite 306, Washington 5, D C

A MEETING of the British Biophysical Society will be held in the Laboratory of Molecular Biology, University of Cambridge, on September 27 The programme will include a talk by Dr M F Perutz Further information can be obtained from Dr Herman Watson, Laboratory of Molecular Biology University Postgraduate Medical School, Hills Road Cambridge

THE sixth plenary meeting of the World Power Conference will be held in Melbourne during October 20–27 Further information can be obtained from the British National Committee, World Power Conference, 201–2 Grand Buildings, Trafalgar Square London, W C 2.

THE QUEEN'S UNIVERSITY OF BELFAST

NEW PHYSICS BUILDING

THE old physics building at the Queen's University of Belfast, which was built shortly before the First World War, has become increasingly inadequate for the needs of the Department since 1945. Temporary extra accommodation has been provided in two groups of three huts and a house, but these were scattered and have shortly to be taken down or used for other purposes. They have now been replaced by a substantial extension to the old building, which was opened by H.M. Queen Elizabeth, the Queen Mother, on April 6.

The new building, which is seen from the north in Fig. 1, is joined to the old building by two curved corridors (beyond the left) at ground-floor and basement levels. It occupies most of the space along the city park boundary between the University's main assembly hall (at the right) and the old physics building. The principal part is a four-storey L-shaped structure concave to the boundary, with, under part of its area, a large sub-basement boiler house serving a number of buildings, and a smaller ventilating-plant room for the basement and ground-floor of the new physics building. There are also tank rooms and a second ventilating plant on the roof, and a new workshop connected to the lower link corridor, between the park boundary and the old building, but structurally separate from the latter. Goods can be delivered from the rear of the building to a two-ton lift giving access to most parts of both the old and new buildings. The main store-rooms are located off the link corridors. Water and gas feeds are generous, and the central switch-room is supplied from an individual 500-kW. sub-station the capacity of which can be doubled later if necessary.

The new building has two lecture rooms. The larger, seating 280, is situated at the south-west end. As it will also be used for general university purposes, it has a separate entrance and separate services from the rest of the building, and contains a number of projection units which are not at present usually considered necessary for physics teaching. The smaller, seating 100, is on the first floor above a ground-floor library and reading room and the boiler house.

The ground, first and second floors will carry uniform loads of approaching 240, 180 and 120 lb./sq. ft. respectively. The bulk of the room heating in all three of these floors and the basement is by hot water circulating in acoustically treated ceiling panels. The layout is such that the uses of most sections of the building can be varied substantially. The initial allocation of space will probably be: basement, research and service rooms; ground floor, office, staff and research rooms; first floor, third- and fourth-year undergraduate teaching laboratories; second floor, second-year undergraduate teaching laboratories.

The old building is to be rehabilitated during the session 1962–63. When it is again available, the

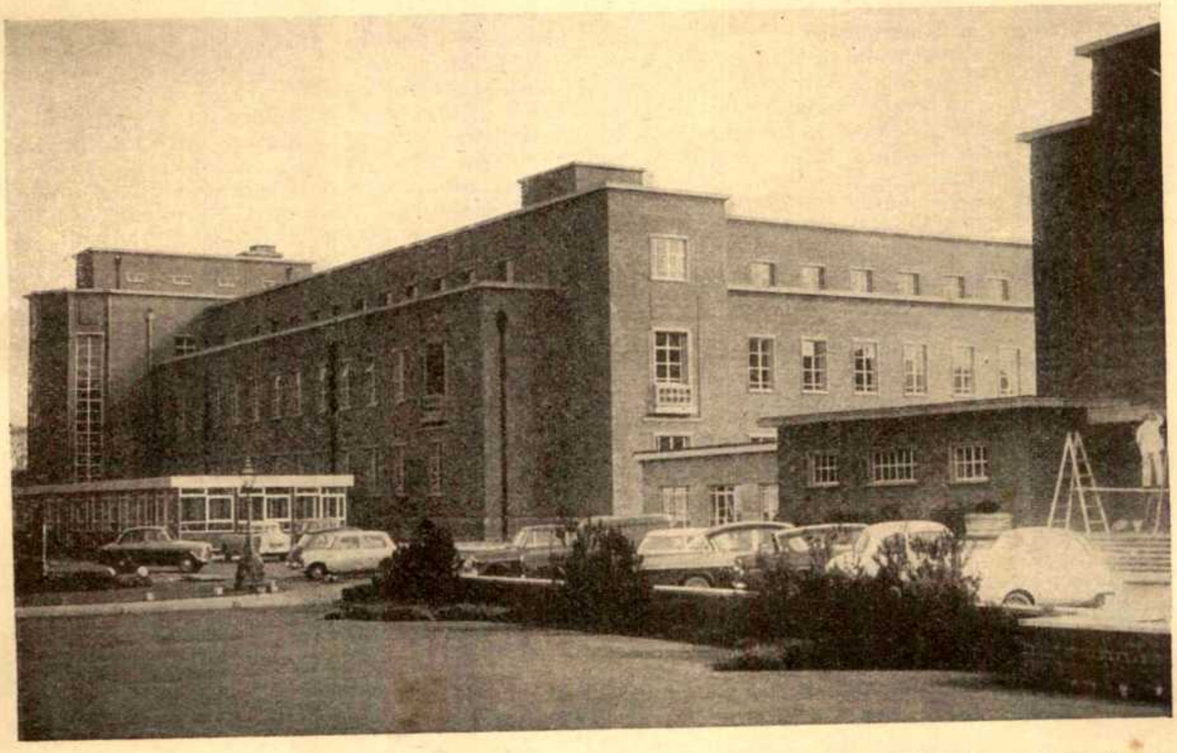

Fig. 1. Physics Extension Building, Queen's University of Belfast. The building to the right is the University assembly hall, the Whitla Hall; the small building between it and the Physics Building, and the small building to the left front of the Physics Building, are a temporary refectory and library reading room, respectively (*Photo: by courtesy of the Northern Whig, Ltd., Belfast*)

first floor of this will be used for the first-year undergraduate teaching laboratory. The total useful floor space in the old and new building will be somewhat over 60,000 sq. ft. The number of physics students in all years has been nearly 1,000 in the session 1961–62; future numbers will depend on the University's policy about limitation.

The architect of the building was Mr. John MacGeagh, the principal contractors Messrs. F. B. McKee and Co., Ltd., and the engineering consultants Messrs. Oscar Faber and Partners. The Department's interests have been attended to by Dr. R. H. Sloane, reader in physics. The total cost has been about £800,000.

EXPERIMENTAL TAXONOMY

THE annual symposium of the group for experimental taxonomy of the Linnean Society of London was held on March 2, at the Society's rooms in London. After a brief introduction from Prof. D. H. Valentine, the meeting began with a paper by Dr. D. M. Moore (University of Leicester), on "Speciation within Chilean Clarkias". The annual genus *Clarkia* contains some 34 species in western North America, and a series of forms from central Chile and adjacent regions of Argentina which are presently included under *C. tenella* (Cav.) Lewis and Lewis. *C. tenella* is tetraploid and has been shown to be closely related to *C. davyi* of coastal California by Raven and Lewis, who suggested that South American plants resulted from trans-equatorial long-distance dispersal in late Pliocene or more recent time. Data were presented to illustrate the pattern of variation within the Chilean species of *Clarkia* and its correlation with the breeding system. It was suggested that the ancestral introduction was close to the large-flowered outcrosser, with $n = 17$, which occurs in southern central Chile; and a scheme was outlined to suggest the derivation of the other South American forms from this type. Some populations of outcrossers, separated from the others and representing the southernmost occurrence of the genus, have lost a pair of chromosomes but are morphologically identical with the 17-chromosome form. Most differentiation was shown to be associated with the change to autogamy, which generally involves no change of chromosome number. Self-pollinated forms have the stamens and style of equal length ; anthers and style mature simultaneously and are in close contact. The most widespread form has small flowers, is markedly self-pollinated and shows a whole range of biotypes usually differing slightly from one another, though occasional more extreme deviants are found. Populations intermediate morphologically between these small-flowered self-pollinaters and the outcrosser are facultatively outcrossing, though primarily self-pollinated, suggesting a gradual change to autogamy. Another entity sympatric with, but temporally quite isolated from, the outcrosser has acquired self-pollination without other marked morphological change. In only one case do self-pollinating plants show a different chromosome number, $2n = 28$; they are also remarkable for their yellow flowers, which are very rare in the genus. This scheme was discussed in relation to the frequent independent origin of self-pollinating from outcrossing forms within *Clarkia*.

The second paper, by Dr. A. J. S. Davies (Chester Beatty Institute), was entitled "A Cytogenetic Study of 28 Species of the Genus *Lathyrus*". The genus as a whole contains some 110 species occupying marginal and open habitats. Many morphological characters are used in the comparison of phenotypes in the traditional taxonomy of the group, and evolutionary trends in some of these can be postulated. The bracts at the base of the pedicel provide a useful indication of whether a species is specialized or not; there is a trend from long leafy bracts, to shorter, to a state where they are represented by a tuft of hairs. The genus is predominantly diploid ($2n = 14$) and gross chromosomal changes have probably not played a large part in its evolution. It is thought that there has been a tendency for chromosome-length to decrease and for the karyotype to become more asymmetrical, and this trend is associated with a decrease in plant size. Attempts have been made to cross fourteen of the annual species; and it has been shown that embryo abortion is the main barrier to interspecific hybridization. Hybrid plants have, however, been raised from four combinations of species. One of these was taken to the F_2 generation, and it was shown that at least four major gene loci are common to the parental species. Two of these genes, however, were modified in their expression in the F_2, indicating differences in the genetic background of the two species involved.

Dr. C. D. K. Cook (University of Liverpool) gave the next paper on "Variation in *Ranunculus* subg. *Batrachium*". He noted that the number of taxa recognized from the time of Linnæus to the present day ranged from 1 to 108; the number of new names per decade follows a normal curve of distribution. Much of the confusion in the group is due to the wide range of phenotypic plasticity shown by the species and the inability of workers to distinguish it, in the herbarium, from genotypic variation. In cultivation, a great deal of variation, both genotypic and phenotypic, is revealed. Thus, the achene number in both British (Isle of Wight) and Finnish (Helsinki) topodemes of *R. baudotii* varied from 10 to 60; but under experimental conditions, they differed in the average number of achenes produced in still and flowing water. Their total plasticity-range was, however, the same, and they are best regarded as two parts of a cline, with different ecological preferences. Extreme phenotypes occasionally become genetically fixed; an example is provided by plants of the alpine state of *R. trichophyllus*, some of which retain their original shape when cultivated in lowland conditions, and some of which revert to normal. The transition between capillary and laminar leaves in the group is always abrupt and is affected by length of day and temperature, but it is primarily under genetic control. Many hybrids are known. Some are infertile but very persistent, for example, some pentaploids in the *fluitans* group. Other species form fertile hybrids which are often unstable and unlikely to persist, but great variability in the F_2s has been demonstrated, and some of these, which differ greatly from their parents, may occasionally be able to invade new habitats.

Dr. A. J. E. Smith (University College, Swansea) then gave a paper on "Variation in *Melampyrum* *pratense*". This semi-parasitic species is difficult to raise from seed, but experiments have shown that it will grow on a variety of woody plants, such as species of *Betula*, *Calluna* and *Rubus*. It is very variable, 28 intraspecific taxa having been described, and much of this variation follows geographical trends in the British Isles. Plants become shorter in the north, and this is correlated with a reduction in the number of leaves and branches. The leaves also become shorter in the north and west. Anther-length is 20–30 per cent shorter in Northern Ireland than elsewhere. Superimposed on this clinal and geographical variation is ecological variation, the leaf width/length ratio divides the populations into those of calcareous and acid habitats, but the distinction is not clear-cut. Population samples were grouped according to habitat types and an analysis was made of the variation, using 8 vegetative and 3 floral characters, all of them quantitative. This showed that there was random distribution of genes or gene combination in the small isolated populations in which the plant occurs, and that similar groups of characters occurred in similar habitats. Some populations had cleistogamous flowers and were effectively inbreeding units. The variation pattern in certain morphological characters was, however, markedly different from that observed on the Continent.

Dr. J. K. Morton (Birkbeck College) gave the final paper on "Introgression in West African *Eulophia* Orchids". Two species, *E. cristata* and *E. millsoni*, were studied in the Accra coastal plains where their hybridization is associated with the disturbance caused by peasant agriculture, although their ecological preferences are different. Populations were analysed in order to discover the extent and direction of the apparent introgressive hybridization which was taking place. Ten characters associated with the structure of the flower and inflorescence were scored, such as height of inflorescence, number of flowers, and angle of lateral lobes of the lip to the horizontal, the introgression was demonstrated by pictorialized scatter diagrams and histograms. A remarkable feature which emerged was the unilateral gene-flow, back-crossing to the *E. millsoni* parent being frequent while none to *E. cristata* was detected. The introgression affected the biology of the populations, the *millsoni* hybrids extending the range of the species. Vegetative features were not included in the introgression studies since the leaves appeared 2–3 months later than the flowers.

The general discussion was opened by Dr. V. H. Heywood (University of Liverpool), who commented that the day's papers illustrated the tendency of botanists to emphasize breeding systems and dispersal mechanisms in consideration of speciation and adaptation, while zoologists place greater emphasis on phenotypic plasticity. He suggested that botanists should pay greater attention to the adaptive role of plasticity: they should not only be concerned in experimental taxonomy with determining genotypes, but also with the way that particular phenotypes may be selected. Given a range of expression of a particular genotype, only certain parts of that range will be selected in particular areas; this was well shown by Dr. Cook's paper on Batrachian *Ranunculi*. The problems of the taxonomic recognition of plasticity have also not been adequately considered.

Other points of importance which arose out of the papers were the effects of changes in the breeding system from outbreeding to inbreeding, the lack of synchronization of karyotype evolution with morphological and other genic evolution, as in *Lathyrus*, and the polytopic origin of taxa, such as had been suggested in the water buttercups and in *Melampyrum*. The question of long-distance dispersal in *Clarkia* was raised, and a discussion ensued, in which Dr. Gillet, Dr. Jones and Prof. Valentine took part. There was also a brisk exchange of views on the likelihood of the polytopic origin of species, especially in polyploid groups.

V. H. HEYWOOD
D. H. VALENTINE

FOOD TECHNOLOGY IN GHANA

To many people nutrition means no more than the study of foods and their use by the living body. But with increasing evidence of widespread malnutrition throughout the world, and of a deteriorating situation in regard to world population and food supplies, much emphasis needs to be placed on the importance of work outside the laboratory on problems associated with providing people everywhere with an adequate diet. In many parts of the world an immediate problem is a means of conveying foods from one place to another. For example, fruit, the sale of which provides a livelihood for some people, may be rotting on the trees because there are no roads on which it can be transported to other people who might benefit by addition of fruit to their diet. Sometimes roads exist, but mechanical transport is inadequate or excessively costly. Lack of adequate means of distribution may seriously limit the use of fish, and while many people suffer the effects of protein malnutrition, fishermen not far away suffer economic disaster because they cannot get rid of harvest gluts. Not only are roads and mechanical transport involved in the distribution of foods but in addition a means of preservation through cooling, drying or other means.

Ghana is a country where transportation and preservation of foods are of great importance. Owing to the geographical features of the country adequate diets can only be ensured when there is a free exchange of foods grown in the north with those grown in the south. The Government of Ghana has wisely taken steps to deal with nutritional problems, and has included food technology in its programme. The Government has called on the World Health and the Food and Agriculture Organizations for assistance in developing a public health nutrition programme, Dr. Francis Aylward, as Food and Agriculture Organization consultant (see also p. 20 of this issue), has recently examined problems of food supplies and nutrition in Ghana and has submitted a report on this subject*.

Dr. Aylward's report is a most useful one, not only for those interested in Ghana, but for many others as well The results of inquiries made under the auspices of United Nations' specialized agencies are in the first instance of a confidential nature and are designed to help the Governments concerned However, many excellent accounts are frequently being composed, which could be most useful to workers in special fields, and to administrators in many countries Such a report is that under discussion, and it is to be hoped that if the Government of Ghana receives requests for copies of the report it will be willing to assent to its distribution

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* *Report to the Government of Ghana on Foods and Nutrition*. By Francis Aylward. ETAP Rep. No. 1449. (Food and Agriculture Organization of the United Nations, Rome, 1961.)

Dr Aylward has dealt systematically with many aspects of food technology and its relation to the provision of adequate diets He has wisely emphasized inherent economic and social dangers of thoughtless introduction into a developing country of new techniques Such is the perversity of man that valuable methods of preserving much-needed food may be diverted to less needy ends Thus one of the most important recent changes in Ghana noted by Dr Aylward is the introduction of ice-plants which provide refrigeration for the storage of animal products, but refrigeration is now also being used to an appreciable extent for cooling commercially prepared soft drinks This is not necessarily to be deplored, but administrators must be aware that when they sponsor the introduction of a given technique for one end, it may be used also for less-worthy, and sometimes undesirable, purposes

Usually modern techniques lead to the production of foods which are more expensive than the original materials When the product enables some people to obtain foods necessary for their well-being and which they would not otherwise obtain the cost must be ignored But very often a new product, especially if it is commercially advertised, is regarded as of particular value and may be purchased at unwarranted expense by those not specially in need of it In developing countries this is unfortunate and may be a factor leading to malnutrition among sophisticated urban groups Any means, therefore, of producing food products cheaply is to be encouraged Perhaps one of the most significant advances in this connexion has been the use of plastics for food packaging Not only is this kind of material cheaper than metal containers, but also it may provide a better means of preservation than some alternatives Dr Aylward directs attention, for example, to the satisfactory use of plastics in the storage of fish meal produced in Ghana

Proposals have been made for the introduction into Ghana of 'filled milk' composed of skimmed milk powder, water and coconut oil This poses a troublesome problem for public health administrators Though such a milk product may be produced relatively cheaply in some circumstances, thus leading to its widespread use among the poor and needy, there is not yet enough evidence of the effects, especially on infants, of repeated ingestion of coconut oil which contains a high proportion of saturated fatty acids, and also a good deal of lauric acid It is often the case that new preparations of food such as filled milk are readily accepted by the public before administrators are in a position to control their use should this be necessary

While it is true that the application of scientific methods in the growth, storage and preparation of foods carries possible hazards, it is of the greatest importance, as Dr Aylward states, to realize that food technology has developed in order to meet the urgent problems associated with feeding ever-enlarging urban communities, modern food industries are essentially a response to public needs "And it is precisely for this (as well as other reasons) that the food scientist and technologist has a role to play in Ghana" G R WADSWORTH

CHOOSING FOREST SPECIES

WORLD requirements for wood and wood products are steadily increasing, though the forms in which it is being used and the kinds of timber most in demand are constantly changing World forest resources, which are badly distributed in relation to centres of consumption, are becoming inadequate to meet requirements, and acute shortages are already being felt At the same time, many natural forest types have relatively low production, and the kinds of wood produced are often not those most in demand

This is particularly true of vast areas of tropical forest where the luxuriance and variety of the growth make them economically a poor proposition Many of the species in them are hard, heavy and difficult to season, and are unsaleable Even in Europe, the large areas of oak forest, often of low quality, which used to meet demands for fuel and heavy constructional timber are becoming a burden on the market If future demands for wood are to be met in the quantities and in the kinds of product required, it will be necessary to improve the productive efficiency of forests all over the world, and to grow more intensively those particular species that will be most in demand The kind of species most likely to be favoured have recently been discussed by Prof M V Laurie of the University of Oxford (*Span*, 5, No 1, 1962)

It is difficult to estimate what will be wanted half a century or more ahead, but this is what the forester has to try to do when formulating his objects of management and considering what species he ought to be growing Heavy timbers have largely disappeared from constructional uses On the other hand, softwoods are still used in large quantities for house building and other structural purposes, and recent developments in laminated construction have provided new outlets for softwood timber in short lengths and of relatively low quality

Present indications are that softwoods will be in greater demand than hardwoods for most purposes, and that general purpose timbers, which at the same time can be used for core stock or laminated construction and can be chipped or pulped, are most likely to be wanted in large quantities in the future This trend is already reflected in the large programmes for planting conifers in many parts of the world Although it may not be difficult to envisage the kind of trees that are likely to be needed to meet future requirements, the question of what can be grown on the sites available is more difficult to answer Here the limitations of climate, soil and biological agencies are crucial Different species vary greatly in their tolerances of conditions differing from those of their native habitat, so much so, in fact, that it is impossible to say with any degree of certainty whether exotic species will succeed or fail purely from a comparison of conditions in their native land with those in the locality of introduction

As an example, the Monterey pine, *Pinus radiata*, from a restricted area in California, has been outstandingly successful in New Zealand, many parts of Australia, in South Africa, Spain and Chile, and it even grows well in the highlands of Kenya Again, the Serbian spruce, *Picaea omorika*, which grows on limestone mountains in a very restricted area in Yugoslavia, grows remarkably well on acid peats in Scotland In other cases, the reverse may be true, and many species which have a wide natural range, like the Douglas fir or European larch, have developed local strains which are susceptible to the conditions under which they are grown The only safe way of selecting a species for introduction is to try it, and all large-scale plantings of exotic trees have been preceded by species trials in arboreta or small trial plots where suitability to the locality is tested, followed by larger plot tests on a more limited range of sites where performance can be estimated

There are many doubts and risks in introducing an exotic species into a new habitat, and it should always be a principle first to see whether any indigenous species will fulfil the objects in view The principle that one should not consider introducing an exotic species on a large scale if there is an indigenous species that will meet requirements is generally true In countries like Britain, however, where the only native timber-yielding conifer, the Scots pine, grows relatively slowly, there is a good case for introducing species that will give higher yields It is fortunate that some of the conifers from the west coast of North America, Sitka spruce, Douglas fir, western hemlock and lodgepole pine, as well as some from Europe, such as Corsican pine, Norway spruce, European larch and, more remotely, the Japanese larch, have proved so successful in Britain Trials have shown that many species, particularly those from the eastern parts of North America, suffer so seriously from late-spring frosts in Britain as to be not worth growing Most species of *Eucalyptus* are found to be too susceptible to frost to be grown safely in Britain, except in sheltered parts of Devon and Cornwall Corsican pine, when moved to northern British latitudes, is found to be restricted in the elevation at which it can be grown Teak does not grow well on very heavy soils or on laterites in the tropics and needs some lime in the soil, it seems to require a definite dry season when it is leafless No amount of information can enable a species to be accepted as safe for large-scale planting without extensive trials

It frequently happens that a particular species is so superior in performance to all others tried that there is a tendency to rely on it alone in large plantation programmes This has occurred, for example, in New Zealand and parts of Australia where *Pinus radiata* has been planted extensively. So far there has been no serious trouble, but there is always the danger that something may go wrong It is advisable to have one or more alternative species to fall back on in an emergency

In South Africa, for example, *P radiata*, which showed great promise to start with, has more recently suffered severely from a fungal disease which kills the leading shoot There is now a tendency to prefer *P patula*, another fast-growing pine, which does not suffer from this defect

ERADICATION OF MALARIA IN EAST AFRICA

In the annual report of the East African Council for Medical Research[*], the director of the East African Institute of Malaria and Vector-Borne Diseases, Dr G Pringle, describes how a draft plan has been prepared for the eradication of malaria from East Africa, based on a combined attack with drugs as well as insecticides The plan has already received critical examination by a meeting of experts held in Tanga, and further inter-territorial consultations are planned

In the Taveta-Para area, where spraying with dieldrin was discontinued early in 1959, a close watch on malaria incidence has been kept *Anopheles gambiae* has shown a marked increase in numbers, although house catches have still been less than pre-spraying densities The longevity of this vector is still reduced, indicating the presence of some continuing insecticidal effect *A funestus*, which was almost certainly eradicated from the area, has not reappeared Although parasite densities are not back to former levels, there has been a major resurgence of the disease in the swampside villages Studies of the serum γ-globulin levels in schoolchildren also indicate that an effective immunity has yet to be acquired

The feeding preference of *A gambiae*, in areas where man and cattle are both attacked, has been studied with the view of finding out whether the individual mosquitoes show a consistent tendency to feed on one or other host Results in one area are still inconclusive, but in another district the presence of normal infection-rates in insects caught feeding on cattle clearly showed that the same mosquitoes had fed at different times on both available hosts Thus, so far, no evidence has been found of two co-existing strains of *A gambiae*, differing in their feeding habits, and hence probably in their response to insecticides

Among potential secondary vectors of malaria, infections have been found in *A pharoensis* and *A squamosus*, but none in the more abundant and ubiquitous members of the *A coustani* group Age-grouping studies indicate that, in all these species of exophilic mosquitoes, longevity is greatly reduced compared with the main house resting vectors

A flourishing colony of the coastal salt water form of *A gambiae* has been established Crossing experiments with two strains of fresh water *A gambiae* have shown that, although occasional fertile offspring are produced, a formidable barrier to gene exchange exists Thus reproductive isolation of the two groups in Nature seems likely, although proof of this has yet to be obtained in the field

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[*] East African High Commission. The East African Council for Medical Research—Annual Report, 1960/61 Pp ii+16 (Nairobi Government Printer, 1961) 2s

DETECTION OF THE MICROWAVE ν_{27^-} LINE OF MOLECULAR OXYGEN PRODUCED IN THE HIGH ATMOSPHERE

By W. KAHAN

Columbia Radiation Laboratory, Columbia University, New York

RADIOTELESCOPE observations of atmospheric absorption of solar millimetre wave radiation have resulted in the detection of the ν_{27^-} telluric molecular oxygen line at 53,070 Mc /s Furthermore, measurements made on different days have shown that the peak of the ν_{27^-} molecular oxygen absorption varies in intensity, presumably as a result of temperature variations in the Earth's high atmosphere where the pressure is low enough to produce the line peak observed Because the wings of the line are primarily due to high-pressure absorption, high-altitude temperature changes could greatly vary the vertical atmospheric absorption at the centre of the line without appreciably affecting the off-centre portions

Atmospheric absorption in the 0 5 cm wave-length region is largely produced by the oxygen molecule, which in its ground-state, $^3\Sigma$, has an electronic spin $S = 1$ which couples to the odd integral angular momentum, N, of the end-over-end rotation of the dumbbell molecule to produce states of $\mathbf{J} = \mathbf{N} + \mathbf{S}$ (ref 1) The permitted transitions $J = N \rightarrow J = N + 1$ and $J = N \rightarrow J = N - 1$ give rise to lines at frequencies denoted respectively by ν_{N^+} and ν_{N^-} which lie primarily near 60 kMc /s There are 34 molecular oxygen lines between 51 kMc /s and 68 kMc /s which combine to produce a highly attenuating frequency region for microwave transmission through a vertical column of the Earth's atmosphere

The central frequency portion of the attenuating region is almost completely opaque because the high intensity and pressure broadening of the oxygen lines bring the vertical atmospheric transmission down to less than 10^{-6} over an extended frequency interval Consequently, it is not feasible to use the Sun as a blackbody radiator and to measure, with a radiotelescope near sea-level, an individual telluric molecular oxygen absorption peak in the opaque region where receiver intensities corresponding to antenna temperatures of 10^{-3} ° K and 10^{-8} ° K would be typical for respectively the wings and the peak of an absorption line A millimetre-wave radiometer is unable to distinguish between such low intensities Away from the central opaque region, the molecular oxygen transitions originate from progressively higher rotational quantum states and are less intense as a result of the decrease of state population with increasing N

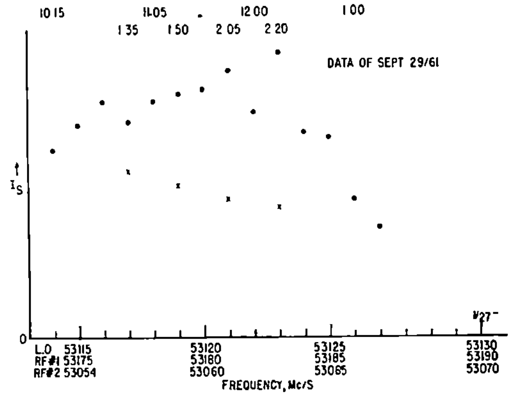
The experiments described in this article were done on the ν_{27^-} line, where N is high enough for the detection of an isolated molecular oxygen absorption peak to be possible The frequency ν_{27^-} had not been previously measured, and its value was computed from theoretical expressions of $\nu_N\pm$ as a function of N (refs 2 3, 4 and 5) which were derived from the frequencies $\nu_N\pm$ which had been measured for many values of N (refs 4, 6, 7, 8 and 9) According to the formula given by Miller and Townes, $\nu_{27^-} = 53{,}070$ Mc /s

Measurements of vertical atmospheric absorption in the vicinity of 53,070 Mc /s have been made with a Dicke radiometer which had an input band-width of 1 Mc /s , an intermediate frequency of 60 Mc /s and an output integrating time of 25 sec The resulting curves of solar radiation received, I_s, versus frequency are shown in Figs 1, 2 and 3 The abscissæ show the local oscillator frequency as well as the two radio-frequencies received simultaneously by the radiometer The local oscillator frequency was measured by beating it with the sixth harmonic of an X-band stalo which, in turn, was checked periodically with a harmonic of a 100-Mc frequency standard In order to cancel radiometer and atmospheric low-frequency noise, the points shown were obtained by alternately pointing the radiotelescope antenna on and off the Sun and subtracting from an 'on-reading' the average value of the two adjacent 'off-readings'

Fig 1 shows the results obtained on September 29, 1961, where a strong absorption is evident near $\nu = 53{,}070$ Mc /s This frequency is marked ν_{27^-} on all the figures The results in Fig 2, taken on October 11, 1961, show no definite absorption at ν_{27^-}, but an absorption does appear at ν_{27^-} in the data of October 19, 1961, shown in Fig 3 The centre frequency of the absorption of Fig 3 agrees within experimental error with the value of ν_{27^-} calculated from the formula of Miller and Townes Fig 5 shows the results taken at a frequency away from ν_{27^-} on September 5, 1961, and is presented for comparison with the other figures

As shown in the figures, the points were taken during different times of the day, and consequently different lengths of atmosphere attenuated the Sun's radiation That the resulting differences in atmospheric transmission do not account for the changes of oxygen absorption shown on the three curves can be seen from Fig 1, where the four X's are points taken in succession after the highest frequency point was taken The Sun was lower in the sky, yet the radiometer readings are higher and show a definite frequency-dependent absorption which is absent from the points of Fig 2 Fig 4 shows the points taken during the 'off' portion of the radiotelescope on September 29, 1961 As ν_{27^-} is approached, the atmosphere becomes more absorbing and produces a higher antenna temperature Here too the X's are points taken in succession after the highest frequency point and corroborate the frequency dependent absorption shown in Fig 1

The graphs of Figs 6 and 7 showing zenith atmospheric molecular oxygen transmission as a function of radiometer local oscillator frequency are the results of calculations using oxygen-absorption formulæ[10,11] with the assumption of an isothermal atmosphere with an exponential decrease in pressure The intensity of the ν_{27^-} lines calculated for atmospheric



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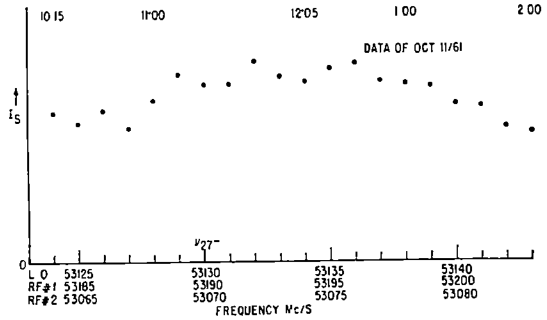

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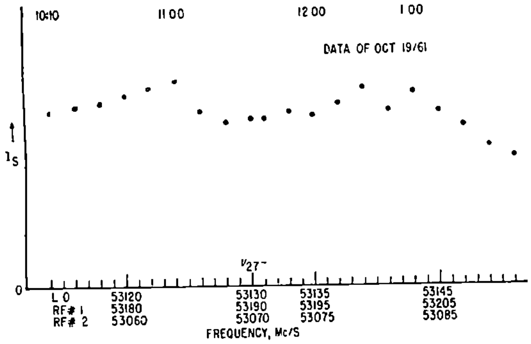

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Figs 1, 2 and 3 show the intensity of solar radiation received, I_s, as a function of frequency The topmost numbers are the time of reception for the points denoted by dots Times for the points denoted by X's are given on the next to the topmost line All times are in E S T The units of I_s are arbitrary The antenna temperature of the higher points was approximately 70° K

temperatures of 280° K and 260° K is of the same magnitude as the measured intensity shown in Fig 3 Further calculations showed that if the temperature of the atmosphere were 260° K up to a height of 30 km and 240° K above 30 km, the ν_{27}- line of Fig 7 would not be detectable by the radiotelescope used in the experiments described here

It appears that the temperature of the high atmosphere, to which the peak of the oxygen line is sensitive, may vary enough over different days to produce the changes of oxygen absorption which have been measured Further experiments are being set up to study the ν_{27}- line and other oxygen telluric lines to learn how the intensity of oxygen absorption varies with time and to correlate this variation with other atmospheric variations so that the molecular oxygen line may be used to measure atmospheric conditions such as temperature and pressure

I thank Prof J Charney and Prof C H Townes for their helpful discussions on the temperature variations of the high atmosphere and the resulting effects on oxygen absorption I wish to acknowledge the advice and encouragement that I have received throughout the research from Prof Townes, who initially suggested to me the study of the oxygen telluric millimetre wave spectrum

This work was supported in part by the Air Force Office of Scientific Research under grant number *AF–AFOSR*–62–50 and in part by a Joint Services

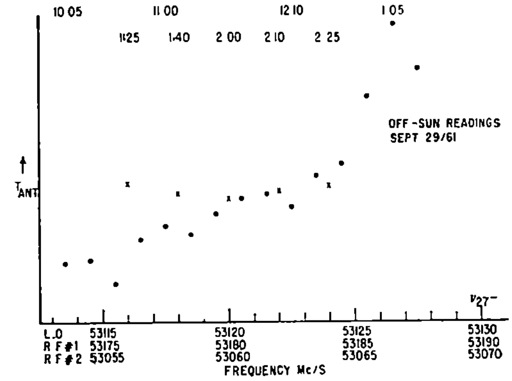


Fig 4 is a plot of radiometer readings versus frequency taken when the antenna pointed off the Sun on September 29, 1961, the day the data of Fig 1 were gathered The units and zero point of T_{ant}, the antenna temperature, are arbitrary

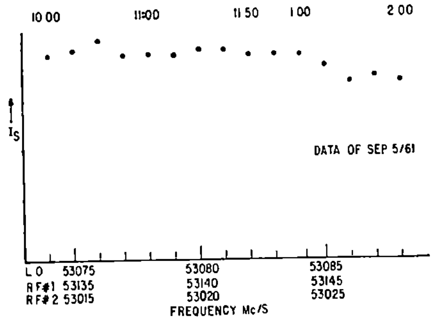
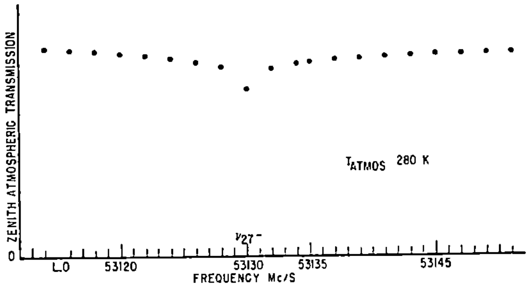


Fig 5 shows the intensity of solar radiation received on September 5, 1961, at a frequency away from ν_{27}-



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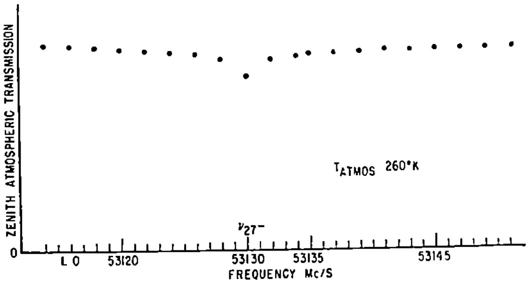

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Figs 6 and 7 are plots of the calculated atmospheric vertical transmission as a function of frequency for an isothermal atmosphere with a temperature of 280° K and 260° K, respectively The units of the transmission are arbitrary

Contract with the U S Army Signal Corps, the Office of Naval Research, and the Air Force Office of Scientific Research

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FUSION OF SOIL DURING METEORITE IMPACT, AND THE CHEMICAL COMPOSITION OF TEKTITES

By Dr S R. TAYLOR

Department of Geophysics, Institute of Advanced Studies, Australian National University, Canberra

A RECENT proposal by Schwarcz[1] that tektites originate by fusion of soil at meteoritic impact sites raises several interesting issues A principal conclusion is that a mixture of soil and 3 5 per cent of the silicate phase of chondrites is close to the chemical composition of tektites

There are some well-known difficulties in matching the composition of tektites to that of common crustal materials, the major problem being to provide about 2 per cent magnesium oxide (about four times that of crustal material of similar silica content) Cherry and Taylor[2] first suggested a mixing hypothesis to solve the enigma of high magnesia and silica contents A suitable match for the major elements was produced by mixing arkose (which combined a high silica content with a low alkali concentration) with 7 per cent of the silicate phase of chondrites (selected to represent the composition of non-volatile material in comets) When this was proposed, no modern determinations for such critical elements as nickel or chromium were available

Recent determinations of nickel in tektites have been published by Ehmann[3], and I[4] have made a study of the chemical composition of the australite group of tektites, with determinations of trace elements on 24 specimens, and of major elements on 17 specimens (by H B Wiik) Preliminary results have been published[5-7] The average nickel content of australites is 29 p p m , and the chromium average is 77 p p m These data place severe restrictions on the amount of admixture of meteoritic material during tektite formation, and it is the purpose of this article to list some of the difficulties inherent in the proposal by Schwarcz[1]

(1) Iron meteorites, with several per cent nickel, provide no magnesium, and embarrassing amounts of nickel if any mixing occurs The average chondrite contains 1 34 per cent nickel This is mainly contained in the metal phase It seems difficult to restrict mixing in of the silicate phase only Chao[15] has reported the presence of iron–nickel spherules in a philippinite, and this must be considered strong evidence for an impact origin The question of place of impact, and whether by meteorite, asteroid or comet, remains undecided In the present connexion, the inclusion of iron spherules seems to rule out the restriction made by Schwarcz[1] of using only the silicate phase of chondrites

(2) A calculation has been made of the effect of the addition of 3 5 per cent chondrite, and this is shown in Table 1 Column 1 gives the average composition of chondrites[4] and column 2 represents 3 5 per cent of column 1, or the amount added in the calculation of Schwarcz[1] to soil Column 3 gives the average composition of australites (24 specimens)[4] The first seven elements, silica, aluminium, iron, magnesium, calcium, sodium and potassium, are given in per cent, and the remainder in parts per million (p p m) A comparison of column 2 (the meteoritic contribution) with the australite average in column 3 shows that nickel is the outstanding anomaly, being too abundant by a factor of 15, if such an amount of chondrite is added to any other parent material Cobalt likewise would be over-abundant by a factor of two, while 100 per cent of the chromium content is added, and nearly 40 per cent of the phosphorus content of australites

Table 1 COMPARISON OF COMPOSITION OF CHONDRITES (COL 1) WITH AUSTRALITES (COL 3)

Column 2 represents 3 5 per cent of the chondrite composition By adding such an amount to tektite parent material, 470 p p m nickel, 77 p p m chromium and 28 p p m cobalt are added

		1 Average chondrite (ref 4)	2 3 5 per cent of 1	3 Average australite (ref 4)
Per cent	Si	17 8	0 62	34 22
,,	Al	1 32	0 046	6 10
,,	Fe	25 1	0 88	3 56
,,	Mg	14 4	0 50	1 24
,	Ca	1 39	0 049	2 50
,,	Na	0 67	0 024	0 95
,	K	0 082	0 0029	1 89
p p m	P	1,420	50	130
,,	Ga	5 3	0 18	8 1
,,	**Cr**	**2,200**	**77**	**77**
,,	Li	2 7	0 10	42
,,	Ti	790	28	3,960
,,	**Ni**	**13,400**	**470**	**29**
,,	**Co**	**800**	**28**	**15**
,,	Cu	90	3 2	8 1
,,	V	39	1 4	78
,	Zr	33	1 2	400
,	Mn	1,900	67	770
,	Sc	9 4	0 32	13
,	Sr	10	0 35	210
,,	Ba	3 4	0 12	630
,	Rb	4	0 14	81
,,	Cs	0 13		2 5

(3) I[8] have recently considered the effect on tektite composition of an origin by meteoritic splash, using the new trace element data for australites[4,5] The australite composition indicates that the amount of admixture of material from iron meteorites cannot exceed about 0 03 per cent, or from chondrites, about 0 1 per cent Australites possess an embarrassing nickel/chromium ratio of about 1/3, compared with 2,000 for irons (about 35 if average amounts of troilite are present) and 6 for chondrites If the total amount of 29 p p m nickel is provided from chondritic sources, only 5 p p m chromium is added, leaving 72 p p m chromium to be derived from non-meteoritic sources Terrestrial material with 72 p p m chromium can be expected to contain about 25 p p m nickel, so that it is difficult to bring more than a small fraction of the nickel from the chondrite

The problem is not eased by recourse to the group of high nickel tektites from Java, southern Indo-

Table 2. CORRELATION COEFFICIENTS (r) AND LEVELS OF SIGNIFICANCE FOR MAGNESIUM OXIDE AND OTHER MAJOR CONSTITUENTS IN 17 AUSTRALITES[4]

	r	Level of significance
MgO–SiO_2	−0.973	>0.01
MgO–Al_2O_3	+0.887	>0.01
MgO–FeO	+0.767	0.04
MgO–Na_2O	+0.784	0.02
MgO–K_2O	+0.829	>0.01
MgO–TiO_2	+0.851	>0.01
MgO–CaO	+0.267	Not significant

china, Billiton and Borneo, which contain an average of 295 p.p.m. nickel and 375 p.p.m. chromium[9]. The high nickel content may readily be provided from meteoritic sources, but not the chromium. It must be regarded as a curious coincidence that those tektites high enough in nickel to make an origin by meteoritic impact reasonable are also notably rich in chromium.

(4) If most of the magnesium were contributed from a chondritic source, an inverse relation should exist between it, and constituents, such as potassium, drawn mainly from the soil[1]. In fact, magnesium shows a good positive correlation with the other major constituents[4,10,11]. Care must be used in interpreting correlations in a closed array[12,13] and "in small samples, a correlation must be very strong to be significant"[12]. The correlation coefficients and levels of significance for 17 analyses of australites by H. B. Wiik[4] between magnesium oxide and the other major constituents are given in Table 2. Except for calcium oxide, all correlations are significant at the 0.04 per cent level, and most are significant at less than 0.01 per cent. The negative correlation with silica, the lack of correlation with calcium oxide and the positive correlations with the other constituents have been interpreted as indicating a mixture of quartz and shale, with some calcite, as the most likely parent material[14]. This conclusion is strongly reinforced by the trace element data[4–7]. It may be noted that the correlation of magnesium and potassium in tektites is the reverse to that observed in igneous rocks of similar silica content (68–80 per cent) and it is the identical behaviour of these two contrasted elements which is of such significance for parent material of tektites.

(5) A terrestrial meteorite impact of sufficient energy to distribute tektites over an area of 2×10^6 sq. miles (for example, australite distribution) is unlikely to involve only the upper layer of soil. To account both for the wide distribution and the restrictions on admixture of meteorite material[8], a meteorite collision at close to the maximum velocity of 72 km sec^{-1} seems required. Such an event would produce an observable crater, and underlying rock, as well as soil, would be involved.

(6) The calculation of Schwarcz[1] results in an average of 1,300 p.p.m. phosphorus. From the analyses of H. B. Wiik[4], the average content of phosphorus in australites is 130 p.p.m., a factor of ten lower. This difference is probably significant, although the precision in the older analyses used by Schwarcz[1] may be poor at this level, where relative deviations between different analysts may be of the order of 50 per cent[16].

Table 3. COMPARISON OF COMPOSITION OF TEKTITES WITH LOESS

	1*	2*	3*
SiO_2	73.72	73.45	75.02
Al_2O_3	12.34	11.53	12.32
FeO	4.73	4.54	2.98
MgO	2.07	2.05	1.66
CaO	2.70	3.50	2.85
Na_2O	1.31	1.28	1.33
K_2O	2.36	2.28	2.89
P_2O_5	—	0.03	0.10
TiO_2	0.83	0.70	0.98
Σ	100.06	99.76	100.13

*1 Average composition of 85 tektites (ref. 1).
*2 Average of 17 australites (ref. 4).
*3 Average loess (ref. 18). Less volatile matter at 1,000° C.

The suggestion by Schwarcz is of value in directing attention to widespread surficial deposits as possible parent materials. I[4] have suggested that loess might be a likely source. The composition given in Table 3 is very close to tektites. Magnesium oxide is slightly low (1.66 per cent) compared with tektites (2.07 per cent), and this difference, although small, may be significant. Phosphorus is somewhat high as well, although much closer to tektites than the soil average used by Schwarcz[1]. In addition, sodium was determined by difference in the analyses quoted[18], and only semi-quantitative trace element data are available, so that rigorous comparisons with tektite composition are not possible until this is remedied. Loess is, however, very much closer in composition to tektites than other common terrestrial material[19], the use of which implies only surficial melting (without deep excavation of a crater) as envisaged by Urey's comet hypothesis[17].

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PACKING OF BINARY MIXTURES OF SPHERES AND IRREGULAR PARTICLES

By Prof. S. YERAZUNIS, J. W. BARTLETT and A. H. NISSAN
Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York

THE packing characteristics of binary mixtures of spheres and irregular particles have been explored during an investigation of the occurrence of 'critical points' in the drying of impervious particulate solids in random packing. Initial investigations with equal glass spheres, 0.05 mm in diameter, using a container-to-sphere aspect ratio of about 750, led to an estimate of a packing fraction of 0.636 which

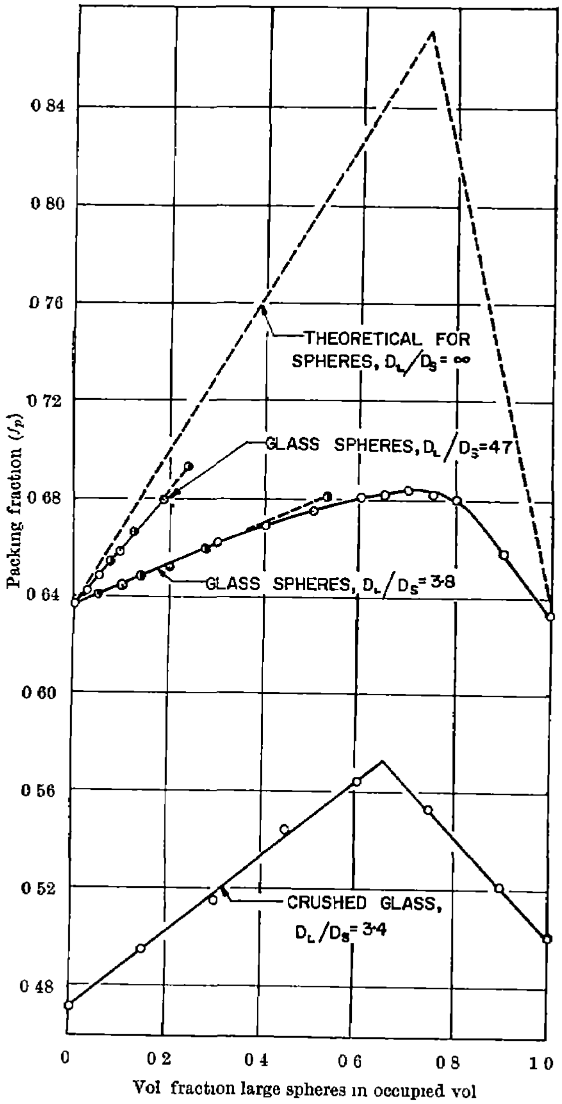

Fig 1 Packing fractions in binary mixtures ○, Experimental, ◑, theoretical using equation (4) with $K = 0 48$

compares favourably with Scott's[1] determination of 0 637 and Rutgers's[2] subsequent estimate of 0 641

The packing fraction of binary mixtures of spheres was found to be a function of the composition of the mixture and the ratio of sphere diameters The experimental results (Fig 1) for a diameter ratio of 3 8 show that a maximum value of the packing fraction of 0 684 is obtained at a composition of 0 735 volume fraction of large spheres in the occupied volume These results can be partly explained in terms of the theoretical behaviour of binary mixtures of an infinite diameter ratio In one extreme, the addition of a large sphere to a small sphere régime results in the replacement of small spheres producing an increase in the packing fraction by excluding the micro-voids associated with the small sphere régime which is replaced This replacement process may be continued only up to that point at which the large spheres are themselves arranged in dense random packing At the other extreme, small spheres can be added to an existing random array of large spheres, resulting in the partial filling of the existing voids This addition process may be continued until the macro-voids formed by the large spheres are entirely filled by small spheres in dense random packing For the case of spheres, the maximum packing fraction resulting from either the replacement or addition process will be $1 - (1-0\ 637)^2 = 0\ 868$ The packing fraction at intermediate compositions will be a linear function of composition for the infinite diameter ratio since both the small and large sphere régimes will correspond to infinite container-to-particle aspect ratios

For a finite diameter ratio, the distortion of the small sphere régime due to the large sphere surfaces must be taken into account An estimate of the effect can be obtained through the following arguments In the binary mixture, the small spheres can be imagined to occupy domains of size and shape determined by the number and the arrangement of the large spheres The average volume of the small sphere domains will be given by $(1 - V_LN_L)/N_L$, where V_L is the volume of the large sphere and N_L is the number of large spheres per unit volume of the mixture A characteristic length of the small sphere domain can be defined as D_D equal to $[(1 - V_LN_L)/(N_L)]^{1/3}$

The local aspect ratio, that is, particle-to-domain dimension, is approximately $[D_s][N_L/(1 - V_LN_L)]^{1/3}$, where D_s is the diameter of the small sphere

From Scott's measurements, the packing fraction of the small sphere domain, f_p^s, may be expressed in terms of the aspect ratio by

$$f_p^s = f_{p\infty} - KD_s[(N_L)/(1 - V_LN_L)]^{1/3} \qquad (1)$$

where $f_{p\infty}$ is the packing fraction for dense random packing of equal spheres in an infinite container and K is an empirically determined constant

Since the degree of distortion of the small sphere packing would be expected to be related to both the aspect ratio and the extent of the 'wall' (surface of the large sphere), equation (1) cannot be applied directly On the average each of the small sphere domains is associated with one large sphere It is proposed that an effective packing fraction f_{pe}^s be defined so as to reflect the area of the large sphere relative to a sphere of diameter corresponding to the characteristic domain length, D_D, that is, a function of $[D_L/D_D]^2$ The simplest assumption is to modify K in equation (1) as follows

$$f_{pe}^s = f_{p\infty} - KK'(D_L/D_s)^2D_s[(N_L)/(1 - V_LN_L)]^{1/3} \qquad (2)$$

where K' is a constant of proportionality which on further simplification can be absorbed in K Since the packing fraction for the mixture, f_p, is

$$f_p = V_LN_L + (1 - V_LN_L)f_{pe}^s \qquad (3)$$

it follows that

$$f_p = V_LN_L + [1 - V_LN_L]\left[f_{p\infty} - KD_sD_L^2\frac{N_L}{1 - V_LN_L}\right] \qquad (4)$$

In (4) K' was assumed unity, in other words, absorbed in K Theoretical packing fractions were calculated by (4) for the two sets of experimental data shown in Fig 1 (*a*) $D_s = 0\ 05$ mm, $D_L/D_s = 3\ 8$, and (*b*) $D_s = 0\ 13$ mm, $D_L/D_s = 47$ The constant, K, was originally estimated as 0 41 using Scott's[1] measurements for equal spheres in dimpled cylinders While the predicted and observed results were not in exact correspondence, the comparison was not unsatisfactory, a very good fit was obtained for K taken as 0 48 The correlation is excellent for the 3 8 diameter ratio up to a volume fraction of large spheres of 0 40

Above this concentration, it appears that the characteristic domain length is too small for equation (2) to represent the small sphere régime distortion adequately.

This analysis indicates that the mixture packing fraction for large sphere concentrations less than that corresponding to maximum compaction is determined by two opposing factors (*a*) the replacement of small sphere arrays and their associated microvoids by the large spheres; (*b*) the ensuing distortion of the residual small sphere domains determined by the characteristic length of the domain and the surface provided by the large spheres.

The compaction process for concentrations exceeding that associated with the maximum packing fraction has not been explained quantitatively for mixtures of finite diameter ratios. However, for the case of the 3·8 diameter ratio, it would appear to be a consequence of the distortion of the large sphere array due to the insertion of the smaller spheres. In this case, there exist very few voids in the random packed large sphere system capable of accommodating the smaller spheres. An expansion of the large sphere arrangement would be quite in order leading qualitatively to the observed behaviour.

Furnas[3] had previously inferred relations between the void fraction, size ratio, and composition of binary mixtures of broken solids. Although the experimental results presented in his and these investigations are in approximate agreement, the shape of the theoretical functions is dissimilar. Furnas's theoretical packing fraction curves are concave upwards in contrast to the predictions based on equation (4), which are very nearly linear. The discrepancy cannot be explained since Furnas did not report the basis for his theoretical curves.

Shown also in Fig. 1 are the packing fractions of mixtures of crushed glass particles. The material was obtained by crushing glass rod in a dry ball mill and sizing in a U.S. Standard Sieve Series. The resulting particles were highly irregular and of complex polyhedral shape; the nominal particle sizes were comparable with those of the spheres. The packing fraction of these mixtures followed the trends for spheres, suggesting that the irregular particles may be thought of as incomplete spheres. The maximum packing fraction occurred at a composition of 0·66 volume fraction occupied by the large particles, which compares favourably with the theoretical value of 0·68 based on the packing fractions of 0·50 and 0·47 determined experimentally for the large and small sizes, respectively.

This work was supported by grant *G*–10185 of the National Science Foundation made to one of us (A. H. N.).

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INFLUENCE OF MECHANICAL AGITATION ON THE NUCLEATION OF SOME AQUEOUS SALT SOLUTIONS

By Dr. J. W. MULLIN and Dr. K. D. RAVEN

Department of Chemical Engineering, University College, London

NUCLEATION constitutes the first, and probably the least-understood, stage in the production of a new phase. Modern theories of nucleation stem from the work of Gibbs[1], Volmer[2] and others, who considered the conditions necessary for the equilibrium of a small spherical particle located within a larger mass of a different phase. This equilibrium, defined by the balance between surface tension and pressure forces, is an unstable one, since a slight increase or decrease in the size of the particle leads to indefinite growth or decay.

It is postulated by the Gibbs–Volmer theory that embryos of varying size occur within a metastable phase, and that any which exceed the critical equilibrium size grow spontaneously as particles of the new, stable phase. The energy, ΔG, required to form a critical nucleus can be calculated, and used in a general rate equation of the form

$$J = \nu \exp\left[-\Delta G/\mathbf{k}T\right]$$

Where J is the nucleation-rate, and ν is a constant for the system. This relationship leads to nucleation-rates in fair agreement with those obtained in practice.

Mechanical effects can profoundly influence the onset of nucleation, and as long ago as 1911, Young[3] and Berkeley[4] independently investigated the effect of mechanical shock on supersaturated aqueous systems. No systematic study of this aspect has been reported since their work, although several authors have referred to isolated investigations.

It is generally agreed that mechanical disturbances will enhance or even initiate nucleation, but no quantitative assessment of the effect has been made. However, during the course of some investigations made by the authors in a pilot-scale Oslo crystallizer, it became apparent that the relationship between agitation and nucleation was not a simple one. It was found that whereas nucleation was more prominent in disturbed than in quiescent systems, as generally supposed, an increase in the intensity of agitation did not necessarily lead to a continuous enhancement of the nucleation process.

A preliminary report[5] of this new phenomenon, which was investigated in more detail in a nucleation cell, was concerned only with ammonium dihydrogen phosphate solutions over a limited range of temperature. The work has now been extended to a number of other aqueous salt systems, and an explanation of the results is proposed.

The nucleation cell used for this work consisted of a 'Pyrex' glass beaker 60 mm. diameter × 80 mm. deep, equipped with a 1 × ¼ in. glass T-stirrer. A verticle 'Perspex' baffle, 10 mm. wide, protruded from the wall of the cell, and a thermometer placed diametrically opposite approximated to a second baffle.

Solutions were cooled at a constant rate in the cell at known agitation-rates and the temperature at

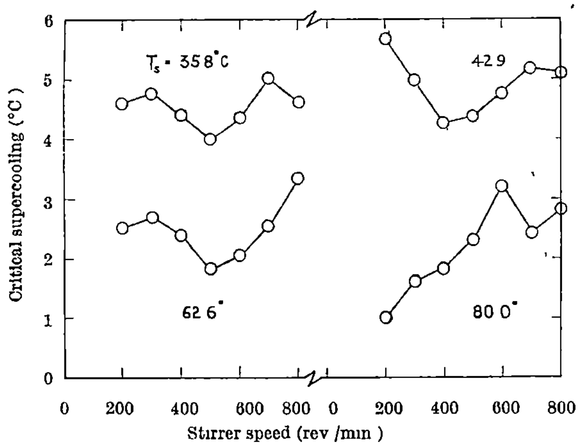

Fig. 1. Nucleation of ammonium dihydrogen phosphate solutions (T_s, saturation temperature)

which nuclei were first observed noted. Reproducible results could not be achieved unless the solutions were very carefully protected from inoculation by air-borne dust particles. A thick layer of liquid paraffin above the solution was found suitable for this purpose. The range of stirrer speed used was 200–800 rev./min., with a constant cooling-rate of 0.1° C./min. Nuclei were detected by visual observation, using oblique lighting, when they appeared as bright 'spangles'.

Some typical results obtained with aqueous solutions of ammonium dihydrogen phosphate are plotted in Fig. 1 as critical supercooling versus stirrer speed. By critical supercooling is meant the supercooling, in deg. C., below the saturation temperature, T_S, at which nucleation is first detectable. For saturation temperatures of about 40° C., increasing agitation at first reduces the supercooling required for nucleation, but further increase in agitation actually retards nucleation. Finally, at the highest usable rates of agitation, nucleation again increases.

At higher temperatures, the behaviour remains qualitatively the same, but the portion of the agitation range where increased agitation inhibits nucleation becomes larger until, at temperatures around 80° C., increased agitation inhibits nucleation throughout the agitation range available. (See also Fig. 3.)

It was not possible to determine nucleation temperatures accurately under quiescent conditions, but at all temperatures nucleation was much less pronounced in non-agitated than in agitated systems.

Data obtained from aqueous solutions of magnesium sulphate and sodium nitrate (Fig. 2) show that qualitatively their behaviour is similar to that of ammonium dihydrogen phosphate, but the temperature dependence of the relationship between nucleation and agitation is less clearly shown by sodium nitrate than by ammonium dihydrogen phosphate. It was not possible to draw any conclusions as to the influence of temperature on the nucleation behaviour of magnesium sulphate because temperatures were limited to below 45° C. to avoid transition to lower hydrates.

A few results have been plotted in Fig. 3 in terms of calculated values of critical supersaturation, rather than measured values of supercooling. Supersaturation is here defined as the ratio of the solution concentration to the equilibrium saturation concentration. Expressing the results in this way gives a more direct measure of the concentration driving force, and so of the speed at which nucleation and growth may be expected to proceed in different systems.

Nucleation is considered to occur by diffusion of solute particles to an embryo, followed by a pseudo-chemical reaction when the particle joins the lattice structure. The resultant dependence of the nucleation-rate, J, on agitation may be expressed by an equation of the form

$$J \propto (\text{rev./min.})^n$$

The index, n, should vary between zero, for conditions where the surface reaction step controlled the nucleation rate, and unity for a diffusion-controlled process. The fact that nucleation is more likely in agitated than in quiescent conditions has in the past led to the conclusion that nucleation is diffusion controlled. But the diffusional mechanism alone cannot account for the results reported here where increasing agitation is shown, under certain conditions, to reduce nucleation.

Experimental determinations of viscosity and diffusivity for saturated solutions of the salts used, at various temperatures, failed to reveal the existence of any

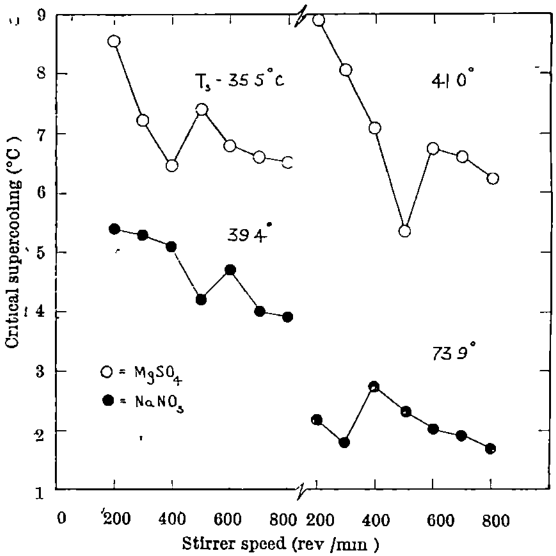

Fig. 2. Nucleation of solutions of magnesium sulphate and sodium nitrate (T_s, saturation temperature)

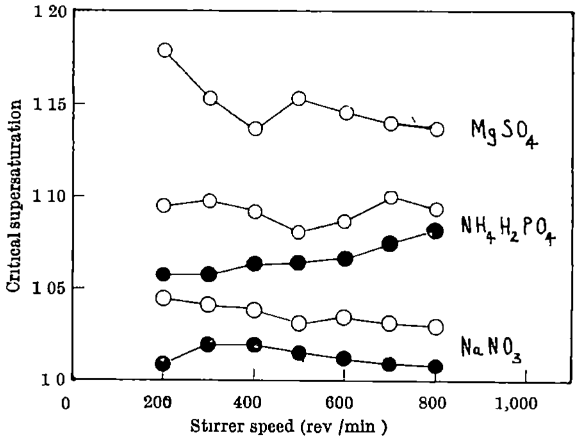

Fig. 3. Critical supersaturation versus stirrer speed: comparison of three aqueous salt systems. ○, T_s ~ 35° C.; ●, T_s ~ 70° C.

irregularities that could account for anomalous behaviour for a diffusional process.

Viscosities were determined in a standard Ostwald viscometer, immersed in a thermostat bath, and showed that viscosity was a smooth function of temperature for saturated solutions of each of the salts used. Viscosity was also a smooth function of temperature, for any given solution, showing no discontinuity at the saturation temperature. Diffusivities of saturated ammonium dihydrogen phosphate solutions were determined with a Zeiss diffusion interferometer; values varied smoothly from 3·38 to 4·81 × 10^{-6} cm^2/sec over the range 20–50° C.

Since these results offered no explanation of the nucleation effects reported here, other possible mechanisms that could inhibit nucleation were considered. It has frequently been suggested, and as often disputed, that attrition effects can operate between macro crystals in agitated crystallizers. The possibility that a similar disruptive effect, on a smaller scale, might be responsible for inhibiting nucleation was therefore considered.

On the macro-crystalline scale, attrition increases the nucleation process, as the broken fragments or disturbed surface nodules are generally of nucleic size. However, the fracture of nuclei, either by mutual collision, or impact with the vessel or stirrer, would yield fragments of less than nucleic size, and thus reduce the net rate at which embryos grow into nuclei.

At least two practical difficulties in the investigation of this latter possibility are immediately obvious. First, damage to true nuclei could not be studied as they are too small for any damage to be detected. Secondly, under the metastable conditions existing in supersaturated solutions, attrition effects would be masked by the normal growth and decay mechanisms operating. Accordingly, it was decided to investigate attrition among small macro crystals suspended in an inert medium, under the same agitation conditions as prevailed in the nucleation experiments.

Ways were considered for detecting damage to such crystals. Straightforward microscopic examination of a sample of the crystals before and after periods of agitation seemed possible, but to detect any but the severest of damage, the initial crystal mass would have to consist only of perfect, undamaged crystals, and the problems of producing quantities of such crystals of a small, fairly uniform size appeared formidable.

It was considered that damage arising from impacts between the stirrer and the crystals would be at least as serious as damage from inter-crystal collisions, and accordingly only the former effect need be studied. Furthermore, if the stirrer itself were of the same consistency as the crystals, it too would be damaged by such impacts, and this damage would be roughly equivalent to the total damage thereby suffered by the crystal mass.

A number of large single crystals were grown for use as stirrers in the following manner. A small needle crystal of ammonium dihydrogen phosphate was cemented to the end of a glass rod with epoxy resin to yield a miniature T-stirrer. This was left revolving gently in a slowly cooling saturated solution containing a stagnant mass of larger crystals to limit the supersaturation-level.

After cooling by about 8° C over a period of 24 hr., crystals grown in this way were approximately 1 in. long and ¼ × ¼ in. in cross-section, externally undamaged, and of almost perfect form, apart from slight asymmetry of the pyramidal ends occasioned by differing growth-rates due to the continuous rotation of the crystal in one direction.

The attrition experiments were conducted in the nucleation cell using one of these grown crystals in place of the stirrer, to which they approximated in size, to agitate a suspension of crushed ammonium dihydrogen phosphate crystals (< 40 mesh) in kerosene. Runs were conducted at various agitation-rates and for various times, and a qualitative assessment of the erosion of the leading edges of the stirrer crystal was made after each run by visual observation and photography.

The results showed that there was a threshold stirrer speed of about 400 rev./min., below which negligible attrition occurred. This threshold corresponded, roughly, to the degree of agitation at which inhibition of nucleation had first been noted. Attrition effects then increased markedly in extent up to the limit of the agitation range used (~ 1,000 rev./min.). No attrition of the crystal stirrer occurred when it was operated for prolonged periods of time in kerosene alone at the highest rate of revolution.

The nucleation experiments had shown that, at the higher available rates of agitation in the cell, increasing agitation resulted in an increased tendency to nucleate. To account for this reversion to 'conventional' nucleation behaviour would require an upper limit at which attrition reached a maximum, but this could not be demonstrated as no accurate measure of attrition was available.

However, the existence of the threshold suggested that attrition would not occur unless the impact velocity exceeded some minimum value sufficient to fracture the crystal lattice. The velocities of the embryos in an agitated system will not be uniform, but will have a spread which may be assumed to approximate to a Gaussian distribution. Accordingly, the probability of attrition occurring for any given impact depends on the probability of the impact velocity exceeding this minimum value.

A plot of mean particle speed against the proportion of particles having a speed in excess of some given value is, for a Gaussian distribution, given by an S-shaped cumulative distribution curve, or ogive. Since mean particle speed may be assumed to be proportional to stirrer speed, the relationship between attrition effects and stirrer speed should also follow an ogive relationship (Fig. 4b). The influence of agitation on diffusional and surface effects can be

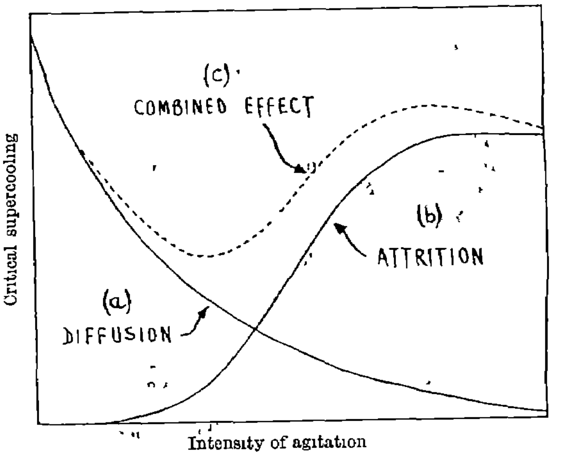

Fig. 4. Construction of a theoretical curve relating the intensity of agitation of a solution to its tendency to nucleate

predicted as described here, from a power law (Fig 4a). A combination of these two relationships yields a theoretical 'nucleation versus agitation' curve. While the relative magnitudes of the two conflicting effects cannot at present be predicted, the general form of this plot will be as shown in Fig 4c.

This composite plot may be compared with the experimental graphs shown in Figs 1 and 2. At low temperatures the agreement is quite good, but at higher temperatures the experimental plots tend to deviate from the theoretical curve. The prominence of nucleation inhibition at elevated temperatures may possibly be due to the thermal weakening of the metastable embryo lattices and the consequent lowering of their resistance to attrition.

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A HYPOTHESIS CONCERNING THE WAY OF CODING INTERACTION BETWEEN TRANSFER RNA AND MESSENGER RNA AT THE LATER STAGES OF PROTEIN SYNTHESIS

By Dr Zh. A. MEDVEDEV

Department of Agrochemistry and Biochemistry, Timiriasev Agricultural Academy, Moscow, A 8, U S S R

IT has been shown during the past few years that the linking of activated amino-acids to the terminal adenosine of special low-molecular forms of so-called 'transfer' RNA (or *s*-RNA) is the preliminary stage of protein synthesis. Molecules of these 'transfer' RNA's carry the activated amino-acids to the ribosome particles where the amino-acid moieties are incorporated into newly forming peptide chains.

This interaction between 'transfer' RNA and ribosomal RNA has become a central point of some present hypotheses concerning the possible mechanism of reproduction of amino-acid sequences in proteins[1–8].

Though differing in some details, all these hypotheses have suggested that the polynucleotide chains of different molecules of 'transfer' RNA's, consisting of some 100 bases, are complementary (or have some restricted three-nucleotide complementary sequences) to the different parts of template RNA. It has been suggested, therefore, that among many collisions between 'transfer' RNA and ribosomes some can be effective in producing exact interaction between complementary nucleotide sequences and result in amino-acid transfer in accordance with the template 'code'[9].

However, the mechanism of protein synthesis now seems to be more complicated especially in the ribosomal stage. The discovery of a special RNA-messenger of genetic information which acts as a real informative template of protein synthesis[10–12], the finding that only a proportion (about 10 per cent) of 70 *S* double ribosomes are active as the sites of protein synthesis[13], and that the disintegration of these 'active' 70 *S* ribosomes into two particles stops protein synthesis which resumes after re-integration[14], and finally the interesting results demonstrating that such disintegration releases some polypeptides and peptides with high specific activity[15,16] (in experiments with amino-acids labelled with carbon-14) suggested the possibility that the amino-acids are incorporated in the 'active' 70 *S* ribosomes (containing the 'messenger' RNA as activator) in the groove between 30 *S* and 50 *S* particles[13].

In addition, the finding in cells of special so-called 'transfer' enzymes responsible for the transfer of *s*-RNA–amino-acid complexes to ribosomes[17–20] makes it somewhat difficult to explain the interaction between this *s*-RNA and the template on the basis of random collision only. If one takes into consideration that 'transfer' RNA is used repeatedly for protein synthesis without intermediate breakdown[21], that its 100 nucleotide strand is enriched by 'unusual' additional nucleotides, especially pseudo-uridine[22–25], and that the 'messenger' RNA consists of some 1,000 bases, then it becomes clear that all these data have to be considered in any coding speculations concerning the transfer of information from DNA and RNA to direct protein synthesis.

The recent achievements in the experimental attack on the coding problem[26–33] must be discussed, of course, not only from theoretical or statistical points of view. when the nucleotide code for amino-acids becomes a reality it must be considered as part of the complicated system of protein synthesis.

The following general characteristics of the amino-acid code seems now to be the best-established as a result of new experiments: (*a*) The code has a triplet nature (three nucleotides code one amino-acid). (*b*) The code is of the non-overlapping type. (*c*) The code is degenerate and one amino-acid can be coded by several triplets of bases.

But though these suggestions in the genetic code are now widely known, one can see that the evidence which supports them is indirect and some other speculations on the nature of the code are still possible.

Here we have devised an overlapping type of code which can explain the facts concerning the mutants of tobacco mosaic virus which have been used as the main argument for the existence of non-overlapping coding triplets[32,33]. The non-overlapping code was deduced from the fact that the alteration of a single nucleotide leads, as a rule, to the alteration of a single amino-acid, though if the code is overlapping the same alteration would produce a change in two or three adjacent amino-acids in the polypeptide chain.

But if one suggests that the coding nucleotide triplets are composed of non-adjacent nucleotides in the template and the transfer RNA, then the same result of mutation could be explained in the case of

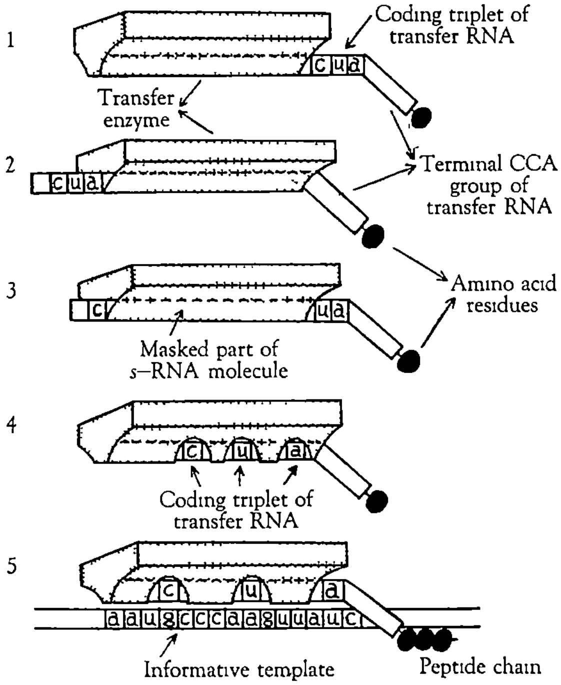
1
2
3
4
5
Coding triplet of transfer RNA
c u a
Transfer enzyme
Terminal CCA group of transfer RNA
Amino acid residues
Masked part of s–RNA molecule
Coding triplet of transfer RNA
a a u g c c c a a g u u a u c
Informative template
Peptide chain

Fig 1. Outline of different possible coding interaction between transfer RNA–amino-acid–transfer enzyme complex and messenger RNA as a template

an overlapping code, if the complete length of polypeptide chain is not bigger than the two or three lengths of transfer RNA Alternatively, the specific nucleotide triplets may be located not far from the terminal position (Fig 1) If only three adjacent nucleotides are necessary for coding, it would be difficult to understand the role of so long a polynucleotide of transfer RNA (80–120 nucleotides) To solve this difficulty some possibilities have been suggested concerning the mechanism of the elimination of the main part of transfer RNA polynucleotide chain from binding with the template (breakdown of transfer RNA before binding[2,4], formation of the protein along a microsomal phospholipid membrane[7] sited at a distance equal to the length of transfer RNA chain next to the triplet coding sequence and at the end of the chain and some others)

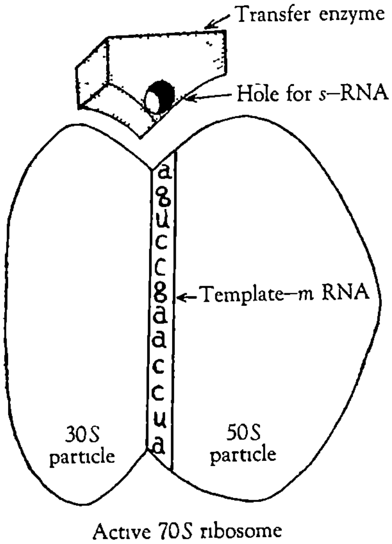

Fig 2 Outline of interaction between 'transfer' enzyme and 70 S ribosomes

In my model of the later stages of protein synthesis a new possibility of such elimination is suggested—the masking of the greater part of the transfer RNA chain by its entering into a hole or groove in the transfer enzyme molecule (some analogy with the location of RNA in an intact virus particle) I think that the pseudo-uridine nucleotide can probably serve for the necessary fixation of the polynucleotide chain position in the hole Fig 2 shows a possible mode of interaction between transfer enzyme, 70 *S* ribosome and messenger RNA

I favour the idea of this overlapping code not because it seems to be more economical, but because it can better explain the parallelism of distances between nucleotides and amino-acid residues and because the deletion or addition of a single nucleotide means in this case a point mutation only, but not the wrong reading of all the genetic information and the production of completely nonsense protein molecules

I thank Dr P N Campbell, Dr M B Hoagland and Dr G Webster for useful discussion of my suggestions before publication

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VISUAL PIGMENT 544, A PRESUMPTIVE CONE PIGMENT FROM THE RETINA OF THE PIGEON

By Dr C. D B. BRIDGES

Visual Research Division, Ophthalmological Research Unit (Medical Research Council),
Institute of Ophthalmology, London, W C I

ALTHOUGH iodopsin has so far been extracted only from the retina of the chicken, it has been held to be the mediator of photopic vision in a number of vertebrates Thus, Wald, Brown and Smith[1] have written " it seems probable that it is the major pigment of cone vision in the frog, snake and cat" This view has arisen because of a resemblance between the photopic sensitivity curves for these animals and the extinction of iodopsin This resemblance, however, does not extend to the photopic sensitivity[2] of the chicken, which is maximal some 20–30 mμ higher than the extinction maximum of iodopsin (562 mμ) This discrepancy is often considered to be due to the filtering action of the coloured oil-droplets in the cones In recent years the extinction spectrum of iodopsin has been compared not with the photopic sensitivity of the chicken, but with the more widely studied and more accurately known function for the pigeon With the view of determining whether this comparison is justified on the basis of the pigeon's visual pigments, I have prepared extracts of photosensitive material from the retinæ of dark-adapted pigeons, these extracts have been analysed by the method of partial bleaching[3]

Owing to the kind assistance of the Medical Officer of Health for Westminster, pigeons which had been trapped alive for the purposes of pest control were dark-adapted overnight and were then killed by breaking their necks in darkness The dead birds were brought to this laboratory in a light-tight bag Within 1½–3 hr of death the whole retinæ together with the adhering pigment epithelium had been removed under normal dark-room conditions The subsequent procedure was similar to that described by Wald, Brown and Smith[1] for their chicken extracts The retinæ from 18–25 birds were shaken with 50 per cent w/v sucrose in McIlvaine's *p*H 7 0 buffer, centrifuged at 4,000 relative centrifugal force, and the resulting supernatant suspension of photoreceptors was then precipitated by dilution with McIlvaine's *p*H 7 0 buffer and centrifuging at 35,000 relative centrifugal force After washing once with distilled water (*p*H 6 6) the precipitated material was freeze-dried and extracted five times with petroleum ether, b p 60°–80° These extracts contained the yellow colouring materials of the oil-droplets Residual traces of petroleum ether were pumped off and the dried residue was extracted for about 17 hr at 4° C with 4 per cent digitonin in McIlvaine's *p*H 6 6 buffer

The curve describing the filled circles in the upper part of Fig 1 is the extinction spectrum of an extract prepared in this manner The remaining spectra were obtained during the course of an experiment, typical of the series, designed to test the homogeneity of the extracted photosensitive material The spectrum represented by the dotted curve in Fig 1 was measured after 45 min exposure to deep red light of dominant wave-length 720 mμ and that by the dashed curve after 25 min exposure to light of wave-length 620 mμ A subsequent identical exposure to λ720 mμ light produced no further change of extinction but when the solution was exposed to white light the spectrum altered to that shown by the open circles The two difference spectra representing extinction changes due to the initial deep red and final white irradiations are shown in the lower half of Fig 1 Clearly the solution contains two photosensitive pigments, for the material bleached by red light has a maximum loss of extinction at about 545 mμ (difference spectrum shown by the crosses), whereas the loss of extinction caused by the final white irradiation was maximal at 505 mμ (difference spectrum shown by the open circles)

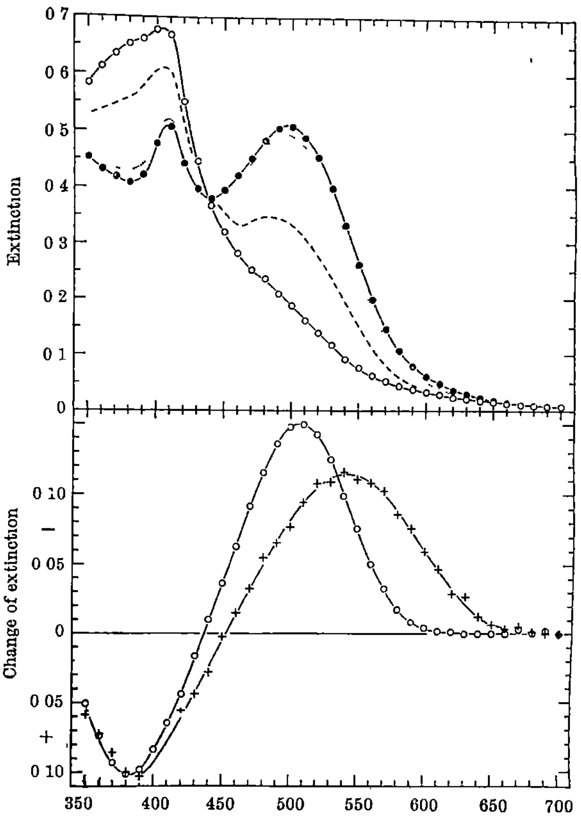

Fig 1 A typical experiment designed to test the homogeneity of pigeon visual pigment Upper half ●, initial spectrum, , after 45 min exposure to λ 720 mμ light (from a Unicam *SP* 500 monochromator, exit slit at 1 0 mm, Ilford filter No 206) ––––, after 25 min exposure to λ 620 mμ light (exit slit at 0 6 mm Ilford filter No 204), a subsequent exposure to λ 720 mμ (identical with the first one) produced no appreciable change of extinction, ○, after 10 min exposure to white light (from a 15-W lamp) Lower half +, difference spectrum due to the initial λ 720 mμ irradiation (×5), ○, difference spectrum due to the final white irradiation *p*H 6 6, all extinction spectra measured at 25 ± 2° C on a Unicam *SP* 500 spectrophotometer, extinctions refer to a 1-cm optical path

In all the experiments carried out, it was found that the photo-sensitive pigment remaining after exhaustively irradiating the solutions with red light yielded identical difference spectra with λ_{max} at 504–505 mμ. From 490 to 620 mμ these difference spectra were identical with the extinction spectrum of visual pigment 502 (ref. 4), and it is therefore concluded that one of the pigeon pigments is a rhodopsin with λ_{max} at 502 mμ. The same pigment has been extracted from the retina of the domestic duck (my own observation).

What is the λ_{max} of the red-sensitive pigment? In view of Wald's[5] reference to some unpublished work of Brown in which she had "extracted iodopsin from the pigeon retina" and found it had "virtually the same spectrum as chicken iodopsin", it was at first thought possible that the difference spectrum maximal at 545 mμ merely represented a mixture of iodopsin, λ_{max} 562 mμ, contaminated by the bleaching of some of the large excess of rhodopsin present. In order to test this assumption, it was necessary to obtain the rhodopsin free from the red-sensitive pigment and then see what proportion of this rhodopsin could be bleached by an identical red irradiation. The results of such an experiment are illustrated in Fig. 2. The solution was first irradiated for 1 hr with 720 mμ light. The difference spectrum for this operation (open circles) has λ_{max} at 544 mμ and ΔD_{max} of 0.0483. All the red-sensitive pigment was then removed by irradiation with light of wave-length 620 mμ. This operation left about 60 per cent of the estimated initial amount of rhodopsin. A second deep-red irradiation identical in all respects with the first caused no detectable bleaching, only a slight rise of extinction amounting to 0.0030, probably unconnected with the irradiating light, was observed. This is shown by the dashed curve in Fig. 2. Since the light which produced the initially observed difference spectrum was incapable of bleaching any of the rhodopsin present it must be concluded that this represents the true difference spectrum of the red-sensitive pigment. Similarly, in none of the experiments carried out was it possible to find any evidence that bleaching operations which yielded difference spectra maximal at 540–546 mμ had caused any concomitant rhodopsin bleaching.

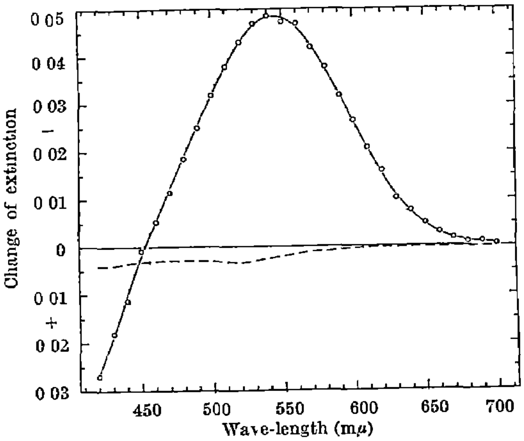

Fig. 2. Experiment showing that the red-sensitive difference spectrum represents the bleaching of a pure pigment, and is not contaminated by any concomitant rhodopsin bleaching. o, difference spectrum due to an initial exposure of 1 hr to λ 720 mμ light (exit slit at 1.0 mm, Ilford filter No. 206). The red-sensitive material was then removed by irradiation with λ 620 mμ light, and the solution was again exposed to an irradiation identical in all respects to the first. The difference spectrum shown by – – – – gives the change in extinction due to this irradiation. *p*H 6.3, other details as for Fig. 1

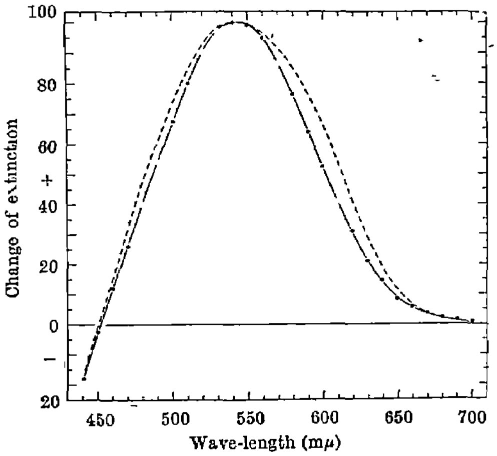

Fig. 3. An attempt at simulating the difference spectrum of the red-sensitive pigment by adding the difference spectra of pigeon rhodopsin and chicken iodopsin. – – – –, the summed difference spectra of pigeon rhodopsin (1 part) and chicken iodopsin (2.3 parts); ●, difference spectrum of the red-sensitive pigment (mean of five results on three separate extracts)

In any event, it is not possible to simulate the difference spectrum of the red-sensitive material by adding together the difference spectra of pigeon rhodopsin and chicken iodopsin. In Fig. 3 an attempt has been made to do this: the dashed curve represents the summed difference spectra of these two pigments (one part of rhodopsin to 2.3 parts of iodopsin), while the filled circles represent the red-sensitive difference spectrum. Although the λ_{max} of these two curves are both 544 mμ, the composite difference spectrum is obviously much too broad to fit the experimental results.

Further evidence that the red-sensitive component is a single pigment is provided by considering the shape of its extinction spectrum. This has been derived in the same manner as that used by Wald, Brown and Smith[1] for iodopsin. A difference spectrum represents the difference in extinction between a visual pigment and its photoproducts: in the present case, at *p*H 6.6, these photoproducts are probably retinene and the protein moiety of the visual pigment molecule. If the extinction spectrum of this mixture were known, then by adding it to the observed difference spectrum the extinction spectrum of the visual pigment should result. Wald, Brown and Smith[1] have published the spectrum of a mixture of retinene and the protein moiety of chicken iodopsin (at a *p*H of 6.5), and in the absence of data corresponding to the present investigation, this has been used to derive the extinction spectrum shown in Fig. 4. The figure shows that the extinction spectrum of the red-sensitive component is the same as that for a hypothetical pigment, with the same λ_{max} of 544 mμ, derived from Dartnall's nomogram[4]. This agreement would be impossible if a mixture of rhodopsin and iodopsin were used instead of the experimental results.

These considerations show beyond reasonable doubt that the second pigment of the pigeon retina is not chicken iodopsin but is a new pigment with λ_{max} at 544 ± 3 mμ (visual pigment 544). In conformity with every known visual pigment, its extinction spectrum is in agreement with that predicted by Dartnall's nomogram. In view of this conclusion, Wald's[5] reference to the extraction of iodopsin from the pigeon retina is puzzling. Visual pigment 544 only accounts for 15 per cent of the total photopigment, and a possible explanation would be

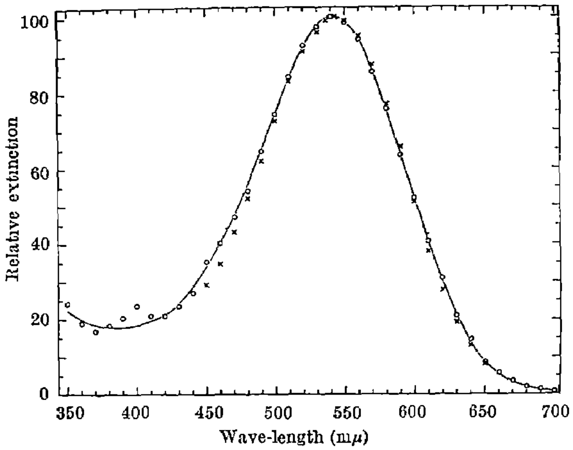

Fig. 4. Extinction spectrum of the red-sensitive pigment. O, extinction spectrum of the red-sensitive pigment (calculated as described in the text); ×, visual pigment 544 according to Dartnall's nomogram

that the results were similar to those reported here, but that the difference spectra obtained by exposure to deep-red light were regarded as a mixture of rhodopsin and iodopsin. Further discussion of this discrepancy must be deferred until Mrs. Brown's results have been published.

Is it possible to determine whether visual pigment 544 is a rod or a cone pigment? In one experiment concerning this question, each isolated retina was very gently stirred in a tube of 0.9 per cent saline prior to placing in the sucrose solution. It was thus hoped that the more fragile cone outer segments would be washed into the saline, leaving the retina relatively cone-free. A digitonin extract from the retina was prepared as previously described. The saline, which to the naked eye contained a number of detached pieces of pigment epithelium, was centrifuged and the precipitated material was dried and extracted first with petroleum ether, finally with digitonin. Analysis of the photosensitive material in each digitonin extract revealed that tissues which contained 82 per cent of the total retinal visual pigment 544 had been washed into the saline. Only about half (52 per cent) the total retinal rhodopsin was found in the 'saline' fraction.

This experiment shows, therefore, that visual pigment 544 is present in receptors which are more fragile or more deeply embedded in the pigment epithelium than those which contain the rhodopsin, and consequently the results suggest that visual pigment 544 is a pigment primarily of the cones.

The discovery that the pigeon has a visual pigment some 18 mμ below that of chicken iodopsin raises a number of questions concerning the interpretation of spectral sensitivity curves. It is proposed to consider these in a paper to be published shortly[6].

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ACTION OF 7,12-DIMETHYLBENZ(ALPHA)ANTHRACENE ON THE MITOTIC ACTIVITY OF NORMAL AND MALIGNANT RAT FIBROBLASTS *IN VITRO*

By V. B. STARIKOVA and J. M. VASILIEV

Institute of Experimental and Clinical Oncology, Moscow, U.S.S.R.

CARCINOGENIC hydrocarbons as well as some other carcinogenic agents (for example, ionizing radiation) can inhibit the growth of various normal and neoplastic tissues both *in vivo* and *in vitro*$^{1-3}$. For the better understanding of mechanisms of chemical carcinogenesis it is important to know whether the sensitivity of cells toward the growth-inhibiting action of these hydrocarbons is changed in the course of neoplastic transformation.

The aim of our experiments was to examine the sensitivity of the primary cultures of normal rat connective tissue and of rat sarcomas to the action of very strong carcinogenic hydrocarbon-7,12-dimethylbenz(α)anthracene (DMBA).

Three types of normal connective tissue were used in these experiments: (1) Granulation tissue developed in adult rats around subcutaneously implanted 'Cellophane' films, the specimens of tissue were taken for cultivation 14 days after the implantation of films. (2) Subcutaneous connective tissue of rat embryos about 18–20 days after gestation. (3) Heart fibroblasts of rat embryos.

Only primary induced tumours were used in the experiments with neoplastic tissue.

Two types of sarcomas were tested: (1) Those induced by DMBA implanted in pellet form subcutaneously, each pellet contained 1 mgm. of hydrocarbon, tumours usually formed 4.5–6 months after the implantation of pellets. (2) Those induced in the rat connective tissue by subcutaneously implanted 'Cellophane' films. Tumours usually formed 10–16 months after the implantation of films (see ref. 4 for details of method). Each of the normal and malignant tissue types was used in five separate experiments. Tissue specimens in each experiment were taken from a new animal.

Thus, ten various induced sarcomas were used in all experiments, histologically all these tumours were fibrosarcomas and pleomorphic-cell sarcomas.

Tissue fragments were cultivated on the plasma clot in Carrel flasks in the synthetic medium 199 supplemented with 10 per cent calf serum. DMBA was dissolved in acetone and then added to the culture fluid medium so that fine suspension of the hydrocarbon was formed. Control experiments showed that even the highest concentration of acetone added to the medium in our experiments (1 per cent) did not change the mitotic activity of the cultures. Various concentrations of DMBA (ranging from 0.25 to 0.000025 mgm. per ml. of medium) were added to the various groups of explants in each experiment. The minimal number of explants in

Table 1. INFLUENCE OF DMBA ON THE MITOTIC ACTIVITY OF THE PRIMARY CULTURES OF NORMAL RAT CONNECTIVE TISSUES

Concentration of DMBA (mgm./ml. culture medium)	Subcutaneous connective tissue of embryos						Granulation tissue of adult rats				Embryonic heart				
No. of Exp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	18·2	10·0	6·5	17·0	6·2	16·2	5·0	10·0	11·4	12·0	16·4	7·7	10·6	7·2	12·2
0·25	No growth	0 (0·999)	No growth	0 (0·999)	No growth	0 (0·999)	No growth	No growth	No growth	No growth	0 (0·999)	0 (0·999)	No growth	0 (0·999)	0 (0·999)
0·025	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)
0·0025	5·2 (0·999)	0·5 (0·999)	0 (0·999)	0·2 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)	0·4 (0·999)	0·2 (0·999)	Not tested	0 (0·999)	0 (0·999)	0 (0·999)	0 (0·999)
0·00025	6·0 (0·999)	1·5 (0·999)	Not tested	3·6 (0·99)	0 (0·999)	Not tested	Not tested	Not tested	2·4 (0·999)	3·2 (0·99)	Not tested	Not tested	0 (0·999)	1·0 (0·999)	0 (0·999)
0·000025	Not tested	Not tested	Not tested	3·8 (0·999)	3·6 (0·999)	Not tested	Not tested	Not tested	0·6 (0·999)	Not tested	Not tested	Not tested	0 (0·999)	Not tested	2·1 (0·999)

Average numbers of mitoses per thousand cells in the outgrowth zones in the explants of each experimental group are given. The figures in the brackets are P values—probability of the significant difference of the averages in given group and in corresponding control groups.

Table 2. INFLUENCE OF DMBA ON THE MITOTIC ACTIVITY OF PRIMARY CULTURES OF RAT SARCOMAS

Concentration of DMBA (mgm./ml. culture medium)	DMBA-induced sarcomas					'Cellophane'-induced sarcomas				
No. of Exp.	1	2	3	4	5	6	7	8	9	10
Control	23·7	11·6	10·4	5·8	14·2	6·6	11·5	10·2	8·3	14·2
0·25	3·5 (0·999)	0·2 (0·999)	0 (0·999)	8·5 (0·9)	10·7 (0·8)	0 (0·999)	6·5 (0·95)	0 (0·999)	0·2 (0·999)	3·0 (0·999)
0·025	9·2 (0·25)	17·4 (0·7)	8·5 (0·4)	7·0 (0·6)	9·4 (0·8)	4·7 (0·25)	12·2 (0·3)	8·3 (0·8)	7·2 (0·6)	10·0 (0·8)
0·0025	21·4 (0·3)	15·8 (0·5)	Not tested	11·0 (0·99)	20·0 (0·8)	5·5 (0·8)	20·5 (0·9)	6·0 (0·95)	9·3 (0·5)	5·2 (0·99)
0·00025	Not tested	7·0 (0·4)	9·2 (0·3)	8·0 (0·5)	16·3 (0·3)	Not tested	Not tested	Not tested	10·4 (0·9)	10·4 (0·8)
0·000025	Not tested	Not tested	Not tested	8·4 (0·6)	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested

each group was 12. DMBA was present in the medium during the period of cultivation. Seven days after the explantation all the cultures were fixed in Schabadasch mixture[5] and then stained with Meyer's hæmatoxylin. The number of mitoses per 1,000 cells was counted in five various explants of each group. The statistical significance of the differences between average mitotic indices in various groups was tested by the 'Student' Fisher small-sample method. Average culture areas in various groups were also compared. Morphological examination showed that all the normal and neoplastic tissues tested in these experiments gave the outgrowth of cells of fibroblastic type.

Results of experiments are summarized in Tables 1 and 2. As can be seen (Table 1) DMBA in all the concentrations caused severe inhibition of the growth of normal fibroblasts. High concentrations of DMBA prevented completely the outgrowth of the normal cells from the explanted fragments; smaller concentrations of the same hydrocarbon reduced the mitotic activity several times as compared with that of control cultures. DMBA did not cause any characteristic changes in the distribution of relative frequencies of various mitotic phases in the cultures.

Other results were obtained in the experiments with the explants of induced sarcomas. In most of these experiments only the highest concentration of DMBA (0·25 mgm./ml.) caused partial or complete inhibition of mitotic activity; in two experiments even this concentration did not reduce the mitotic activity. Smaller concentrations of DMBA did not cause any statistically significant inhibition of the mitotic activity in the cultures of most sarcomas. In some groups where DMBA was added average mitotic indices were even somewhat higher than those in corresponding control groups.

The changes of the average explant areas caused by DMBA in different groups were in general similar to the changes of the average mitotic activity described here.

Thus, in the *in vitro* experiments normal fibroblasts of rat embryos and of adult rats proved to be much more sensitive to the growth-inhibiting action of DMBA than the cells of rat sarcomas. High resistance of the primary induced sarcomas to the growth-inhibiting action of carcinogenic hydrocarbons was observed by Haddow[6] in the experiments *in vivo*. All these facts are in good agreement with the suggestion that formation of a new cell type resistant to the toxic action of carcinogen occurs in the course of chemical carcinogenesis[1]. It is interesting that increased resistance to DMBA was observed not only in the experiments with the cultures of sarcomas induced by the same hydrocarbon but also in the experiments with rat sarcomas induced by the essentially different carcinogenic agent, that is, by 'Cellophane' film. This observation supports the suggestion concerning the similarity of processes which are induced in the subcutaneous tissue of rats by carcinogenic hydrocarbons and by 'Cellophane' films, and which lead to the development of sarcoma[7]. Further work is necessary to elucidate the mechanism of the increased resistance of sarcomatous cells to DMBA and to discover the role of the phenomenon in tumour development.

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DELETIONS OF ALLOTYPIC γ-GLOBULINS IN ANTIBODIES

By PROF. P. G. H. GELL and DR. A. KELUS

Department of Experimental Pathology, University of Birmingham

MANY attempts have been made to demonstrate structural or chemical differences between antibodies and 'normal' γ-globulins. Recently, Edelman *et al.*[1] have shown that anti-hapten antibodies in guinea pigs—antibodies, that is, directed against a single definable determinant group, in contrast with the manifold determinant groups reacting with antiprotein antibodies—give an individual and characteristic pattern on starch-urea electrophoresis. They were unable to demonstrate this phenomenon with rabbit antibodies. It seemed nevertheless possible that, if indeed antigens selectively stimulate γ-globulin antibodies of a particular structure, some rabbit antibodies might show signs of such selection. A useful 'marker' for such differences between γ-globulin molecules in a single serum is the 'allotype' (a genetically determined group on the molecule demonstrable by means of antisera raised in animals of the same species lacking that allotype[2–4]) since there is reason to suppose that individual molecules in the serum of an animal of heterogeneous allotype (for example, *ADEF* in the notation of Dubiski and Kelus[5]) vary in the particular allotypic marker which they carry. It seemed possible that as a result of the selectivity of the antigenic stimulus, antibody molecules might lack some of the allotypes present in the total γ-globulin from the same animal.

We have prepared antigen–antibody precipitates from the sera of individual immunized rabbits, and examined the redissolved precipitates for the presence of all the allotypes known to occur in the original serum. Twelve antisera from single rabbits were available. Of these, six (Nos. 1–6) were directed against haptenized ovalbumin: they contained antibodies reacting with normal ovalbumin, and also antibodies reacting with conjugates of the appropriate hapten on a non-cross-reacting protein (guinea pig albumin): the specific precipitates from both systems were examined. One antiserum (No. 7) was raised against bovine seralbumin: the antibodies cross-reacting with sheep seralbumin were first separated, and then the more bovine seralbumin-specific antibodies thrown down by reacting the supernatant with bovine serum albumin. One antiserum (No. 8) against *Streptococcus pneumoniae* Type III carbohydrate was also examined. All the precipitates were made at the equivalence point or at slight antibody excess, and redissolved in excess of antigen, or of free hapten, where that was applicable. Solution of precipitates was complete in the latter and almost complete in the former case. Four antisera against sheep cells (Nos. 9–12) were tested by coating sheep-cells with a sub-agglutinating dose of the antiserum, washing, and agglutinating the coated cells with each of the allotypic antisera, on the principle of the antiglobulin test.

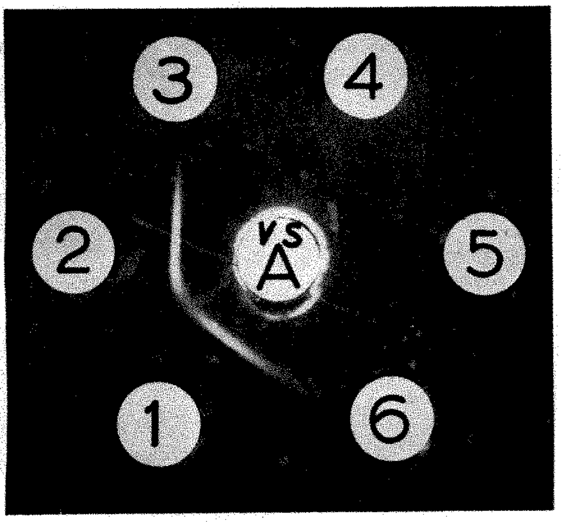

Fig. 1. Reactions with anti-*A* antiserum: 1, rabbit No. 1, whole serum; 2, rabbit No. 1, ovalbumin precipitate redissolved in ovalbumin; 3, ovalbumin; 4, conjugate, *p*-COOH-phenyl NCS guinea pig albumin; 5, free hapten, sodium *p*-amino-benzoate; 6, rabbit No. 1, conjugate precipitate redissolved in free hapten (5)

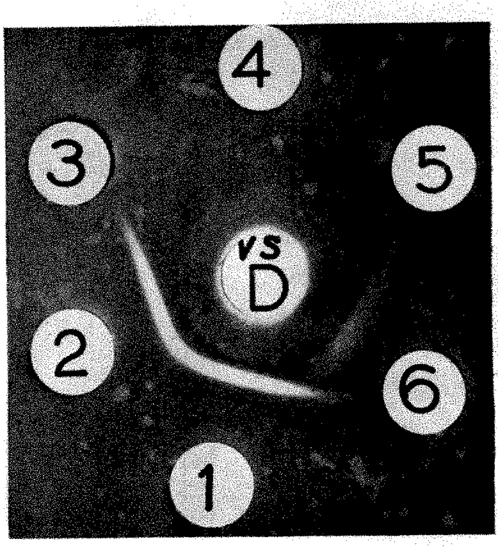

Fig. 2. Reactions with anti-*D* antiserum. For description see Fig. 1

The anti-allotype antisera available were against *A*, *B*, *C*, *D*, *E*, *F* (Dubiski and Kelus[5], and personal communication). Ten of the rabbits were found to be of allotype *DF*, the commonest among English laboratory stock. In all cases, both these allotypes were readily demonstrable in the precipitates (Nos. 2–7) or attached to the cells (9–12). One rabbit (No. 8: immunized to *Streptococcus pneumoniae*, Type III) was allotype *BDF*, and similarly all three allotypes were demonstrable in the antibody redissolved in excess of polysaccharide Type III.

One rabbit (No. 1) was apparently heterozygous at the *A* locus and at the *D* locus, being *ADEF* (*A* and *F*, *D* and *E* appear to be determined by allelic genes). The precipitate with ovalbumin from the serum of this animal, redissolved in ovalbumin,

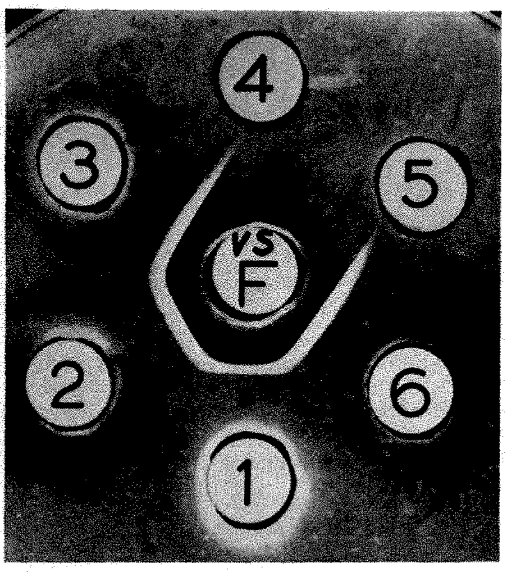

Fig. 3. Reactions with anti-*F* antiserum. 1, Rabbit No. 1, conjugate precipitate redissolved in free hapten (5); 2, rabbit No. 1, supernatant serum after precipitation of 1; 3, rabbit No. 1, ovalbumin precipitate redissolved in ovalbumin; 4, ovalbumin; 5, free hapten, sodium *p*-amino-benzoate; 6, rabbit No. 1, supernatant as in (2) plus free hapten (5)

showed the presence of all four allotypes; but the precipitate with the conjugate (in this case *p*-COOH-phenyl NCS guinea pig albumin) redissolved in hapten (sodium *p*-amino-benzoate) showed elimination of the *A* allotype using six different anti-*A* sera, and probably of the *D* allotype also—the faint line in Fig. 2 with anti-*D* serum is probably due to another allotype system (see Figs. 1 and 2); it was not evident against two other anti-*D* sera. Fig. 3 shows the reaction with anti-*F* serum and demonstrates also that adequate γ-globulin was present in the redissolved precipitate (6); the reaction with anti-*E* serum was essentially similar.

This particular anti-hapten antiserum (No. 1) was a weak one, but neither this nor the exact nature of the hapten is likely to be involved in the anomalous result, since three of the *DF* animals had antibodies against this same hapten, one equally weak in antibody. Clearly a qualitative test of this kind is inadequate to show a complete elimination of one allotypic γ-globulin from antibody, and more extensive quantitative studies will be needed to confirm and generalize any theoretical conclusions. Nevertheless, in the case of antiserum No. 1, the only one with the possible exception of No. 8 where there was a fair range of allotypes to choose from, at both the *A* and the *D* locus, there seems to be evidence for preferential selection of one kind of γ-globulin synthesizing site, so long as a truly 'mono-specific' (anti-hapten) antibody is examined.

If this is a general phenomenon, it confirms that allotypes determined by allelic genes occur on different molecules: its bearing on clonal selection and other theories of antibody production remains to be determined.

We thank the Medical Research Council for financial support for this work.

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INHIBITION OF CELLULAR ADHESION AND PSEUDOPODIA FORMATION BY A DIALYSABLE FACTOR FROM TUMOUR FLUIDS

By Dr. BO HOLMBERG

Cancer Research Division of Radiumhemmet, Karolinska Institute, Stockholm, 60

It has been found that interstitial tumour fluids, in contrast to normal interstitial fluid, contain a dialysable factor deleterious to cultured strain *L* cells. The general observations on this strain as well as on the behaviour of other cell types tested suggest that this factor primarily reacts with the cell surfaces; the induced changes seem irreversible and lead, at least in the case of *L* cells, ultimately to death of the cell. This observation is of interest since it would explain a number of cytolysing effects previously observed in the stromal compartment of tumours[1] and in mixed *in vitro* cultures[2].

The original observations were made with interstitial fluid from Walker rat tumours and solid Ehrlich–Landschütz hyperdiploid (ELD) transplants in *C3H* mice. When strain *L* cells were incubated in such fluids they became cytolysed after 6–12 hr., whereas ELD ascites tumour cell suspensions were unaffected. No hæmolysis of normal mouse blood was noted under these conditions.

The early effects on strain *L* cells by the addition of small amounts of fresh interstitial tumour fluid to the incubation medium involve a nearly complete inhibition of pseudopodia formation and cell adhesion, usually visible shortly after incubation. This could be used as a convenient and rapid test method simply by calculating the frequency of strain *L* cells adhering to the glass by means of pseudopodia after a standardized time of incubation in Carrel flasks[3]. In control flasks containing only the cultivation medium 80–95 per cent of the cells adhere to the glass soon after the incubation (Fig. 1). The deleterious effects on cell growth and cell vitality were estimated in suspension cultures of strain *L* cells by cell counts and Lissamine green permeability tests[4]. A more sensitive test method may in future be worked out using the sedimentation reaction of cells.

Addition of newly collected non-injured strain *L* cells to an incubation medium containing interstitial fluid from solid ELD or mouse mammary carcinomas did not lead to normal cellular adhesion (Fig. 2). The majority of the cells remained rounded and freely floating. This effect could also be obtained by addition of blood plasma from ascites tumour-bearing

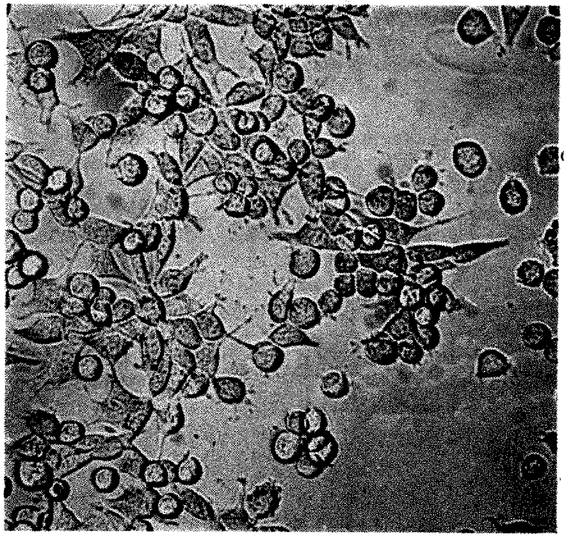

Fig. 1. Strain *L* cells incubated for 3 hr. in Eagle's medium + 20 per cent 0·05 *M* phosphate buffer (× *c*. 660). 80 per cent of cells adhering

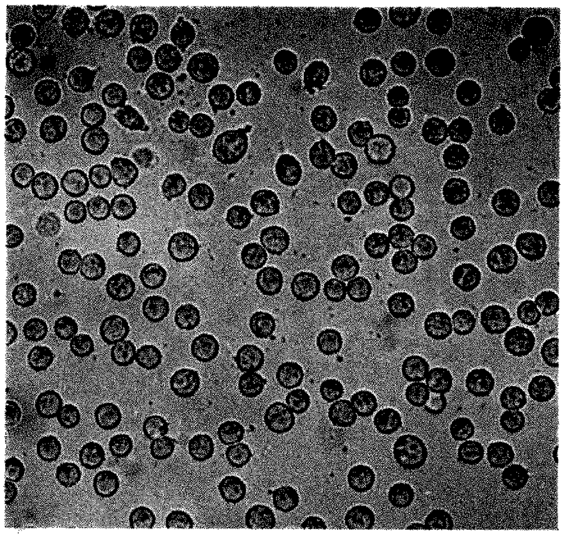

Fig. 2. Strain *L* cells incubated for 3 hr. in Eagle's medium + 20 per cent of the 0·05 *M* phosphate buffer fraction of dialysed ascites fluid (× *c*. 660). 8 per cent of cells adhering

mice, but not with normal mouse plasma. The cell-free ascites tumour fluid obtained at the end of ascites tumour growth was also active and has since been used for isolation of the active component.

Following dialysis overnight of the fresh or slightly autolysed ascites tumour fluid the membrane-active principle was, much to our surprise, found in the dialysate. Serum albumin marked with bromophenol blue was added to the ascites fluid in some cases to ensure that the bags were not defective. The dialysate could be concentrated by evaporation and also freeze-dried without loss of activity. A marked growth inhibition and cytolysing effect was noted when the crude dialysate was added to suspension cultures of strain *L* cells growing in Eagle's medium + 15 per cent horse serum (Table 1).

Table 1. EFFECT OF A CRUDE DIALYSATE* ON STRAIN *L* CELL GROWTH AND VITALITY DURING SUSPENSION CULTURE CONDITIONS IN EAGLE'S MEDIUM + 15 PER CENT HORSE SERUM

Cultivation (hr.)	Controls: Cell conc. 10^6 cells/c.c.	Controls: Cells permeable to LG† (per cent)	Factor added: Cell conc. 10^6 cells/c.c.	Factor added: Cells permeable to LG† (per cent)
0	0·19	3	0·19	3
4·5	0·19	3	0·18	8
21	0·27	3	0·20	9
29	0·30	3	0·16	10
48	0·35	4	0·12	21
72	0·37	4	0·05	70

* Total amount of factor present in the culture medium would correspond to a 4 per cent concentration of ascites fluid.
† See ref. 4.

The active factor was then fractionated from the crude concentrate by means of gel filtration on a small 'Sephadex' column, the active material being eluted with distilled water. The active rapidly moving biuret-positive[5] material was further fractionated on a DEAE-cellulose column and eluted with *p*H 7 phosphate buffers at molarities of 0·046–0·100. Repeated fractionations of the biuret-positive material finally gave a sub-fraction eluted by 0·050 *M* phosphate buffer, which seemed to contain the material active against strain *L* cells (Fig. 3).

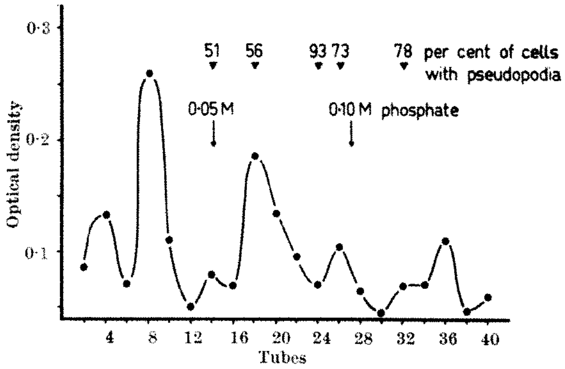

Fig. 3. Chromatographic separation of dialysate obtained from ELD ascites fluid on DEAE-cellulose with phosphate buffer at *p*H 7·0. Optical density refers to the absorption at 750 mμ after addition of the Folin–Ciocalteau's reagent

The ascites fluid activity was apparently associated with a dialysable polypeptide fraction. Its molecular weight was estimated to be 3,000–10,000. Qualitative tests for nucleotides, polysaccharides and lipid material were negative. Cholesterol was absent. Incubation of *L* cells in petroleum-ether extracts of crude dialysates indicated that this lipid material was not active. Following heat treatment of 65° C. for 30 min. the purified fraction still retained an appreciable activity.

The fraction eluted by 0·05 *M* phosphate caused a marked inhibition of pseudopodia formation and of strain *L* cell adhesion both to glass and plastic. This fraction alone, however, did not lead to irreversible cell injury or cell death during the first 2 hr. of incubation. Prolonged treatment of cells growing in suspension cultures is, on the other hand, harmful even at low concentrations.

The isolated fraction further caused withdrawal of pseudopodia in primary glass cultures of ELD ascites tumour cells and Chang liver neoplastic cells established on the glass surface, but without any signs of irreversible cell injury.

2 hr. incubation of strain *L* cells or ELD ascites tumour cells in Krebs–Ringer-phosphate in presence of the active component did not alter the oxygen consumption or the per-cell enzyme activities of lactic dehydrogenase[6], glutathione reductase[7] or cathepsin at *p*H 4·5 (ref. 8).

The sedimentation-rate of normal mouse and human blood cells became slightly retarded by addition of the 0 05 M phosphate fraction, while the sedimentation-rate of blood from ascites tumour-bearing mice or advanced cancer patients was uninfluenced by added factor (presumably due to its presence prior to testing) The sedimentation-rate of L cells, made visible by means of crystal violet staining, was significantly retarded at room temperature by addition of the factor, but the sedimentation-rate of stained ascites tumour cells was uninfluenced

Single intraperitoneal injection into normal mice of the crude dialysate led to a six- to eight-fold rise in the plasma lactic dehydrogenase activity in one week This level of activity was retained for at least 1-3 months The plasma activity of mice injected with factor was not transmittable to untreated mice stored in the same cages The relations if any, to the virus-like agent studied by Riley *et al*[9] and to the polypeptide material purified from the toxohormone[10] remain to be investigated

In some experiments an inhibition of pseudopodia formation could be observed already a few minutes after incubation, suggesting that the active component had reacted with the L cell surface immediately following exposure The subsequent growth inhibition and cell death observed later on cannot be explained at present

Two conditions seem of particular physiological interest One is that the small-size membrane-active factor exists in tumour fluids in a free state allowing diffusion and transportation via the blood stream It may thus have a bearing on the systemic tumour-host relations as yet poorly understood Secondly, the lack of detectable polysaccharide and lipid material seems to imply lack of antigenicity Further investigations regarding the nature and sources of this factor are in progress

This investigation was supported by grants from the Swedish Cancer Society, the Jubilee Fund and the Jane Coffin Childs Memorial Fund

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AMMONIA TOXICITY AND *p*H

By DR. KENNETH S. WARREN

National Institutes of Health, National Institute of Allergy and Infectious Diseases,
Laboratory of Parasitic Diseases, Bethesda

THE deleterious effects of ammonia have been a matter of great interest to investigators in many fields of biological and medical research Its passage into cells and hence its toxicity has been studied in *in vitro* preparations from rhododendron petals to red blood cells, in intact plants from onions to peach trees, in parasites, in fish and finally in mammals from mice to men In many circumstances, however, ammonia appears to be a remarkably non-toxic substance Ammonium compounds not only provide the bulk of the world's fertilizer supply, but are useful therapeutic agents for a variety of human ills Furthermore, when the LD_{50} of ammonium chloride is compared, on an m equiv /kgm basis, to that of many other common drugs the ammonium salt appears to be relatively non-toxic When administered or absorbed at a slow rate, vast quantities of ammonia may be tolerated by most living organisms because of their highly efficient detoxifying mechanisms in which urea, glutamine or asparagine is formed

This relatively benign appearance of ammonia may undergo a radical change, however, simply by an alteration in the hydrogen ion concentration of the medium Ammonia at low *p*H is usually toxic only in overwhelming quantities, whereas at high *p*H much smaller amounts may be lethal This variability in the toxicity of ammonia has been studied by many groups of investigators with great success However, the correlation between toxicity and the dynamic equilibrium of ammonia between intra- and extra-cellular fluid governed by the hydrogen ion concentration has only recently been established in mammalian studies

An attempt will be made here to present some of the physico-chemical factors governing ammonia toxicity, and to discuss the independent work done on this subject in many different fields The common bond among these investigators lies in either a basic interest in the mode of passage of weak acids and bases into cells or on observations of ammonia toxicity in relation to such diverse phenomena as the use of nitrogenous fertilizers, the bacterial contamination of cultures of parasitic nematodes, the effect of polluted waters on fish, the use of urea as a nitrogen source for ruminants, the development of meat intoxication in Eck-fistula dogs, and the occurrence of coma in patients with liver disease

In most biological fluids ammonia exists in two forms, the relative proportions of which are determined primarily by the *p*H of the solution Since toxicity depends on the ammonia which enters the organism, and thence the cell, it is of particular importance that cell membranes are relatively impermeable to one form (ionized ammonia), whereas the other (un-ionized ammonia) passes tissue barriers with ease[1]

The proportion of the total ammonia concentration which is ionized at any given *p*H is governed by the dissociation constant of the molecule The *p*K itself may vary with temperature and type of solution In blood plasma the *p*K*a* of ammonia was found to be 9 02 with a range of from 9 1 at 32° C to 8 91 at 41° C. (ref 2) With the appropriate *p*K value and a knowledge of the total ammonia concentration and solution *p*H, the relative proportions of ionized and un-ionized ammonia can be calculated simply by applying the law of mass action (At *p*K*a* 9 0 the respective percentages of un-ionized ammonia at *p*H 6, 7, 8 and 9 are approximately 0.1, 1, 10 and 50.) These calculations are necessary because prevailing methods of ammonia measurement, which are mainly by microdiffusion, provide only the total concentration. Recently, the concentration of un-ionized ammonia in the blood of dogs was determined by measuring the excretion of ammonia gas by the lungs[3]. The experimental findings agreed with those calculated from a knowledge of the total blood ammonia concentration and the blood *p*H.

Since ammonium ions do not cross cellular barriers at an appreciable rate, and un-ionized ammonia does so freely, an equilibrium occurs which is dependent primarily on the *p*H on both sides of the barrier[1,4]. Under normal conditions, a *p*H gradient exists between the intra- and extra-cellular fluids. When this gradient is changed, by an alteration of *p*H on only one side of the membrane or on both sides in opposite directions, a redistribution of ammonia occurs. However, *p*H changes can occur on both sides in the same direction. Then the redistribution of ammonia is either less pronounced or non-existent, depending on the initial *p*H gradient and difference between intra- and extra-cellular buffering capacity. The following equation expresses these relationships:

$$\frac{\text{Concentration intracellular}}{\text{Concentration extracellular}} = \frac{1 + 10^{(pKa - p\text{H}_{\text{intra}})}}{1 + 10^{(pKa - p\text{H}_{\text{extra}})}}$$

Changes in *p*H on both sides of cellular barriers as thus described can be easily induced. Since hydrogen ion penetrates most biological membranes at extremely slow rates, the *p*H on only one side of a membrane can be altered by the addition of fixed acid or base. If carbon dioxide is either added or removed, however, it crosses membranes quickly and easily, and the *p*H on both sides is changed almost simultaneously. These effects have been demonstrated experimentally by carbon dioxide equilibria[5], the use of natural and supravital indicator dyes[6], the distribution of weak acids such as 5,5-dimethyl-2,4-oxazolidine-dione[7], and recently by direct intracellular electrometric measurements[8].

The investigations reported here have been divided into the following sections: *in vitro*, plants, parasites, fish, ruminants, biomedical research and humans.

In vitro. In addition to his classical experiments on the hæmolytic effects of ammonium salts[9], Jacobs studied ammonia passage into cells in many different *in vitro* systems. By using red hybrid rhododendron blossoms, which contain a natural indicator dye[6], he could observe changes in intracellular *p*H with solutions of ammonia. No change in colour was observed with acid ammonium sulphate, but on the addition of a trace of ammonium hydroxide, raising the extracellular *p*H to 6.2, there was a prompt alkaline colour change. Starfish eggs stained with neutral red were the object of another series of investigations[6]. They were placed in three solutions of the same molarity and of the identical hydrogen ion concentration—*p*H 7.2. There was no change in colour in the eggs in the first solution, which was mainly sodium chloride; those in the second, saturated with carbon dioxide, turned bright pink, indicating a fall in intracellular *p*H, while those in the third, which contained ammonia, turned yellow, showing a rise in *p*H.

Plants. "Ammoniak als Alpha und Omega des Stickstoffumsatzes in den Pflanzen" was the title of one of the early papers by Prianischnikov[10]. To-day, this appears to be particularly true in that ammonia is not only essential for the growth and nutrition of plants but may also be responsible for manifestations ranging from inhibition of growth to death. Ammonium hydroxide is the simplest and cheapest source of nitrogen for growing plants, now that the bulk of the world's fertilizer is synthesized from atmospheric nitrogen[11]. Unfortunately, it has been found that this form of ammonia may be highly toxic, and is probably responsible for a host of plant diseases, including exanthema of lemons and lettuce root rot. The latter disease has been practically eliminated by lowering the *p*H of ammonium hydroxide with nitric acid[12].

Since large amounts of free ammonia in solutions at high *p*H may enter plants so rapidly that their protective mechanisms are overwhelmed, it becomes necessary, in order to attain maximal growth with minimal toxicity, to adjust not only the total ammonia supply but also the *p*H of the solution. Davidson and Shive[13], in their investigations of peach trees grown in sand culture, fertilized with ammonium sulphate, found an average rate of nitrogen absorption, linear growth and increase in wet and dry weight over an entire growing season to be more than double at *p*H 6 than at *p*H 4. Earlier experiments performed at *p*H's 4, 6 and 8 revealed increasing ammonia absorption with each increment in *p*H, although maximal growth was achieved only at *p*H 6.

Parasites. Von Brand and Simpson[14] reported that ammonia toxicity may explain the poor survival of parasitic worms in media contaminated with *B. subtilis* or *E. coli*. The authors found an increase in the concentration of ammonia with time in filtrates of bacterial cultures. When the parasites (*Eustrongylides ignotus*) were placed in the various filtrates, their survival-time was reciprocally related to the ammonia concentration. When sodium hydroxide was added to each of the media, the parasites perished much more rapidly. The authors noted that the toxicity was due to free ammonia, and found that acidified solutions were quite harmless.

Fish. Increased concentrations of ammonia found in polluted waters have been observed to be lethal to fish. The *p*H of the water has been found to be a factor of considerable importance in the development of this form of intoxication. One group of investigators[15] carried out their investigations at both *p*H 7 and 8. They found that ten times as much ammonium chloride had to be added to water at *p*H 7 to achieve a killing-rate comparable with that at *p*H 8. Theoretically, these results were fully expected since in aqueous solution at *p*H 7 only 1 per cent of the total ammonia is un-ionized, whereas, at *p*H 8, 10 per cent is in the freely permeable form.

Wuhrmann *et al.*[16] found that fish maintained in water at a high *p*H containing 10.1 mgm. of ammonium sulphate showed toxic manifestations in 70 min., whereas those in a concentration 16.5 times higher, but in which the aquarium was maintained at a lower *p*H, were unaffected for 170 min.

Ruminants. Ammonia intoxication has been reported in ruminants given urea as a supplementary nitrogen source, and in those fed on spring pasturage, rich in non-protein nitrogen. The ammonia was produced by bacteria in the rumen. Recently, the effect of *p*H on the absorption of ammonia from the rumen was studied. Lewis[17] placed ammonium chloride, ammonium acetate or urea in the rumen, causing, respectively, a fall in blood *p*H, no change, and a rise in blood *p*H. The earliest signs of toxicity were seen in the sheep fed urea, in spite of their having the lowest blood ammonia concentrations. Although similar blood ammonia concentrations were found in the animals given ammonium chloride and acetate toxic signs appeared somewhat earlier in the animals fed the acetate salt.

Hogan[18] examined the effect of *p*H on the passage of ammonia from the rumen of sheep. When an ammonia-containing buffer at *p*H 6.5 was placed in the rumen, transport increased with the concentration gradient. At *p*H 4.5, however, the concentration of ammonia in the rumen did not affect its rate of passage across the epithelium. Net loss of ammonia nitrogen from the rumen at *p*H 6.5 was more than three times that at *p*H 4.5. When carbon dioxide was bubbled through solutions in the rumen there was no change in the rate of transport of ammonia.

Biomedical research. In 1894, Pio Marfori[19] published an investigation in which he administered three ammonium salts to dogs and rabbits. He found that ammonium lactate and tartrate were tolerated better than the carbonate salt. He concluded that this was due to the greater dissociation of ammonium carbonate in blood and to the larger quantity found in the free state. Gyorgy and Kleinschmidt[20] in 1927 noted that meat-fed Eck-fistula dogs which were presumably suffering from ammonia intoxication, improved markedly following the oral administration of hydrochloric acid.

More recently Lawrence *et al.*[21] reported changes in the plasma total ammonia-levels of dogs subsequent to an alteration of blood *p*H. Warren[22] working with mice found a differential toxicity of ammonium salts which was related to their effects on blood *p*H. Further experiments in mice revealed increased passage of ammonia into the brain with alkaline salts[23]. Stabenau *et al.*[4] then emphasized the importance of the *p*H gradient on either side of a semipermeable membrane, in this case, the blood stream and the cerebrospinal fluid, brain and muscle. Nephrectomized dogs were infused with ammonia until the blood level reached a steady state. When the blood *p*H was altered by infusions of acid or base the cerebrospinal fluid *p*H remained relatively constant, bringing about a redistribution of ammonia. When carbon dioxide concentration was altered cerebrospinal fluid *p*H changed along with blood *p*H, and there was no significant redistribution of ammonia.

Human beings. Relman *et al.*[24] have recently reported two cases of severe acidosis caused by the ingestion of huge quantities of ammonium chloride. No signs or symptoms of ammonia toxicity were noted. Ammonium salts have been infused intravenously into human beings in the course of studies on the rate of passage of ammonia into the tissues[25]. Very slight, if any, adverse effects were noted. Ammonium chloride was also infused into several patients with alcoholic cirrhosis of the liver with an adverse effect on only one patient[26]. However when patients with severe metabolic alkalosis due to pyloric obstruction were treated with infusions of ammonium chloride definite signs of ammonia intoxication were observed[27,28].

Recent studies[29] in patients in hepatic coma with high blood ammonia concentrations have shown that infusions of hydrochloric acid or sodium bicarbonate can change the blood-cerebrospinal fluid distribution of ammonia. Experiments with carbon dioxide, undertaken on the assumption that the *p*H effect would be similar to that of fixed acid, resulted in a definite deterioration of the clinical state of the patients[30]. In the previous work in which fixed acid was infused, most of the patients were in a terminal state and no significant change in their condition was noted[29]. Large oral doses of lactic acid, however, have been found to be effective in the management of hepatic coma[31].

The phenomenon of the effect of *p*H on the toxicity of ammonia is one that appears to be universal throughout Nature from the onion to man. This problem has been examined by biologists and medical scientists for more than sixty years. One of the most striking aspects of the accumulated literature is its revelation of the almost complete lack of cross-communication among the botanists, ichthyologists, parasitologists, veterinarians, physiologists and physicians.

The following example of the potential value of such communication might be cited. A recently discovered diuretic, chlorothiazide, has precipitated episodes of hepatic coma, usually thought to be due to ammonia intoxication, by inducing potassium deficiency[32]. It is known that potassium depletion in the mammal results in an intracellular acidosis accompanied by an extracellular alkalosis[33], a situation that should maximally enhance passage of ammonia into cells. Support for this thesis might[34] be found in an earlier study of plant physiology in which ammonium-fed, potassium-deficient tomato plants wilted and died rapidly, whereas those given potassium grew luxuriantly. Further work revealed that the leaves of the deficient plants contained five times as much ammonia nitrogen as their normal counterparts.

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GROWTH ANALYSIS OF MICE SUBJECTED TO HIGH GRAVITY

By CHARLES C. WUNDER, DUANE WOMBOLT and E. N. OBERG

Departments of Physiology and Mathematics, State University of Iowa

CONTINUAL exposure of various organisms to high gravity as simulated by continual centrifugation can result in reduced growth[1-4]. With some forms, this response can be analysed by simple mathematical procedures[2]. However, owing to the complex nature of mammalian growth patterns, such procedures would not be applicable with mice. The primary purpose of this article is to introduce an approach which, although it does not overlook certain complexities, simplifies their analysis.

The response of the mice is the type expected when developing animals are exposed to a detrimental environmental agent. Three phases of this response are measured by the method described here: (1) a temporary decrement in growth-rate which can be so drastic as to cause negative growth (decrease in mass), (2) a gradual recovery of the growth process, (3) a permanent or long-term decrement which demonstrates itself after several weeks of exposure. Those interested in examples of unrefined data are referred to previous publications[4-6]. Such data are not well suited for direct evaluation of the influence of gravitational intensity and of age on these three phases.

Data analysed in this article are for male white mice of the *NLW* strain. These animals were centrifuged from the age of 3, 4, 5, 7 or 9 weeks for the remainder of their lives at fields of 1.5–10 times the Earth's gravity. Approximately six mice were used for each experimental condition. Techniques and equipment are essentially the same as that described elsewhere[4].

Data were simplified by use of a "physiological time-scale"[7]. One physiological week is arbitrarily defined as the average time required for 50 control mice to increase in size by 2 gm. This means that a straight line would be expected when control masses are plotted as a function of this time-scale. Any systematic deviations from a straight line would be attributed to experimental factors. Growth-rates in gm./physiological week for experimental mice were calculated with the aid of a digital computer from daily or weekly measurements of mass. These rates, as a function of physiological time p since onset of exposure, were fitted to an empirical equation by means of an analogue computer.

The empirical equation to which data were fitted is as follows:

$$\text{growth-rate} = -ae^{-rp} + Ye^{-lp}$$

where Y, a, r and l are positive parameters. Control growth-rate is represented by Y. For this equation all values use units of physiological time rather than actual time. However, the values shown in the figures have been converted to those using real time.

It is anticipated that, in future investigations, the integrated form of this equation would be more satisfactory for computer work. This would give measured mass, itself, rather than rates as a function of p.

The experimental constants a, r and l were evaluated separately for each experimental condition. The symbol a is initial decrement in growth-rate during the first phase of response when the exponential terms would be quite small. The acceleration constant r serves as an index of how fast recovery can occur during the second phase of response. The deceleration constant l serves as an index of the speed for onset of the long-term decay in growth-rate during the final phase.

The coefficients as a function of field intensity are shown in Figs. 1 and 2. Division of a by the mass

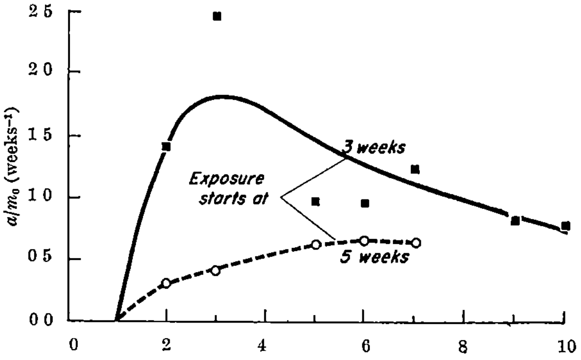

Fig. 1. Temporary decrement in growth constant (a/m_0) versus field intensity. The ratio a/m_0 is used so that the relative magnitude of the decrement can be compared for animals of different sizes. The symbol m_0 refers to body-mass at the onset of exposure

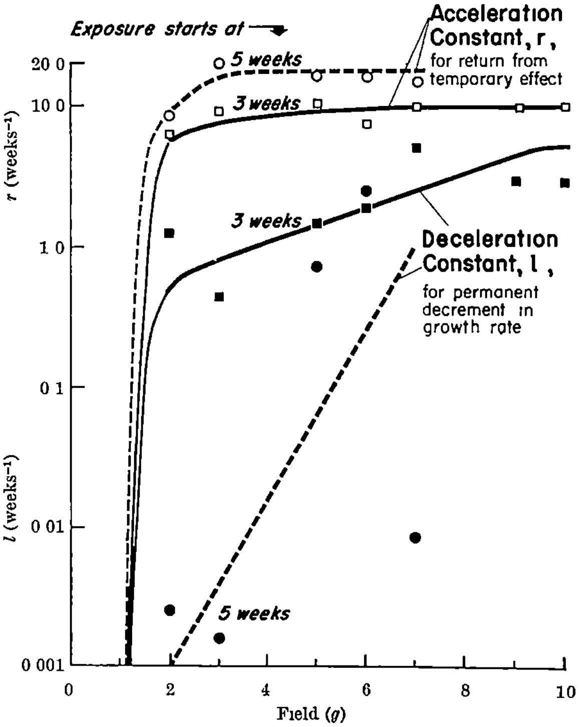

Fig. 2. Influence of centrifugal field intensity on the exponential coefficients

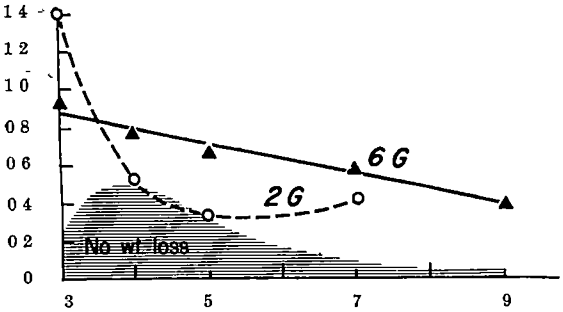

Fig 3 Temporary decrement in growth constant (a/m_0) as influenced by initial age of mouse The height of the shaded areas indicates the magnitude of the control growth constants Y/m_0 When this height just equals that of the experimental points, growth is arrested but no mass loss occurs The fractional decrement in growth constant can be obtained on division of the experimental values by the appropriate vertical extent of the shaded area

m_0 at the onset of exposure gives the initial decrement per unit of body-mass This permits a more meaningful comparison for mice of varying size The ratio a/m_0 can be thought of as the temporary decrement in the growth-constant (growth-rate/unit mass) Although results for newly weaned mice (three weeks old) are somewhat scattered, progressively greater decrement as the field increases is evident for older mice (Fig 1) Fields of six times the Earth's gravity have a more marked effect on the older mice This appears to be contrary to results obtained with fly larvæ[8] However, preliminary computations which involve corrections for changes in control rates Y with age have yielded results which are in better agreement with the data for fly larvæ[9]

On the basis of values for r at fields of 3–10g, recovery-rates are largely dependent on the extent of the decrement (a) rather than being directly dependent on the field intensity (Fig 2)

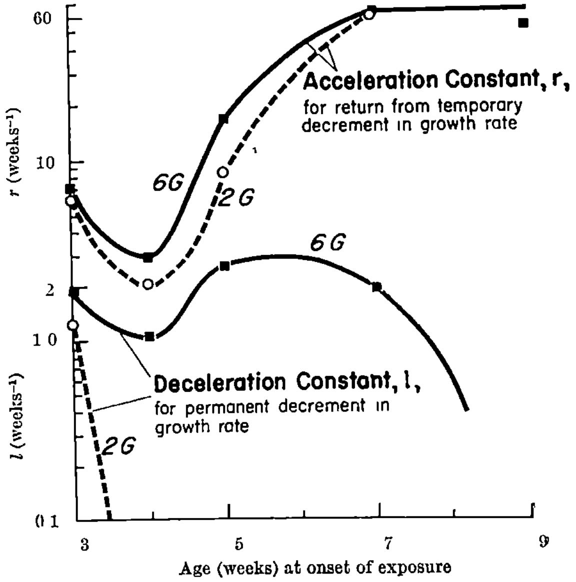

Fig 4 Little or no long-term effect as indicated by values for l could be detected at $2g$ for mice older than 3 weeks

This work was supported in part from U S Public Health Service grant No *RG* 5236 and in part from the 'Clara Duggar Memorial Grant for Cancer Research from the American Cancer Society' We thank Louise Wombolt and William E Reinhardt for their work and advice in the preparation of the computer programmes

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MECHANISM OF SENSITIZATION TO ULTRA-VIOLET LIGHT OF *T1* BACTERIOPHAGE BY THE INCORPORATION OF 5-BROMODEOXYURIDINE OR BY PRE-IRRADIATION OF THE HOST CELL

By PAUL HOWARD-FLANDERS, RICHARD P BOYCE and LEE THERIOT

Department of Radiology, Yale University School of Medicine, New Haven, Conn

THE replacement of thymidine by 5-bromodeoxyuridine (BUDR) in deoxyribonucleic acid (DNA) increases the sensitivity to ultra-violet light of mammalian cells, bacteria, viruses and transforming principle[1–9] Several explanations may be considered. (1) The greater sensitivity to the ultra-violet light wave-length 2537 Å is not due to any increase in the energy adsorbed, as the extinction coefficient of thymine exceeds that of bromouracil at this wave-length (2) The greater sensitivity may in part be due to a greater photochemical effect on the bromouracil than on the thymine in DNA Smith[10] found that BUDR-containing DNA is more sensitive than native DNA to ultra-violet light The rate of loss of bromouracil is 1 9 times greater than that of thymine from the DNA of *E coli B/r* when irradiated *in vivo* (3) Sensitization may also be due to the inhibition of certain enzymes as was shown in experiments with $\overline{T2u}$ and $\overline{T2u^+}$ bacteriophage by Stahl *et al*[8] The action of either the *u*-gene or photoreactivation will eliminate about half the ultra-violet injuries in normal, but not in BUDR-substituted DNA In addition, the pyrimidine analogue can increase the sensitivity of $\overline{T2u^+}$ by as much as two-fold Sauerbier[9] found that BUDR-containing *T1* phage is not photoreactivated, that it is much more sensitive to ultra-violet light, and that host cell reactivation is reduced. The term 'host cell reactivation' refers to the greater survival of ultra-violet-irradiated *T*1 when adsorbed to normal rather than to pre-irradiated *E. coli B* (refs. 11 and 12).

Further insight into the processes of reactivation has been gained as the result of the discovery by Hill[13] of a radiation-sensitive mutant B_s among the survivors of ultra-violet-irradiated *E. coli B*. Ellison, Feiner and Hill[14] found that *T*1 bacteriophage appeared to be more sensitive to ultra-violet light when plated on the sensitive strain B_s than when plated on *B*. The different levels of survival can be explained by the supposition that a factor capable of reactivating certain ultra-violet injuries in DNA is present in *E. coli B*, but is missing in B_s. It is of particular interest that ultra-violet irradiated *T*1 can be photoreactivated after infecting either strain, indicating that the dark reactivation factor in *B* acts on injuries that are not photoreactivable. Moreover, it does not act on ultra-violet irradiated *T*2 or *T*4, as the survival of these phages is the same when plated on either *B* or B_s. It is not clear from these results whether they reflect the action of one or of several reactivation factors.

In this article we report some additional results on the reactivation of ultra-violet injuries in *T*1 bacteriophage. The experimental approach is to examine the sensitization of *T*1 bacteriophage which results from the incorporation of BUDR, and how this is affected by plating on *E. coli B* and on the radio-sensitive strain B_s. Tests are also made on the extent to which the host cell reactivation phenomenon of Garen and Zinder[11] that is found in *E. coli B* also occurs in B_s.

The methods used for preparing the BUDR-substituted phage follow those described by Stahl *et al.*[8] *E. coli B* was grown to a concentration of 10^8 per ml. in medium II containing 20 μgm. per ml. of aminopterin in place of the 10 μgm. recommended. *T*1 bacteriophage were added to a concentration of 3×10^6 particles per ml. and the culture was vigorously aerated. A concentration of about 10^8 phage per ml. was obtained, which was then increased to 1.5×10^9 per ml. by centrifugation. The phage were further purified by centrifugation in a cæsium chloride density gradient so that a dense fraction could be selected. 1 ml. of the concentrated phage suspension was added to 3 ml. of cæsium chloride at 0.8 gm./ml. dissolved in nutrient broth. About 10^7 *T*1 particles grown in normal media and carrying the genetic markers *GeH* were also added to the suspension. The phage were centrifuged in a Spinco swinging bucket rotor *SW* 39 at 25,000 r.p.m. for 26 hr. at about 5° C. The tubes were then pierced at the bottom and the emerging drops were collected in groups of six. This yielded about 35 fractions, of which a dense fraction was selected for use in the experiments with ultra-violet irradiation.

The phage were suspended in buffer and 2-ml. aliquots were placed in a watch-glass with a magnetic stirrer for the irradiation. The phage were irradiated with a partially shielded 15-W germicidal lamp at a distance of 20 cm. The ultra-violet dose-rate measured with a General Electric germicidal ultra-violet intensity meter was 12 ergs/mm.²/sec.

The *T*1 phage stock containing the genetic markers *GeH* and *E. coli B*/1 were obtained from Dr. C. Bresch and the radiation-sensitive mutant *E. coli* B_s was obtained from Dr. R. Hill. The phage were plated on either *E. coli B* or B_s. *GeH* mutants were recognized by plating on *E. coli B* mixed with 0.5 per cent *B*/1.

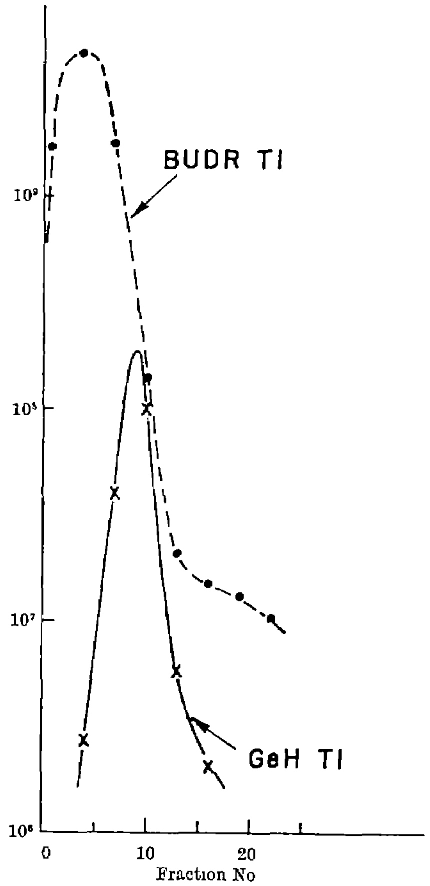

Fig. 1. The number of *T*1 bacteriophage per ml. is plotted against the fraction number from the cæsium chloride density gradient, the first fractions of the greatest density being on the left. Each fraction contains 6 drops from the gradient tube. Fraction number 2 was used in the experiments. The *T*1 phage with the genetic markers *GeH* was added as a density marker and contained no BUDR

The distribution in the numbers of wild-type and *GeH* bacteriophage among the successive fractions from the density gradient centrifugation are shown in Fig. 1. It is seen that at 0.60 gm./ml. cæsium chloride, the density was such that the BUDR labelled *T*1 band was near the bottom of the tube and came out in the early fractions. Samples of normal and the BUDR-containing *T*1 from the second fraction of the density gradient were suspended in adsorption medium at a concentration appropriate for the irradiation experiments. Samples were taken at intervals during the irradiation, diluted and plated on *E. coli B* and B_s. The relations between the number of infective centres surviving and the ultra-violet dose are seen in Fig. 2. It is seen that while BUDR causes a marked increase in the sensitivity of *T*1 when plated on *E. coli B*, it has relatively little effect when plated on B_s.

When ultra-violet-irradiated *T*1 is plated on *E. coli B*, and the logarithm of the number of infective centres surviving is plotted against the ultra-violet dose, as in Fig. 3, a two-component curve is obtained. Fig. 3 also shows the effect of irradiating the bacteria to which the phage were pre-adsorbed before plating. A dose of 240 ergs/mm.² to the host cell increases the steepness and length of the initial part of the curve. At the highest dose tested, 1,440 ergs/mm.², the sur-

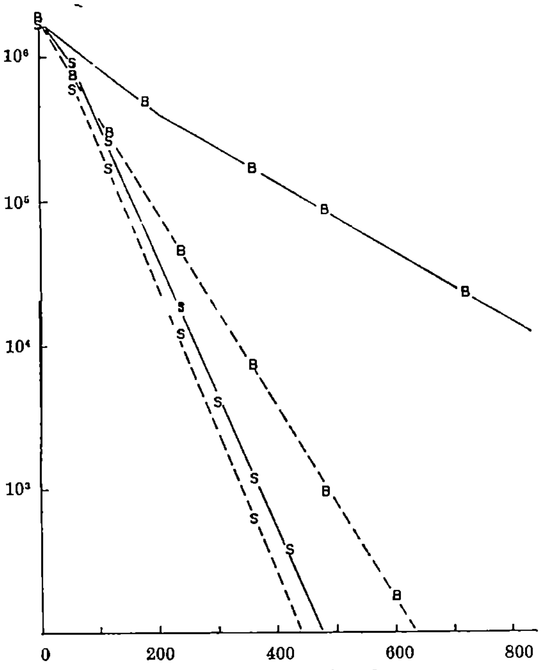

Fig 2 The number of surviving infective centres of *T*1 bacteriophage per ml is plotted as function of the ultra-violet dose delivered to normal and BUDR-containing *T*1 phage when plated on *E coli B* and B_s The dense fraction number 2 in Fig 1 was used in this experiment —, *T*1, – – –, BUDR *T*1, B, *E coli B*, S, *E coli* B_s

vival curve has become as steep as that obtained by plating on *E coli* B_s, and the second component of the curve has been eliminated In similar experiments in which the ultra-violet irradiated *T*1 is pre-adsorbed to *E coli* B_s before plating, a single straight line relationship is obtained and pre-irradiation of the host has very little effect

The results show that the sensitivity of *T*1 bacteriophage to ultra-violet can be increased to about the same extent in any one of three ways (1) by incorporating BUDR in the phage DNA in place of thymidine, (2) by using *E coli* B_s instead of *B* as host, (3) by pre-adsorbing the phage to ultra-violet-irradiated *E coli B* The sensitization produced in three ways is not additive when combined, which suggests that they operate through a common mechanism

The reactivation of ultra-violet injuries without light or *u*-gene action has been demonstrated in other experiments When investigating the loss of ultra-violet-induced mutations in *E coli B* and other bacteria, Witkin[15] and Doudney and Haas[16] found that many factors which increase the time-interval between irradiation and the next cell division also have the effect of decreasing the number of mutants obtained This suggests that the DNA is reactivated if sufficient time is available before the next DNA replication[17] Similarly, there is a reduction in the lethal effects of radiation on bacteria if sub-optimal growth conditions are used[18,19]

If the dark reactivation factor is an enzyme or enzyme system, then like the photoreactivation enzyme[20] it may have an affinity for ultra-violet photo-products in DNA This suggests that the change in the apparent sensitivity to ultra-violet of *T*1 that results from pre-irradiating the host may be due to the enzyme forming a complex with the ultra-violet irradiated DNA of the host cell This would leave none available to act on the ultra-violet-irradiated phage DNA that is afterwards injected It is seen in Fig 3 that an ultra-violet dose to the bacterium of 240 ergs/mm ² is sufficient to inhibit reactivation in the majority of cells, presumably by tying up the reactivation enzyme in this way If only the phage were irradiated, it would take a much larger dose to stop reactivation as there is relatively little DNA in the phage When phage-infected cells are irradiated, the phage DNA is in an unfavourable position to compete for the enzyme, of which much more than 90 per cent might be expected to react with ultra-violet products in the relatively abundant bacterial DNA (and possibly RNA) As a result, there should be little enzyme action on the phage DNA in complexes and therefore, little difference between the sensitivities of the 4 kinds of complexes formed when either normal or BUDR-containing *T*1 phage infect either *E coli B* or B_s This question will be the subject of a further publication

These experiments give little indication of the time taken by the enzyme to reactivate ultra-violet products in DNA Witkin[17] found that the decline in mutation frequency after irradiation occurred in a period of about 10 min The time available for reactivation in *T*1 infected cells is presumably shorter, so that relatively more enzyme may be required if it is to be effective The reactivation enzyme hypothesis affords a coherent explanation of a larger body of data than the proposal by Garen and Zinder[11] and Stent[21] of a mechanism that involves genetic homology and mating between phage and host The presence in the cell of a dark reactivation enzyme that does not act on BUDR-containing DNA must play a part in the sensitization of the bacteria by this thymidine analogue as reported by Greer[3] and Kaplan *et al* [5]

It is concluded that one of the main reasons why *T*1 phage containing BUDR is more sensitive to ultra-violet light is that dark reactivation, presumably

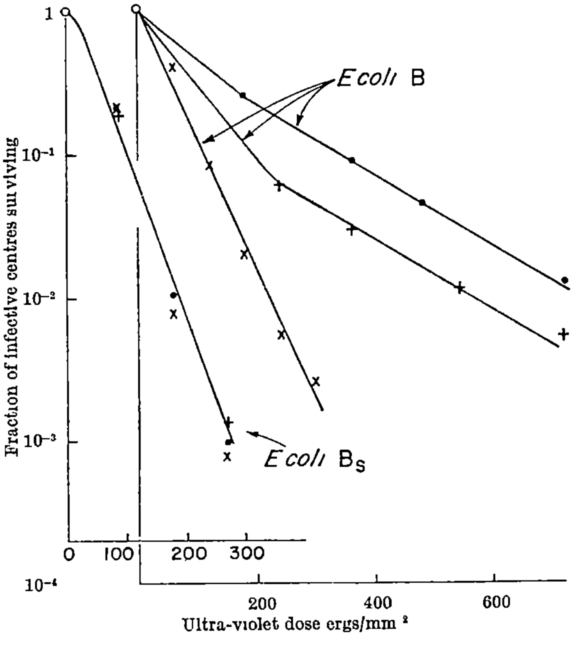

Fig 3 The fraction of infective centres of *T*1 bacteriophage surviving ultra-violet irradiation when pre-adsorbed to normal and irradiated bacteria of strains *E coli B* and B_s The doses given to the bacteria are 0 (●), 240 (+), and 1,440 (×) ergs per mm ²

the work of an enzyme or enzyme system, does not act effectively on BUDR-containing DNA The radiation-sensitive mutant *E coli* B_s (ref 13) lacks the dark-reactivation system

We thank Dr Ruth Hill for the radiation-sensitive mutant *E coli* B_s, and Dr Carsten Bresch for the *GeH T*1 and the *E coli B*/1 This work was supported by grants from the Jane Coffin Childs Memorial Fund and the U S Public Health Service

Note added in proof Other evidence that genetic recombination is not involved in host cell reactivation has just been published by Sauerbier, W , in *Virology*, 16, 452 (1962)

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FOLLICLE PRODUCTIVITY AND GROWTH OF WOOL

By D. A ROSS

New Zealand Wool Industries Research Institute (Inc), Dunedin, New Zealand

SINCE Fraser[1] proposed that wool follicles compete for fibre substrate, a considerable literature on this topic has developed which has recently been reviewed by Fraser and Short[2] Fibres from primary and secondary follicles, or large compared with small fibres, are considered to react differently to changes in nutrition

Over a period of 18 months, wool samples were clipped at monthly intervals from the mid-side position of ten free-grazing full-mouth Romney ewes[3]. Data from only one ewe, which illustrate the relationship between follicle productivity at different levels in the fibre array and the level of wool production in some of these ewes, will be presented in this article

The weight of clean oven-dry wool produced per unit area of skin per day for each month was measured on this ewe and expressed as a percentage of the maximum wool production The minimum level of wool production was 14 per cent of the maximum From each of the 18 monthly samples, 500 fibres were measured for fibre diameter and 150–200 for fibre length The fibre diameter and length arrays were each subdivided into seven groups to enable a study to be made of the effect of changes in the level of wool production at different levels in the array About 15 per cent of the fibres will be of primary follicle origin, and as these will largely be the coarsest and longest fibres they will be principally found in group 1, which consisted of the coarsest or longest 15 per cent of fibres, while group 7 contained the finest or shortest 15 per cent of the fibres Groups 2, 3, 4, 5 and 6 consisted of 15 per cent of the fibres in descending order of diameter or length, except that group 4 contained only the 10 per cent of fibres about the mean Making the reasonable assumption that a fibre occupies the same relative position in an array irrespective of the level of wool production, and that there is a perfect correlation between the diameter and the length of fibres, then three comparisons of follicle productivity in relation to the level of wool production can be made at each of the seven levels in the fibre array These are fibre diameter d, or cross-sectional area d^2, fibre length l, and fibre volume d^2l

Fig 1 shows the regression coefficients, for each group of fibres for these three measures of follicle productivity, for a change of 1 per cent in the level of wool production The values of the regression coefficients for d^2, l and d^2l are plotted against the mean cross-sectional area (d^2) of each fibre group Shown also in Fig 1 are the regression coefficients expressed as percentages of the corresponding cross-sectional areas

The absolute increase in fibre cross-sectional area associated with an increase in the level of wool production increases with increasing fibre cross-section, but expressed as a percentage of d^2 it decreases On the other hand, there was little variation in the fibre length response at the various levels in the array, only a very slight increase in length with increasing fibre diameter (or fibre length) being indicated Again

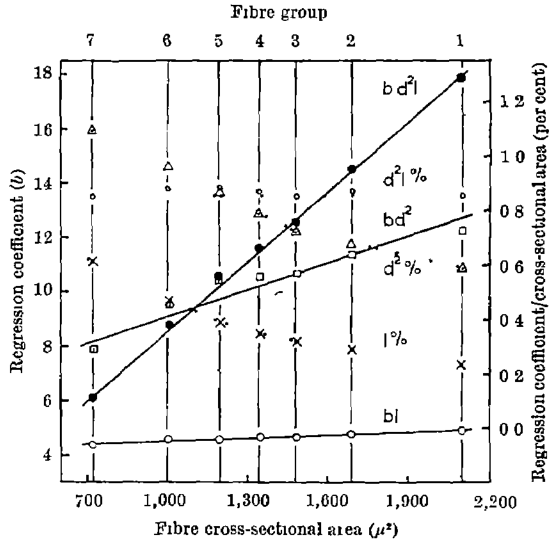

Fig 1 Regression coefficients (b) of d^2 (μ^2), l (μ) and d^2l ($\mu^3 \times 10^{-3}$) for a change in wool production of 1 per cent for each group of fibres, plotted against the mean cross-sectional area (d^2) Also shown are these regression coefficients as percentages of the corresponding d^2

the regression coefficients for the changes in fibre length decrease as a percentage of d^2 (or of l). The volume of fibre produced (with increasing wool production) increases linearly with increasing fibre cross-sectional area within the array, such that the increase *in volume* is a *constant* percentage of fibre cross-sectional area. Per unit area of cross-section, therefore, all fibres, irrespective of size or biological origin, showed the same response in volume to a change in the level of wool production. The wool follicles in this mature Romney ewe therefore have the same 'efficiencies' and do not compete among themselves for fibre substrate.

The following measures of follicle productivity, which have been considered by other workers to indicate follicle competition, arise as a direct result of the relationships which have been found between the level of wool production and d^2, l and d^2l. (1) The ratios of group 1d^2/group 7d^2, group 1l/group 7l or group 1d^2l/group 7d^2l will vary inversely with the level of wool production. Similarly, the ratio of the diameter or length of fibres from primary, compared with secondary, follicles will also vary inversely with wool production. (2) When the follicle productivity of a fibre or group of fibres is considered relative to the mean follicle productivity in the array (for either d^2, l or d^2l), then for fibres above the mean value the follicle productivity will be highest when wool production is lowest, while for fibres below the mean it will increase as the level of wool production rises. (3) The coefficient of variation of fibre diameter will be lowest when wool production is highest.

The interpretation of investigations of the relative follicle productivity of small versus large fibres, based on measurements of either d or l alone, can therefore be misleading. While differences in fibre productivity between follicles have been considered in terms of fibre cross-sectional area, some function of the follicle itself, such as papilla volume or surface area, may be the most appropriate basis for such comparisons.

The results do not indicate that the fibres of group 1, which will be largely of primary follicle origin, respond any differently in this ewe than the other groups which consist of fibres from secondary follicles. As body-weight and consequently skin surface area were approximately constant during this trial, the distance between wool fibres would be at a maximum when wool production was at a minimum, indicating that the spatial relationships of the follicle population of this mature ewe did not affect the relative follicle productivity.

Levels of production in pasture-fed ewes are low compared with the potential maximum level of production. At low levels, the finest fibres cease growing at a diameter of about 12μ in Romneys, while at high levels of wool production the larger fibres may become heavily medullated. As follicle productivity is essentially measured by the mass of fibre produced, in these large medullated fibres, total fibre volume may increase without any accompanying increase in fibre mass or follicle productivity. Five of the ten ewes in this trial contained a high proportion of medullated fibres in the summer months when wool production was high. In these ewes, the change in the volume of fibre produced per unit area of cross-section increased with increasing diameter of the fibres, when wool production increased.

Comprehensive investigations of fibre diameter, length, volume, mass, follicle type and fibre number are necessary before the factors controlling the production of wool, both in the developing and in the mature fleece, are clearly understood.

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SEPARATION OF THE OXYGEN EVOLVING SYSTEM OF PHOTOSYNTHESIS FROM THE PHOTOCHEMISTRY IN A MUTANT OF *SCENEDESMUS*

By Prof. NORMAN I. BISHOP
Florida State University, Tallahassee

THE mechanism of the biosynthesis of chlorophyll[1] and of carotenoids[2] has been elucidated successfully with the aid of mutations artificially induced in certain algae, but little use has been made of this technique to solve quite specific questions concerning the mechanism of photosynthesis. The reason may be that it is relatively easy to produce random mutations of photosynthetic organisms and to concentrate on those which show obvious changes in colour, but considerably more difficult to obtain a mutation the desired characteristics of which have been narrowly defined in advance.

In many strains of algae distributed among several species[3] it is possible to substitute a photoreduction with hydrogen for the normal type of photosynthesis, that is, under these 'adapted' conditions no oxygen is evolved in the light. With certain inhibitors it is possible to make the hydrogen-adapted condition semi-permanent[4]. The question arose whether this effect could be brought about in a more natural way.

The isolation and characteristics of a mutant of the green alga *Scenedesmus obliquus*, strain D_3, which is incapable of evolving oxygen in the light but which behaves quite normally otherwise, are discussed here. The mechanisms for respiration, growth and for a photochemical fixation of carbon dioxide have remained unaltered.

Method of mutant production and isolation. Mutations were induced by irradiating samples of a thin algal suspension with a Philips 25–250 X-ray machine for a period of time sufficient to deliver 60 kr. Afterwards aliquots of the algal suspension were spread uniformly on Petri dishes containing a basal nitrate medium[5] plus 1 per cent glucose–0·5 per cent yeast extract. After 12–14 days the surviving colonies of algae were exposed to light and carbon dioxide labelled with carbon-14 by a method previously described by Levine[6]. With this method colonies of cells were occasionally detected which had not incorporated any $^{14}CO_2$. Such colonies were trans-

Table 1 PIGMENT CONTENT OF WILD-TYPE *Scenedesmus* AND MUTANT 11

Algal strain	(*a*) mgm Chl $a+b$ 50 μl cells	(*b*) mgm β-carotene 50 μl cells	(*c*) Total carotenoids 50 μl cells	a/c	a/b	c/b
Wild-type—Heterotrophic	0.405	0.0115	0.076	5.32	35.2	6.60
Photoheterotrophic	0.630	0.0185	0.97	6.49	34.0	5.24
Mutant 11—Heterotrophic	0.370	0.0098	0.064	5.78	37.8	6.53
Photoheterotrophic	0.240	0.0038	0.032	7.50	63.2	8.42

ferred into separate culture tubes, and after sufficient growth had occurred larger-scale culture flasks were inoculated for either photoautotrophic or heterotrophic growth studies. Those cultures which did not grow photoautotrophically, or only poorly, but developed heterotrophically were further scrutinized.

In this way I have obtained a number of mutants which are unable to photosynthesize aerobically. But among these only one, mutant 11, has the specific characteristics which I sought. *Scenedesmus* D_3, mutant 11, shows no aerobic photosynthesis, no quinone-Hill reaction, but a normal capacity for photoreduction under hydrogen. It has a normal growth-rate heterotrophically in dark or light in contrast to other 'green' mutants which have become light sensitive and survive only in the dark[7]. Furthermore, its respiratory rate and also its ability to decompose hydrogen peroxide are identical to that of the normal 'wild' type *Scenedesmus* D_3.

Pigments of mutant 11. Analyses of the chlorophyll *a* and *b*, total carotenoid, and β-carotene content of dark grown cultures revealed a slight decrease in the amount of total carotenoid synthesized by the mutant. This slight decrease is enlarged several-fold when cultures are grown photoheterotrophically (Table 1). The fact that the mutant grows at all in the light heterotrophically speaks against this alteration in pigment content as being the causative factor in this particular genetic block of photosynthesis. The marked change in content of carotenoid during growth in the light must be due to this particular mutant's inability to dispose of the 'precursors' to molecular oxygen which would accumulate in the light. In the normal alga these substances are dismutated to oxygen but in the mutant accumulate and lead to deleterious side-effects.

No change could be seen in the fine structure of the chloroplast of mutant 11 when cells were examined with the electron microscope.

Capacity of mutant 11 *for photosynthesis, photoreduction and the Hill reaction*. Aliquots of a culture of mutant 11 were used for measuring photosynthesis, photoreduction, and the Hill reaction. A culture of normal cells grown under identical conditions served as control. The capacity for photosynthesis is reduced practically to zero. There is not even a compensation of respiration in the light (Fig. 1). Correspondingly, this mutant does not incorporate carbon dioxide to any extent under aerobic conditions. Chromatographic analysis of $^{14}CO_2$ fixation products clearly shows that light does not alter the pattern or the amount of such substances that become labelled during a control dark experiment. No evidence for the appearance of phosphorylated intermediates common to the photosynthetic cycle were detectable. Furthermore, radioactive tracer carbon is predominantly incorporated into compounds where labelling might occur through exchange and not necessarily by synthesis, that is, aspartic acid, glutamic acid, alanine and malic acid.

Mutant 11 is also incapable of reducing *p*-benzoquinone in the Hill reaction. For example, the $Q_{O_2}^{Chl}$ of a normal culture of *Scenedesmus* was 1,444 µl O_2/hr -mgm chlorophyll, while the highest observed value for the mutant was 22 (highest of five separate determinations). This represents a loss of more than 98 per cent of the capacity for this reaction. Furthermore, we do not know that the slight amount of gas produced was oxygen. The same dubious small gas production was also observed in photosynthesis tests (Fig. 1). The nearly complete loss of Hill reaction activity points to a block in the oxygen-evolving mechanism of photosynthesis. Further evidence for this is the excellent capacity of this mutant to perform photoreduction.

After several washings and a 24-hr period of starvation, aliquots of the normal dark-grown *Scenedesmus* and of mutant 11 were adapted in an atmosphere of hydrogen and 4 per cent carbon dioxide for a period of 16 hr. Then the adapted cultures were illuminated for periods of 15 min at varying light intensities and the amount of gas uptake recorded. Typical results for such an experiment are shown in Fig. 2. The mutant has a light-saturated rate of photoreduction of carbon dioxide which approximately equals that of the control. It is remarkable, however, that the rate of photoreduction at low light intensity is appreciably higher in the mutant than in the 'wild type'. Fig. 2 shows also the effect of 3(3,4-dichlorophenyl)-1-1-dimethyl urea (DCMU). We know that this poison strongly inhibits reactions of the photosynthetic system which depend on the evolution of free oxygen, but not those involved in carbon dioxide fixation[8,9]. In Fig. 2 it is clear that mutant 11 without DCMU behaves exactly like the control with DCMU.

The pattern of $^{14}CO_2$ fixation during photoreduction of the mutant and the 'wild' type *Scenedesmus* when examined chromatographically were found to be identical. During photoreduction the well-known phosphorylated intermediates of photosynthesis were

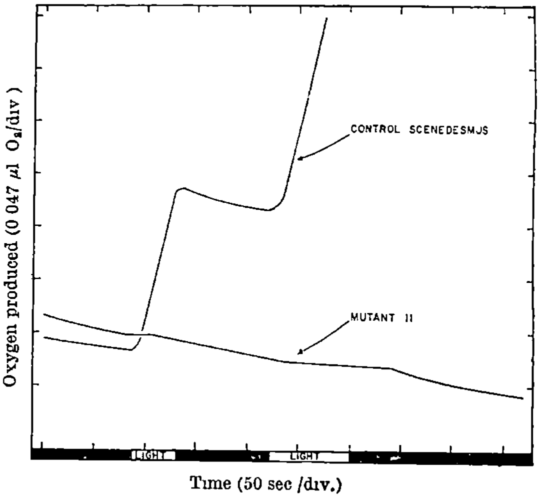

Fig. 1. Polarographic traces of oxygen consumption and production by dark-grown normal *Scenedesmus* and mutant 11. Gas phase = 4 per cent CO_2—96 per cent N_2, temperature = 20° C., cell volume = 15 µl., pH = 6.5, phosphate buffer = 0.05 M, final volume = 2 ml., light intensity = 4 klx.

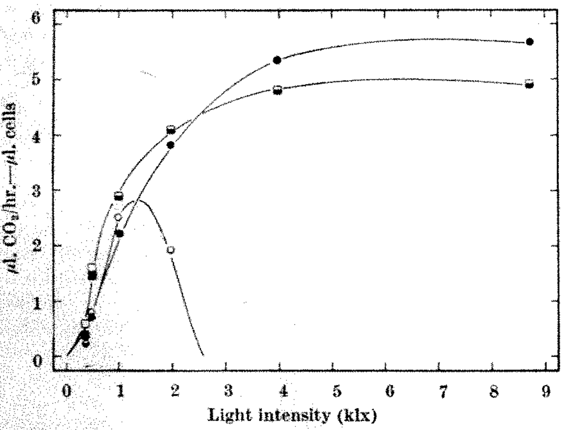

Fig. 2. Rates of photoreduction of dark-grown normal *Scenedesmus* and mutant 11 at various light intensities with and without DCMU (2×10^{-6} *M*). Temperature = 25° C.; phosphate buffer (*p*H = 6·5) = 0·05 *M*; cell volume = 30 μl./vessel. ○–○ = control; ●–● = control + DCMU; □–□ = mutant 11; ■–■ = mutant 11 + DCMU

clearly present, in strong contrast to the $^{14}CO_2$ assimilation pattern observed aerobically. The implications of the lack of photophosphorylation under one physiological condition and its presence in another are of some particular importance, in line of the proposed mechanism of cyclic and non-cyclic photophosphorylation[10]. The point in question is rather obvious: is photophosphorylation in intact photosynthesizing tissue dependent on the integrity of the oxygen-evolving mechanism? This point is at present being investigated with various mutants, including 11.

Analysis of the hydrogenase activity of the mutant in the dark, either with oxygen or sodium nitrite as oxidant, showed that the enzyme was some two- to three-fold more active than that of the 'wild' type. This offers an obvious explanation for the increased rate of photoreduction observed in the mutant. In spite of this higher hydrogenase activity mutant 11 is incapable of any photoproduction of hydrogen as is found normally with algal strains capable of adapting to hydrogen and in some purple bacteria. The implication of this finding is not clear and requires further experimentation.

Certain theories on the evolution of photosynthesis favour the idea that the last major step in the evolution of higher-plant photosynthesis was the addition of a mechanism whereby the oxidant (photoxidant) produced in the photolysis of water could be dismutated directly to molecular oxygen rather than through the energetically inefficient method of reduction via exogenous hydrogen donors. This evolutionary step not only removed the strict dependence of the organism on an organic environment but also opened the way for future Darwinian evolution by providing a means for increasing the oxygen content of the Earth's atmosphere. The addition of this mechanism to the photosynthetic process resulted in a general loss of the enzymes previously involved in the reduction of the photoxidant. The exceptions to this are the few strains of algae which still possess the potential for photoreduction, that is, an adaptable hydrogenase[11].

In mutant 11 we have in a sense reversed the last evolutionary step and again placed the organism into a physiological state where it is dependent on an external hydrogen donor in order to reduce carbon dioxide successfully.

It has been hypothesized that the adaptation process may involve certain subtle changes in an enzyme which in one circumstance functions in oxygen evolution but in the modified form, that is, after prolonged incubation in an anaerobic atmosphere, is temporarily non-functional in reactions leading to oxygen evolution[12]. From this point of view the isolation of a mutant such as No. 11 takes on additional interest. In this mutant the enzyme(s) now function in the more primitive fashion, that is, as hydrogen mediators in the reduction of the photoxidant and not in oxygen evolution. For this reason alone it is of interest to find that in mutant 11 the activity of the hydrogenase is increased several-fold.

In my present experiments I am attempting to determine more specifically the nature of the block to the oxygen-evolving system. So far there are several constituents of chloroplasts which have been implicated in the mechanism of oxygen evolution, that is, cytochromes (b_6 and f), manganese, plastoquinone, and perhaps β-carotene. I am at present investigating whether or not modifications have occurred either in content or requirement for any of these factors.

This investigation was supported by grants from the U.S. Atomic Energy Commission (*AT*–(40–1)–2687), National Science Foundation (*NSF–G*12852), and the Fels Fund.

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RECEPTORS IN THE SUCKERS OF *OCTOPUS*

By Dr. P. GRAZIADEI

Department of Anatomy, University College, London

An octopus can collect with its arms various sorts of information. It is able to discriminate by touch between objects with different surfaces. Moreover, when an octopus walks, the tips of the arms are often used to explore the environment. When two octopuses meet they may examine each other with the tips of their arms. Presumably, chemoreceptors as well as tactile receptors are present. In octopuses experimentally blinded the recognition of prey and the movements necessary to capture it and carry it to the mouth are based and co-ordinated by impulses collected by the suckers. Many experi-

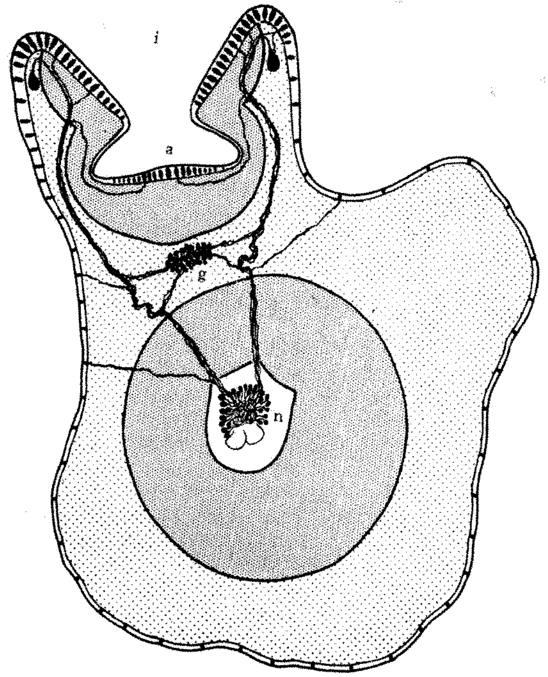

Fig. 1. Transverse section of the arm of *Octopus*. *a*, Acetabulum; *g*, ganglion of the sucker; *i*, infundibulum; *n*, nerve cord of the arm

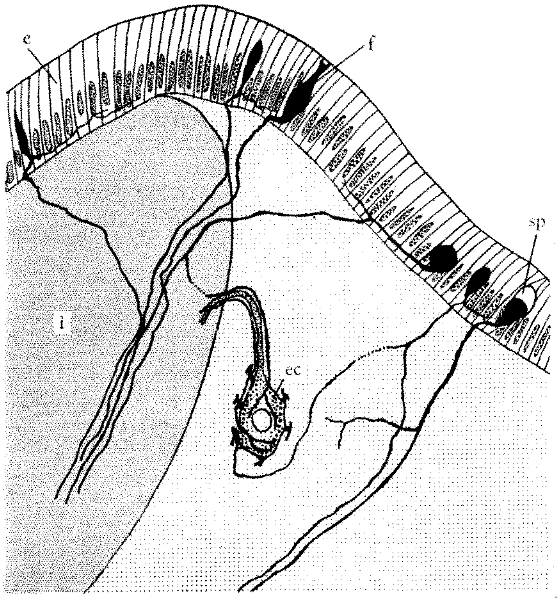

Fig. 2. Detail of the infundibulum of the sucker in *Octopus*. *e*, Epithelium of the infundibular cup and of the rim of the sucker, with numerous primary sense cells; *ec*, encapsulated nerve cell with synapsing fibres; *f*, fusiform-shaped cell reaching the free surface of epithelium where it apparently opens; *i*, infundibulum; *sp*, spheric intraepithelial cell carrying a second sphere of clear cytoplasm

ments have shown that an octopus can discriminate with its arms between objects differing only in small details, such as a smooth surface from one with various degrees of irregularity[1]. All these facts make it especially worth while to examine the receptor apparatus of the arms, and in particular the suckers, in an attempt to find a morphological basis for these findings.

The suckers of several arms of *Octopus vulgaris* (Lamarck) have been used. In each octopus specimens were taken of the suckers at the base and the extreme tip of the arms. The diameter of the suckers, measured at their infundibular openings, varied from 12 to 1 mm. The arms were fixed in 10 per cent neutral formol–sea water and stained modification of Cajal[2] which is particularly suitable for these invertebrates. Other pieces were impregnated by Bielschowsky–Gros.

It is known from previous investigations that the sucker of an octopus has the form of the sections of two cups one on top of the other, having different radii of curvature. The lower one is the acetabulum, the upper one the infundibulum (Fig. 1). A description of the anatomy of the suckers and of the epithelial surfaces has been given in Guérin[3] and by Rossi and Graziadei[4].

In the prismatic epithelium which covers the inside of the infundibulum there are numerous primary sensory cells (Fig. 1). These cells have various forms; in some of them the cell body is round and darkly staining and lies at the base of the epithelial layer, not reaching beyond its lower third (Fig. 2). Others, on top of a deeply staining sphere, carry a second sphere of clear cytoplasm, which does not stain with ordinary histological reagents or with silver (Figs. 2 and 3*b*). Other primary sensory cells have a fusiform shape and reach to the free surface of the epithelium, where their outer ends seem to open by a pore (Fig. 3*a*).

There are thus numerous sensory cells of various types in the epithelium which covers the exterior border of the sucker. The number is greatest around the rim of the suckers and decreases passing down the stalk of the infundibulum, and there are few in the skin of the surface of the arm itself. In that region, in fact, the intra-epithelial receptors consist only of a few cells sending their processes directly to the central nerve cord of the arm (Fig. 1).

The processes of the cells in the suckers often run for a considerable distance along the epithelium itself, finally leaving it with a sudden turn (Fig. 2). During this intra-epithelial course the fibre may be provided with one or two collaterals, which branch and form a fine plexus of fibrils. This plexus extends over the whole of the infundibulum and also receives fibres that originate outside the epithelium and reach the plexus after passing through the basal membrane. Probably these fibres, like the similar fibres seen in *Sepia*[5], arise from ganglion cells, but for the moment

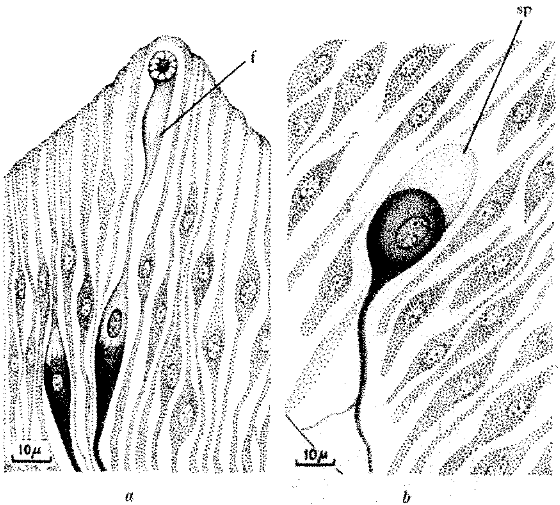

Fig. 3. Lettering as Fig. 2

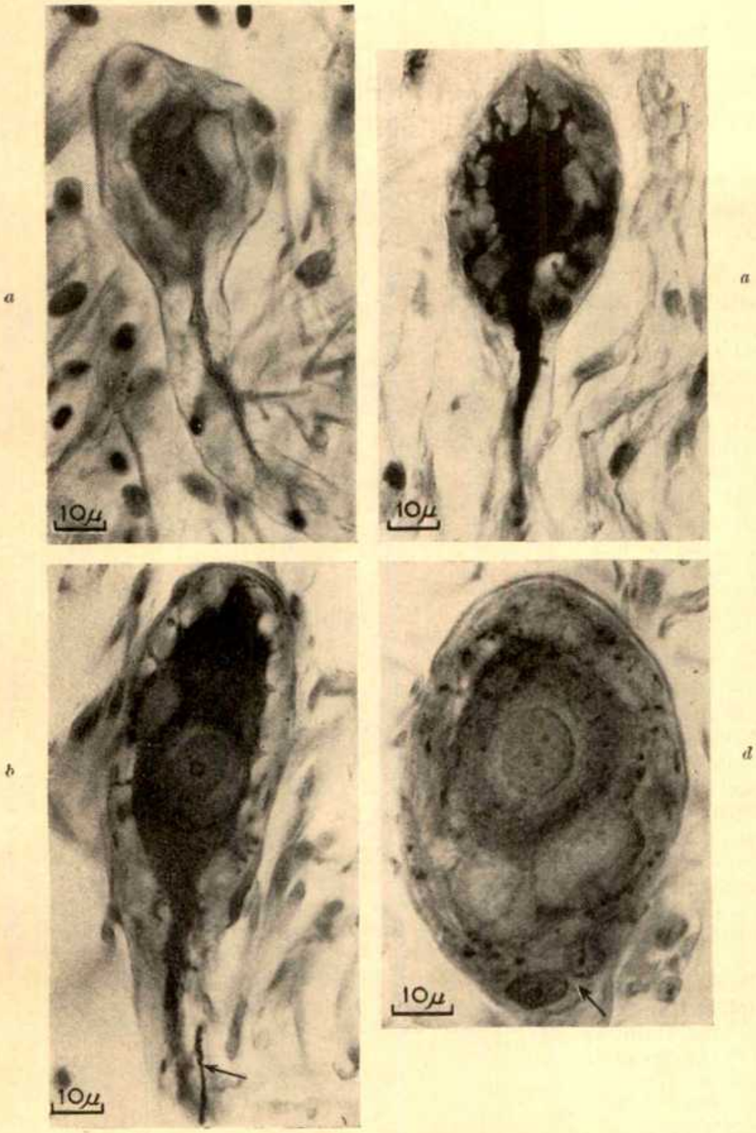

Fig. 4. Encapsulated nerve cell lying in the subepithelial connective of the rim of the sucker in *Octopus*. Bielschowsky–Gros. *a*, Nerve cell with connective tissue capsule surrounding the cell body and axon; *b*, nerve cell with irregular surface and synapsing fibre near the axon (arrow); *c*, nerve cell with stumpy dendritic processes, surrounded by capsule; *d*, nerve cell with vacuoles near the base of the axon (arrow shows nuclei of sheath)

it is not possible to say exactly where these cells are situated.

In addition, these regions contain another type of nervous element, situated more deeply below the epithelium of the sucker. These are ovoid encapsulated nerve cells lying in the sub-epithelial connective tissue at the sides of the muscular walls of the infundibulum (Figs. 2 and 4). These cells, never previously described, show many interesting features. They are numerous in the suckers at the tips of the arms but are less frequent in the large ones near the base. In the larger suckers of 15 mm. diameter, nerve cells reaching 40μ in diameter were found, but in the suckers of 2–3 mm. diameter the comparable cells only reach up to 10μ. The following description refers only to the larger cells. They have an ovoid or globular form and their axon enters the muscles and then leaves them again on the way to the central nerve cord of the arm (Fig. 1). This is not the shortest route, which would run outside the musculature of the sucker.

The nerve cell body and its axon are covered by a nucleated connective tissue capsule. The cytoplasm has numerous vacuoles in its outer region, which are particularly large and numerous at the pole of the cell where the axon arises. The surface of the cells is not smooth but has stumpy dendritic processes. Sometimes these are branched and roughened with ridges, which greatly complicate the appearance of the cell surface. The axon that originates from such cells sometimes shows one or two very fine collaterals. These run among the satellite cells of the sheath that surrounds the axon. It has not been possible to follow these collaterals beyond the entrance of the axon to the sucker musculature. Possibly they run to neighbouring nerve cells.

Around the cell body of each of these cells the silver methods show fine fibres ramifying and branching. These fibres reach the cell from various directions and presumably originate from several sources, including sensory cells of the epithelium (Fig. 2). The character of the fibres when they arrive at the cell, and the fact that some of them originate in the epithelium, suggest that they synapse with the cell. It cannot be excluded, however, from the structure of these cells that they are not themselves receptors.

Thus there is an elaborate receptor apparatus localized in the epithelial covering of the infundibulum and rim of the sucker, indicating that these are areas particularly specialized for receiving stimuli. The conspicuous variety of the shapes of the intra-epithelial receptors provides the possibility for the collection of different sorts of information. In particular, the structure of the cells illustrated in Fig. 3 suggests that there are chemoreceptors as well as tactile receptors in the suckers. The peripheral receptor cells are connected in two ways. There are collaterals in the epithelium and synaptic connexions with nerve cells below the epithelium. The cytological characteristics of these elements and their complicated set of dendrites within a capsule give the impression of a system for the correlation of impulses from various nearby regions.

I thank Prof. J. Z. Young for his help and advice. The work was done at the Stazione Zoologica, Naples, and my thanks are due to Dr. P. Dohrn. Mr. J. Armstrong took the photomicrographs. The work was aided by a grant of the Italian Consiglio Nazionale delle Ricerche.

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DISRUPTIVE SELECTION, POLYMORPHISM AND SYMPATRIC SPECIATION

By J. MAYNARD SMITH

Department of Zoology, University College, London

BY disruptive selection is meant selection which favours those members of a population which, in respect of some measurable characteristic, are well above or below the population mean, at the expense of the more typical members of the population. The recent papers by Thoday *et al.*[1-5] have re-aroused interest in the role, earlier discussed by Mather[6], of disruptive selection as an agent which may produce polymorphism and perhaps lead to sympatric speciation. This article discusses the following questions: (1) In what circumstances can disruptive selection maintain a stable polymorphism ? (2) Could such a stable polymorphism be followed by reproductive isolation between the morphs ? (3) Of what situations in wild populations are Thoday's experiments adequate models ?

Consider a pair of alleles A and a at any locus. The familiar genetic basis for a stable polymorphism is that the heterozygote Aa should be fitter than either homozygote. Haldane[7] has recently pointed out that a stable polymorphism can also arise if AA is fitter than aa in one sex, and aa than AA in the other; but this is possible only if the selective advantages are large. Apart from these two cases, stable polymorphism requires that the fitnesses of the various genotypes should be functions of their frequencies in the population. In particular, a stable polymorphism can be maintained if the fitness of particular genotypes increases as their frequency in the population decreases. Batesian mimicry (Mather[6]) and disease resistance (Haldane[8]) are examples of such a situation. If disruptive selection is to maintain a stable polymorphism it must do so by increasing the fitness of genotypes as their frequencies decrease.

If the effects of A, a on the phenotype are additive, then disruptive selection implies that AA and aa are both fitter than Aa; this would certainly not lead to a stable polymorphism, but to a genetically homogeneous population containing only A or only a. But it is possible to imagine a situation in which disruptive selection could lead to a stable polymorphism.

Suppose that the environment is divided up into two or more sub-environments, which, for want of a better word, I shall call 'niches'. Suppose further that the factors responsible for maintaining the population density are such as to ensure that the total density N is made up of n_1 individuals in niche 1 and n_2 individuals in niche 2. In phytophagous insects the niches might be different food plants, in tits they might be nesting boxes and holes in trees, and in man they might be slums and palaces.

Let the relative fitnesses of the various genotypes in the two niches be:

	AA	Aa	aa
Niche 1	$1 + K$	$1 + K'$	1
Niche 2	1	$1 + k'$	$1 + k$

It will be assumed that mating is at random, and that there is no habitat selection (that is, the probability that an individual will live in a particular niche is independent both of the genotype of the individual and of the niche in which it was raised). Then if p is the frequency of allele A among the adults in one generation, the proportions of surviving adults in the two niches in the next generation are:

	Niche 1	Niche 2
AA	$(1 + K)p^2$	p^2
Aa	$(1 + K')2pq$	$(1 + k')2pq$
aa	q^2	$(1 + k)q^2$
Total	$1 + Kp^2 + K'.2pq$	$1 + kq^2 + k'.2pq$

Hence, since the total number of individuals in niche 1 is n_1, the number of AA individuals in niche 1 becomes $n_1(1 + K)p^2/(1 + Kp^2 + K'.2pq)$, and so on. At equilibrium the overall frequency of A in this adult population is p. Consider first the simple case of additive inheritance of fitness in each environment; that is, $K' = \frac{1}{2}K$, $k' = \frac{1}{2}k$. At equilibrium:

$$p = \frac{n_1}{n_1 + n_2} + \frac{n_1K - n_2k}{(n_1 + n_2)Kk} \qquad (1)$$

If an equilibrium is to be possible, p must lie between 0 and 1. This will clearly be the case if $n_1K = n_2k$, so an equilibrium is possible for any values of n_1 and n_2 provided that the selective advantages are nicely adjusted to the sizes of the niches. But if K and k are small, an equilibrium is in practice unlikely. For example, suppose $n_1 = n_2$, the requirement for an equilibrium to exist becomes:

$$\left|\frac{K - k}{Kk}\right| < 1$$

Thus if $K = 0{\cdot}1$ (10 per cent selective advantage), k must lie between 0·09 and 0·11; but if $K = 1$, there will be an equilibrium provided that k is greater than 0·5.

It is easy to show that these equilibria, if they exist, are stable. It can also be shown that similar stable equilibria exist if the alleles show simple dominance, that is, if AA and Aa have the same fitness in both niches.

If in both niches the heterozygote is as fit as the fitter homozygote (that is, $K' = K$, $k' = k$), there will be a stable equilibrium for all positive values of K and k. But this is merely a special case of the familiar phenomenon of a stable polymorphism due to the superior average fitness of the heterozygote. In this particular case the superior fitness arises because the heterozygote is assumed to be adapted to a wider range of environments.

The main conclusion to emerge from this analysis is that disruptive selection can maintain a stable polymorphism in the absence of heterozygous advantage, but will do so only if: (1) the two morphs or extremes are adapted to different ecological niches, and the factors determining the population density are such as to ensure that the numbers of individuals occupying the two niches remain constant or approximately so, (2) the selective advantages are large, or, less plausibly, are by chance nicely adjusted to the relative numbers of individuals in the two niches.

The force of the latter requirement is obvious enough, but the relevance of the former may be made clearer by an entirely hypothetical example of a form of disruptive selection which would not lead to a stable polymorphism. Suppose that predators took predominantly the medium-sized members of a prey population, because the larger members were too big for them to tackle and the smaller members escaped their notice. This would, for the prey species, amount to disruptive selection, but it would lead to a population consisting entirely of large or entirely of small individuals, it could lead to a stable polymorphism only if there were some additional mechanism ensuring that the population contained a fixed number of large individuals and also a fixed number of small individuals. Now in laboratory experiments the selection procedure itself may provide such a mechanism. It will do so if, for example, a fixed number of 'high' and 'low' parents are chosen in each generation. Since the selection pressures in such experiments are usually high, they would be expected to lead to stable polymorphisms. But it is difficult to judge how often the rather severe requirements for an equilibrium will be met in wild populations.

It was assumed here that there was no habitat selection and no assortative mating. Habitat selection can take several forms, it may involve a choice by an individual of its own environment, or of an environment in which to lay its eggs, and in both cases the choice may depend on the genotype of the individual or on the environment in which it was itself raised. Habitat selection of any of these kinds will favour a stable equilibrium, or, to put the same thing in other words, given a degree of habitat selection a wider range of values of K and k will lead to a stable equilibrium. Conversely, given that a stable equilibrium exists, there will be selection in favour of habitat selection.

In discussing the possible role of disruptive selection in sympatric speciation, the vital question is the relation between disruptive selection and assortative mating. There are in fact two questions first, given that there is a degree of assortative mating, will this favour the establishment of stable polymorphism ?, and secondly, given that a stable polymorphism exists, will selection favour assortative mating ? The answer to both these questions is in the negative unless, because of habitat selection or for other reasons, the mean fitness of heterozygotes is less than that of homozygotes.

Consider first the effect of assortative mating on the stability of the equilibrium. Assuming that assortative mating is complete, then only AA and aa individuals will exist. If the fitnesses in the two niches are as given here, then p, the overall frequency of AA individuals, is given by equation (1), in other words, assortative mating does not alter the requirements for an equilibrium. Since complete assortative mating corresponds to the case of two sympatric species, it may seem surprising that the requirements for coexistence are so severe. But the requirements would in fact be severe in the absence of habitat selection.

Consider now selection in favour of assortative mating, again in the absence of habitat selection. At equilibrium, both for additive inheritance and for simple dominance, the average fitnesses of the three genotypes are equal, consequently the number of progeny left by an individual is unaffected by the genotype of its mate, and so there will be no selection favouring assortative mating. But if there is habitat selection, the mean fitness of the homozygotes will be greater than of the heterozygotes, and there will be selection for assortative mating. Therefore, if polymorphism based on disruptive selection is ever the first step in sympatric speciation, it seems likely that the next phase would be the evolution of some degree of habitat selection, and only then would reproductive isolation arise.

Yet Thoday and Gibson[5], in an experiment in which only flies with the highest and the lowest numbers of bristles were used as parents, observed the establishment of reproductive isolation between the two groups with what they themselves describe as remarkable rapidity. It is indeed difficult to understand the rate of the response they obtained, in view of the comparatively slow progress achieved by other workers[9] selecting for reproductive isolation between mutant strains of *Drosophila*. But leaving aside the rate of response, the system investigated by Thoday and Gibson was one in which there might well be selection for assortative mating. The conclusion reached here that there would be no selection for assortative mating was based on the assumption that in each niche the inheritance of fitness was either additive, or showed simple dominance, the heterozygote always resembling the same homozygote. But if selection is sufficiently intense, it is possible that the fitness of the heterozygote will, in both environments, equal that of the less fit homozygote. For example, suppose that bristle number is determined by only a single pair of alleles. Then it might be that all individuals selected as 'high' would be AA, and all selected as 'low' would be aa, if so, the heterozygotes would have zero fitness, and there would be selection for reproductive isolation.

Thus if the experiment of Thoday and Gibson is a model of a situation which might arise in Nature, it is of a situation in which (1) there is some mechanism ensuring that the extreme forms shall occur in equal numbers, or at least in numbers bearing a fixed relation to each other, (2) the mean fitness of heterozygotes is lower than of homozygotes.

These requirements would be met by the two-allele situation investigated in this paper if in each niche the heterozygote had a fitness equal to the less fit homozygote, that is, if $K' = k' = 0$.

Then at equilibrium

$$\frac{n_1 K p}{1 + K p^2} = \frac{n_2 k q}{1 + k q^2} \qquad (2)$$

This equation is algebraically intractable. But clearly if $n_1 = n_2$ and $K = k$, an equilibrium exists when $p = q = \frac{1}{2}$. Is this equilibrium stable ? If p_{x+1} and p_x are the frequencies of A in two successive generations, then

$$p_{x+1} - p_x = \tfrac{1}{2} p_x q_x \left\{ \frac{K p_x}{1 + K p_x^2} - \frac{K q_x}{1 + K q_x^2} \right\}$$

and considering a small displacement from equilibrium, $p_x = \frac{1}{2} + \Delta p$ where Δp is small

$$p_{x+1} - p_x = \frac{K}{4 + K}\left(1 - \frac{2K}{4 + K}\right)\Delta p \qquad (3)$$

For stability, $p_{x+1} - p_x$ must be negative when Δp is positive. From equation (3) it follows that the equilibrium is unstable for $K < 4$ and stable for $K > 4$. In other words, if reproductive isolation is to be a consequence of disruptive selection, then the fitness of heterozygotes must be low if selection is to favour assortative mating, and this in turn requires that the intensity of disruptive selection must be very high if a stable polymorphism is to be maintained for long enough for isolating selection to operate.

In conclusion, it seems that if disruptive selection is to lead to stable polymorphism, and *a fortiori* if it is to lead to reproductive isolation, the conditions which must be satisfied are severe. But they are of a kind which may often be satisfied in laboratory experiments, and which may perhaps sometimes be met with in Nature.

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EXPERIMENTAL VARIABILITY IN *FUSARIUM MONILIFORME* SHELD. LEADING TO THE FORMATION OF GIBBERELLINS

By Prof. A. A. IMSHENETSKI and O. M. ULIANOWA

Institute of Microbiology, Academy of Sciences, Moscow, U.S.S.R.

FROM year to year micro-organisms thriving under natural conditions continually reveal new physiological features. Recently, numerous investigators have paid attention to the gibberellins. The wide range of tests with gibberellins proves that this group is finding a broad field of practical application, for example, in grape cultivation and in floriculture.

Hence, it was of interest to learn whether mutations can be induced in *Fusarium moniliforme* experimentally, and to explore whether the mutations produced reveal higher activity than the initial wild culture of this fungus. Theoretically, this possibility seems quite feasible owing to the fact that the gibberellins do not belong to the basic products and the formation of the latter is not related to the energy metabolism of the micro-organism. Similar to the antibiotics and other physiologically active substances, gibberellins are produced in comparatively small quantities and their biosynthesis has no direct relation to the energy supply processes.

The experimental material selected was cultures of *Fusarium moniliforme* Sheld. A suspension of fungal conidia filtered through a sterile plankton net and containing 7 million conidia per 1 ml. of sterile water was irradiated with bactericide lamps of the *BUV*-15 type which emits primarily rays with a wave-length of 2537 Å. The time of exposure varied from 1 to 20 min., and this corresponded to the quantity of radiation energy ranging from 1,800 ergs/mm^2 to 36,000 ergs/mm^2. The water-suspension of irradiated or non-irradiated conidia was plated on potato or Czapek glucose agar. An inoculation of different nutrient media was made from each colony grown after the inoculation of the exposed material irrespective of whether the colony in its external appearance was like the colony of the initial culture or strongly different from it.

The cultivation of the initial *Fusarium* and various ultra-violet mutants of it was carried out on several solid and fluid media. The solid media were potato agar, Czapek glucose agar, agarized Raulin–Thom medium, Stodola agarized medium in which ammonium chlorate was substituted by sodium nitrate and malt agar 7° Balg. The liquid media were Stodola medium with an addition of trace elements according to the method of Lilly and Barnett, Raulin–Thom medium with trace elements and Darken medium.

To test the ability of *Fusarium* to form gibberellins, yeast autolysate, milk hydrolysate and a solution of glutamic acid were added to liquid media. The quantitative determination of gibberellins was carried out according to the Muromtsev and Nestjuk colorimetric method. Other biological tests were also applied.

Exposure of the initial *Fusarium* culture to various ultra-violet doses showed first of all that increasing the exposure results in an abrupt decline of the number of colonies growing from the exposed conidia. If the inoculation of the non-irradiated (control) conidia on to the potato agar led to the development of 4,400 (100 per cent) colonies, only 100 (2.3 per cent) developed after exposure to 30,600 ergs/mm^2. The conidia of one particular strain of *Fusarium* proved to be resistant to the effect of the ultra-violet rays and the application of relatively large doses of irradiation equal to 36,000 ergs/mm^2 revealed 0.45 per cent of viable conidia.

120 ultra-violet mutants were obtained altogether, most of them exhibited changed morphological and physiological peculiarities. All mutants obtained when grown on solid media formed colonies of smaller size than of the initial culture. This difference in the size of colony was especially great on potato agar and was less pronounced on other solid nutrient media. Moreover, most of the mutants differed from the initial culture in having a shorter aerial mycelium, a less fluffy surface to the colonies, more acutely pronounced folded pattern of growth, a changed shape of the colonies and different colour of the aerial and substrate mycelia.

For example, on Czapek agar the colonies of the initial strain were white with a slight lemon-yellow shade, whereas the colonies of active variants 3_6 and 5_6 were shaded pink and lilac. Apart from the Czapek agar the change of shape and coloration in colonies was distinctly revealed on malt-agar on Raulin–Thom agarized media and on Thom media, it was less pronounced on potato agar. The liquid nutrient media revealed a changed pattern in the growth of films and change of colour in the culture liquid of most variants.

Table 1. Formation of Gibberellins by the Mutants of *Fusarium moniliforme*

Groups of ultra-violet mutants	Activity of mutants	No of ultra-violet mutants	Per cent of the total No of ultra-violet mutants	Doses by which the mutants were produced (ergs/mm ²)
1	Exceeding the initial	2	1 7	5,400 (600)*
2	Equal to the initial strain	35	29 1	at all doses of 900–32,400
3	Less than initial	78	65 0	mainly 23,400–25,200 and higher
4	Complete loss of activity	5	4 1	30,600–36,000

* Recurrent dose irradiation is indicated in brackets.

The action of large doses of ultra-violet rays led to the formation of variants with little pronounced fluffiness in colonies and these cultures produced 'bare' leathery films at the top surface of liquid media, these films resembled bacterial films

The ultra-violet doses equal to 21,000–25,000 ergs/mm ² led to the formation of mutants which produce sporodochia and pionnotes in great numbers, this may be ascribed to a considerable change in metabolisms The initial fungal culture has never produced any similar formation in any of the media tested Microscopic examination of the mutant preparations has also revealed several changes in the shape and size of the macro- and micro-conidia and in the mycelium threads

The results presented in Table 1 show that the 120 mutants can be subdivided into four groups in accordance with their ability to produce active gibberellin-like substances

Formation of gibberellin in the *Fusarium* cultures developed very slowly, therefore we examined the dynamics of its accumulation in the cultures of the initial strain and in some of the mutants (Fig 1) Experiments have shown that active variants 3_6 and 5_6 produced a somewhat greater amount of gibberellin than the initial culture and that an acceleration of gibberellin biosynthesis was observed in mutants 12_1, 3_6 and 5_6 since the latter formed a maximum quantity of gibberellin 5–7 days before it was noticeable in the control strain In more active mutants the constructive metabolism was somewhat upset, and they accumulated a biomass which was considerably less than that of the control strain However, their ability to synthesize gibberellin exhibited a sharp increase, and 1 5–3 times more gibberellin was produced per unit dry weight These changes in the physiological features of the mutants most probably support the hypothesis that the production of gibberellin is not related to the metabolism and appears to be a 'by-synthesis', and in ultra-violet mutants this process may be intensified

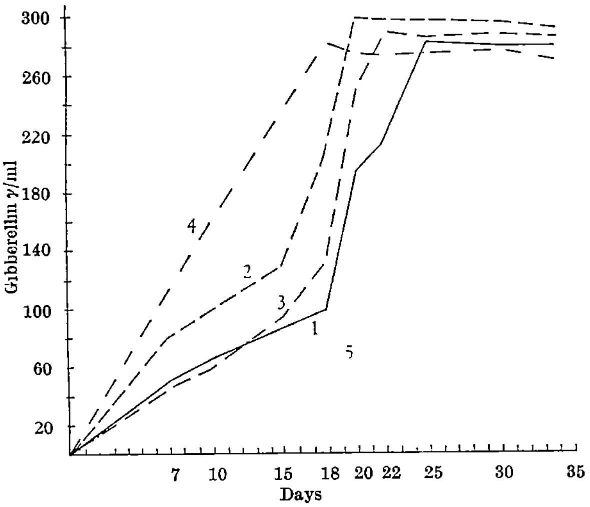

Fig 1. Formation of gibberellin in the initial strain of *Fusarium* and in mutants 5_6, 3_6, 12_1 and 16_1 in Stodola medium with 3 per cent glucose, trace elements and glutamic acid
1, initial strain, 2, mutant 5_6, 3, mutant 3_6, 4, mutant 12_1, 5, mutant 16_1

Active variants, as compared with the initial culture, consumed glucose at a greater rate, this resulted in a more rapid decrease in *p*H of the medium Less citric and gluconic acids and more acetic acid and ethyl alcohol were produced (Table 2) Consequently, the metabolism in those mutants was subjected to similar changes, it should be emphasized that the reduced product, that is, the ethyl alcohol, was being formed more intensively

Table 2 Production of Organic Acids and Alcohol in the Initial *Fusarium* Strain and in Ultra-Violet Mutants (Time of Experiments, 25 Days)

Compound	Amount in mgm /200 ml medium The initial strain	Mutant 3_6	Mutant 5_6	Mutant 12_1
Citric acid	984 57	804 75	645 35	769 5
Gluconic acid	474 24	437 76	414 96	383 04
Oxalic acid	0	0	0	0
Acetic acid	160 0	175 0	220 0	237 5
Ethyl alcohol	75 90	100 67	158 7	104 65
Malic acid and succinic acid (qualitative reactions)	present	present		present

The physiological effect produced by the filtrate of the cultural media of active variants of *Fusarium* on the growth and development of different higher plants has shown that it has a stronger stimulating effect on the growth of shoot and root than the cultural filtrate of the initial strain

The dwarf peas (varieties De Grass and Pioneer) responded strongly to the action of gibberellin and attained the height of common peas under the influence of stimulators A distinctly pronounced stimulating effect was observed in the surface parts of plants when the latter were treated with cultural liquid of the active mutants This accelerating influence was noted in corn, cucumbers, garden peas, soya beans, but it was less pronounced in barley, oat, rye and wheat In dwarf peas and lettuce the lengths of the roots increased 2–3 times under the effect of the cultural filtrate of the active variants as compared with the initial strain

The stimulation of shoots and roots developed along with the intensification of the synthetic processes, owing to this the dry weight of plants treated with cultural filtrate of active mutants was higher A greater increase in dry weight was observed in plants which failed to reveal considerable elongation Excessive 'gigantism' in plants under the influence of stimulators sometimes exhibited a pathological condition, in this case the dry weight of the plant was sometimes even lower than that of control plants In our experiments the dry weight of peas, cucumbers and soya increased relatively more than in other plants

The stimulating effect of the mutant strain cultural filtrate cannot be ascribed solely to the action of gibberellin present in the fluid The formation of this stimulant in the mutant cultures is not solely responsible for the considerable difference observed in the action of the cultural filtrate of the initial strain Most probably, the mutants have developed and intensified the biosynthesis of other substances formed by *Fusarium* and stimulating plant growth.

LETTERS TO THE EDITOR

RADIOPHYSICS

Very-Low-Frequency Discrete Emissions received at Conjugate Points

In a recent article[1] describing a geomagnetic conjugate point experiment an accurately synchronized pair of spectrograms was shown. As shown in Fig. 1 of that article a sequence of six noise bursts in the 5–6 kc./s. frequency region was observed at both Knob Lake, Canada (68° N., geomagnetic), and at Byrd Station, Antarctica (70° S., geomagnetic), but the sequence began at Knob Lake 0.8 ± 0.1 sec. before it began at Byrd.

However, close inspection of Fig. 1 indicates that in each case the sequence is not six separate bursts but a pair of bursts seen three times. In support of this it will be noticed that the shapes and separation of the two bursts in each pair are similar and in each case the first burst occurs at a slightly higher frequency than the second. Furthermore, the time separation between successive pairs (using the time-scale provided) is 1.6 ± 0.1 sec., which is just twice the delay between Knob Lake and Byrd. Thus at intervals of 0.8 sec. this pair of bursts appeared alternately at Knob Lake and Byrd.

This phenomenon cannot be due to successive reflexions in opposite hemispheres of electromagnetic energy because the observed time delays are too short. Whistler observations in these latitudes show 5 kc./s. propagation times between 1.5 and 2.5 sec. for a single hemisphere to hemisphere trip[1]. Even then these times refer to lower latitudes because geomagnetic field lines terminating at latitudes greater than about 62° do not allow 5 kc./s. propagation of this type[2]. Thus if this phenomenon were due to whistler type echoes the delays would be several times longer.

However, such a sequence of alternate reception at conjugate points was predicted in a theory proposed by me[3] for the production of very-low-frequency discrete emissions. In fact such a conjugate point experiment was suggested as a test for the theory.

According to this theory Doppler-shifted cyclotron radiation is emitted by a small cloud or bunch of electrons spiralling along a field line. Only the downward-shifted frequency can propagate so that radiation is only emitted backwards. If the bunch should survive several trips along the field line from hemisphere to hemisphere, being reflected at the ends by magnetic mirror effect, then an observer at each end of the field line would receive an emission each time the bunch was travelling away from him. Thus for each observer the time between successive emissions would be the complete (there and back) oscillation period of the cloud and the delay between the sequences observed in opposite hemispheres would be half this period[3].

The complete oscillation period of 1.6 sec. from Fig. 1 (ref. 1) would correspond to 60 keV. electrons (for minimum helical pitch angles of 20°) if the guiding field line was that connecting the two observing stations or 15 keV. electrons if the guiding field line was typical of those (around 60°) producing nose whistlers observed at Byrd[2]. These electron energies deduced from the oscillation period (15–60 keV.) are close to those (5–25 keV.) required by my theory to produce the detailed frequency-time shape of observed discrete emissions[3].

I thank Prof. G. R. A. Ellis, of the Physics Department, University of Tasmania, for advice.

R. L. DOWDEN

Ionospheric Prediction Service
and Department of Physics,
University of Tasmania,
Hobart.

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Dowden suggests that the six bursts of noise shown in Fig. 1 of our article consist of a pair of bursts seen three times at each end of the path, and that this interpretation provides support for a new theory of very-low-frequency emissions which he has published elsewhere.

We agree that this interpretation is entirely consistent with the data reported in our article. As a matter of fact, further study of the original tape recordings has shown additional noise bursts which can be fitted into a repetitive or echo pattern, in which the phase is opposite at the two ends of the path. The echoing of noise bursts between stations in opposite hemispheres is a common occurrence, and is the subject of a detailed study now in progress at the Radioscience Laboratory, Stanford University. Results of this work will be reported in the near future.

Although we can support the 'echo' interpretation of Fig. 1, we question Dowden's conclusion that the phenomenon cannot be due to successive reflexions in opposite hemispheres of electro-magnetic energy travelling in the whistler mode. His argument is based on the fact that whistler observations at these latitudes show 5 kc./s. propagation times between 1.5 and 2.5 sec. for a single hemisphere-to-hemisphere trip (one-hop), while the observed one-hop delay of the noise bursts is only 0.8 sec. (one-half of observed period of 1.6 sec.). It is implied that both the path of propagation and the electron density in the magnetosphere are about the same in the two cases. Neither of these assumptions is justified, as we shall see.

Whistler-mode path latitude bears little relation to the latitude of the receiving station. For example, data analysed by Smith[1] show a variation in the nose frequency (closely related to path latitude) at Byrd from 4.5 kc./s. to 12 kc./s. For the model of electron density derived by Smith, the corresponding minimum gyro-frequencies at the top of the path are about 11.2 kc./s. and 30 kc./s., respectively. The corresponding effective geomagnetic latitudes are 61° and 55°, respectively. Other data show that even lower latitude paths are often observed at Byrd Station[2].

Another factor affecting propagation time is the electron density along the path of propagation. Carpenter has shown that electron densities are often markedly depressed during magnetic storms[2]. One-hop delays at 5 kc /s can drop as low or lower than 0 8 sec at Byrd following the onset of a magnetic storm The record in question was obtained on the day following a magnetic disturbance in which the 3-hr K_p index reached a maximum of 6 Thus it is quite possible that the delays observed are in fact consistent with the whistler-mode interpretation

The fact that we cannot rule out the whistler-mode echo explanation does not, of course, disprove Dowden's hypothesis that the noise bursts were generated by bunches of electrons bouncing back and forth between magnetic mirror points Our comments are intended only to suggest the inherent complexity of the problem and the need for more complete data on the relationship between whistler-mode propagation and very-low-frequency emissions

R A HELLIWELL

Radioscience Laboratory,
Stanford University,
Stanford, California

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GEOPHYSICS

Age of the Earth's Crust and Lead Model Ages

OVER the past few years the observed time-dependence of the isotopic composition of lead in ore deposits has been used in attempts to estimate a 'model age' for each deposit In the main, two general lines of attack appear to have been used On one hand Canadian writers[1] have adopted a simple model, with a single growth curve, the parameters of which have been determined empirically; whereas the European workers[2] have set up models which allow for a greater variety of relative abundances of uranium–lead–thorium in the parent material, and where the parameters are physical constants which can be determined by independent methods These two methods of attack have seemed to give different values of the age, and the further suggestion has recently been made[2] that the two models furnish very different values for the age of vein deposits

In calculations which I have carried out in connexion with some results to be published elsewhere, I have shown that the major part of the discrepancy in model ages obtained by the two methods rests on the value of t_0, the 'age of the earth's crust'

In the Holmes–Houtermans equation (2)

$$\frac{y - y_0}{x - x_0} = \frac{1}{137\ 8} \times \frac{e^{\lambda_2 t_0} - e^{\lambda_2 t}}{e^{\lambda_1 t_0} - e^{\lambda_1 t}}$$

where x, y, are the ratios $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ for the sample, x_0, y_0, the corresponding values for 'primordial lead' (average of Patterson's[3] values for Canyon Diabolo and Henbury troilite leads), and λ_1, λ_2, the decay constants for ^{238}U, ^{235}U (ref 4), three values now used of t_0 have yielded three very different values for the model age calculated for the one (x, y) (Table 1)

Table 1

t_0 × 10^9 yr	4 50 (ref 2)	4 510 (ref 1)	4 550 (ref 3)
age × 10^6 yr	1669	1640	1524

These compare with a value 1,524 million yr for t_{6-7} of Russell and Farquhar[1], whose empirical equation yields an independent value for t_0 = 4 56 × 10^9 yr Over quite a variety of specimens from Precambrian localities, which range from 'conformable' leads through vein deposits to those distinctly anomalous, close agreement has been obtained between the t_{6-7} model age and that calculated according to the Holmes–Houtermans model, using $t_0 = 4\ 55 \times 10^9$ yr , with the standard deviation of the ages of a set of comparable specimens slightly less in the first case than in the second

Thus it would seem that both models yield very similar conclusions, and that any discordance which has appeared in the past has resided almost entirely in the value chosen for t_0 The large error magnification exhibited here would appear to be an inevitable consequence of the exponential function used in all lead calculations, of which the equation quoted is a typical example Where t_0–t (or in the general case t_1–t_2) is small, this magnification should prove to be less than unity, which is perhaps one reason why this effect has not been remarked on previously

J R RICHARDS

Department of Geophysics,
Australian National University,
Canberra.

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PHYSICS

Direct Conversion of Fission Heat to Electric Power

EXPERIMENTS have been performed in the reactor *Pluto* as a stage in the development of cæsium-neutralized diodes for the direct conversion of fission heat to electric power This work is similar to that being carried out in the United States, and the first fission-operated diode was reported by Grover at Los Alamos in 1959[1] Due to its greater cathode area, the present device has produced a larger value of output current than hitherto reported and incorporates a number of new features Measurements were made with two rigs, the object being to test the fuel rod material and method of mounting, to determine temperature distributions and heat transfer data and to study the performance of the diodes as generators

Each diode (Fig 1) contained a cylindrical cathode of UC/ZrC solid solution brazed to a tantalum support, and a stainless-steel anode which was extended to form a catchpot and a cæsium reservoir The radial spacing between cathode and anode was 0 5 cm In this intermediate design the emission current circulated via the extended anode casing and cathode support, so that a ceramic/metal insulating seal was not required The output was determined by monitoring the voltage across the extended anode casing above the ceramic spacer The cathodes were 5 7 cm long and 1 0 cm in diameter, enriched to contain 8 3 gm uranium-235 over the lower 3 0 cm , the remainder containing natural uranium The composition of the UC/ZrC was 45/55 mol per cent with a melting point more than 3,000° C Cathode temperatures were monitored by means of a W/WRe, beryllium oxide insulated, tantalum sheathed thermocouple situated at the junction of the natural and enriched material Other thermocouples of chromel/

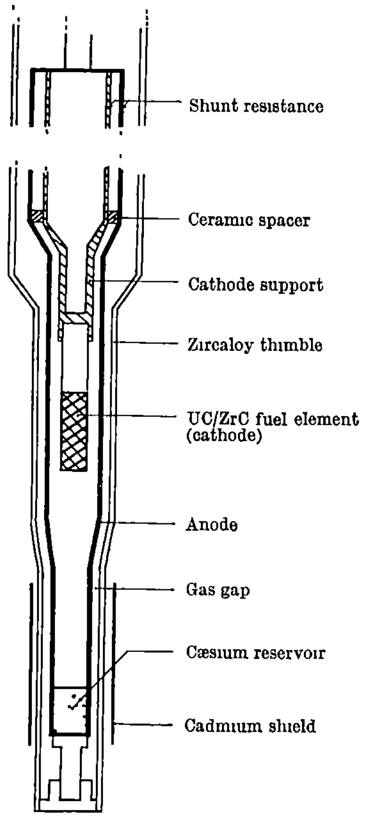

Fig 1 Inpile diode (first rig)

alumel measured the temperature distributions over the catchpot, casing and cathode support

The catchpot was designed to hold the cathode in the event of its failure by fracture or melting It was lined with tantalum and also contained a tantalum thimble located over the cæsium capsule The catchpot region was shielded by a stainless-steel sheathed cadmium cylinder which reduced the neutron flux in this region to about 5 per cent of the undisturbed value Cæsium was inserted in a sealed silica capsule which was burst by heating to about 200° C after the chamber had been sealed

A concentric zircaloy thimble surrounded the anode with a nominal gas gap of 0.010 in containing helium The bulk of the anode heat was transferred to the reactor heavy water flow through the helium gas gap and the zircaloy thimble

In the first rig (Fig 1) the temperature of the cæsium was controlled in conjunction with the anode surface temperature by varying the pressure of helium in the gas gap between the limits of 1 atmosphere and 8 torr Thus, in this case, the cæsium vapour pressure and the collector surface temperature varied together In the second rig the cæsium reservoir was surrounded with a 250-W 'Pyrotenax' heater (not shown in Fig 1) which yielded some measure of independence in cæsium vapour pressure and anode temperature

The highest value of current achieved with the first (variable gas pressure) rig was 140 amp (14 amp / cm ^{2}) when the voltage across the load was 0.3 V The corresponding cathode and anode temperatures were about 2,100° C and 687° C respectively and the cæsium vapour pressure 0.1 torr This output of 42 W was obtained at an efficiency of about 3 per cent Similar results were obtained from the second rig

The first rig yielded the above output after a life-test of 38 hr operation time with cathode temperatures in the region of 1,950° C and the output currents of about 70 amp During this test there was no clear evidence of any decrease in performance

In this intermediate design the current was not fed to a matched load, and furthermore not all the output voltage appeared across the load An allowance for these effects would lead to an efficiency in the 5–10 per cent range

P D Dunn
G Rice
L G Sanders
D A Watt

Direct Conversion Group,

J Adam
R J Hill
J G Purchas
C C H Wheatley

Metallurgy Division,
Atomic Energy Research Establishment,
Harwell

J A McWilliams

Boucher and Co , Ltd.,
Kidderminster, Worcs

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Quasi-Trapping of Low-Energy Electrons in Water Vapour

The interaction of low-energy (less than 1 eV) electrons with water vapour has been examined by means of a swarm method[1-3] in which water vapour at pressure f_1p was added to ethylene, carbon dioxide and methane, at pressures f_2p It was found that when f_1p ranged from 1 to 16 mm mercury, and f_2p was of the order of 100–200 mm mercury, no electron capture occurred for an E/P [V cm $^{-1}$ (mm mercury)$^{-1}$] ranging from 0.01 to 4.0 (An exception must be made for the case of water–carbon dioxide where a very small capture cross-section was observed and may be due to the formation of carbonic acid) However, an interesting and significant increase in the electron transport time was observed and is illustrated in Fig 1 for mixtures of water and ethylene In Fig 1, w^{-1} is the transport time per cm of travel in the field direction, and it is observed that (1) w^{-1} increases linearly with the ratio f_1/f_2, (2) w^{-1} is independent of the total pressure p for the range 100–400 mm mercury These results suggest that electrons make collisions of long duration with water and that the lifetime of the 'quasi-trap' $[H_2O]^-$ is limited by collision with ethylene for the pressure-ranges already quoted

The notion of long-duration collisions suggests immediately that the observations by Bradbury and Tatel[4] of electron capture in pure water vapour can be interpreted in terms of the formation of electron traps, that is, successive collisions of quasi-traps with water molecules to form $(H_2O)_n^-$, where n, the number of water molecules in the negatively charged cluster, becomes of such magnitude that electrons become permanently trapped by polarization forces The existence of such local self-consistent quantum states has been shown to be theoretically possible by Pekar[5] in the case of ionic crystals, and these states (polarons) have, in fact, been invoked for the case of

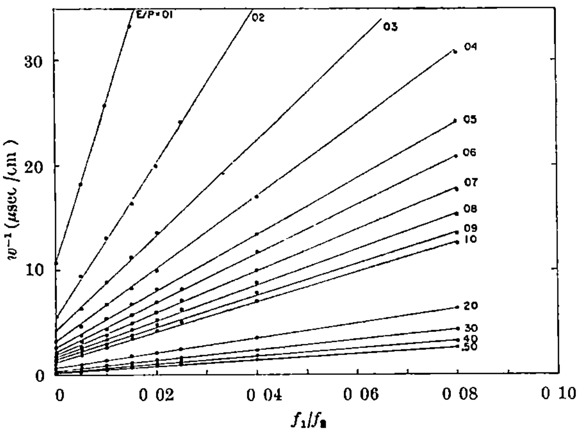

Fig. 1. Electron transport time per cm , w^{-1}, for mixtures of water (mole fraction f_1) and ethylene (mole fraction f_2) versus f_1/f_2 for various values of E/P

low-energy electron trapping in liquid water by Weiss[6] The present ideas of quasi-traps are not in contradiction with the fact that $[H_2O]^-$ has not been observed in mass spectrometers, since the lifetime of an isolated $[H_2O]^-$ may be much shorter than the flight time (typically 10^{-6} sec) in a mass spectrometer The 'polaron' would, of course not be observed in the mass spectrometer since the vapour pressure is too low to permit its formation Thus the simple mechanism discussed here suffices to interpret four sets of observations, that is (1) our observation that electrons are not captured when the pressure of ethylene is about 100 mm mercury, (2) our observation of increased transport time (Fig 1), (3) the observations of an increasing capture cross-section with increasing pressure of pure water made by Bradbury and Tatel, (4) the failure to observe $[H_2O]^-$ in the mass spectrometer

The nature of the force involved in the primary collision process is thought to be that of an electron-permanent electrical dipole interaction for the following reasons (1) when other molecules having permanent electrical dipoles are substituted for water, an effect similar to that in Fig 1 is observed, while little correlation is found when molecules merely having large polarizabilities are substituted, (2) at distances r greater than 3 Å , the energy of interaction of an electron with the permanent dipole of water is considerably greater than its energy of interaction with the induced dipole in water , (3) the slope of the curves in Fig 1, $S(E/P)$, can be accounted for when a v^{-2} cross-section (associated with the r^{-2} interaction energy of a permanent dipole) is assumed, and cannot be accounted for when a v^{-1} cross-section (corresponding to the r^{-4} interaction energy of an induced dipole) is assumed

We wish to emphasize some essential differences in the quasi-trap, $[H_2O]^-$, and the 'polaron' The quasi-trap, $[H_2O]^-$ (1) results from the trapping of an electron by a single molecule, (2) has a limited lifetime (for example, 10^{-8}–10^{-7} sec) which is governed by a statistical process in which some kinetic energy of interaction is transferred to an internal degree of freedom (for example, vibration or rotation) On the other hand, the polaron (1) results from the interaction of an electron with a group of polarizable molecules, (2) a stationary quantum state is formed Thus, the quasi-trap, according to earlier comments on the observations of Bradbury and Tatel, may grow into a polaron by further interaction with polarizable molecules

G S Hurst
L B O'Kelly
J A. Stockdale *

Health Physics Division,
Oak Ridge National Laboratory †,
Oak Ridge,
Tennessee

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* Australian Atomic Energy Commission, Research Establishment, Lucas Heights, Sydney

† Operated by the Union Carbide Corporation for the U S Atomic Energy Commission

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Re-entrant Motion in Special Relativity

Consider two inertial co-ordinate systems, S and S^*, such that S^* moves relatively to S along the direction of their coincident x-axes at the constant speed $v = \lim_{\Delta x\to 0} (\Delta x/\Delta t) = \lim_{\Delta x^*\to 0} (-\Delta x^*/\Delta t^*)$ The Lorentz transformation requires that, for any two events characterized by separations $(\Delta x, \Delta t)$ in S and by $(\Delta x^*, \Delta t^*)$ in S^*

$$\Delta x^* = \frac{\Delta x - v\Delta t}{\sqrt{1 - v^2/c^2}} \qquad (1)$$

Consider an idealized, thin, circular, disk of radius r, made of infinitely strong material, rotating with uniform angular velocity ω relative to the laboratory system S about a fixed axis through the centre and perpendicular to the plane of the disk It is sometimes thought in such a case that the edge speed is $v = r\omega$ It is shown here that this is true only as an approximation, valid for small v, and that in fact $r\omega$ can exceed c, the speed of light, while v can never do so

We suppose the outer edge of the disk to be marked with numerous point-markers that divide it into n equal segments (Alternatively, the circular disk might be replaced by an n-sided regular polygon) If n is very large the segment rest-length, s_0, will be very nearly equal to $2\pi r/n$, and this approximation will become exact as $n \to \infty$ Consider S^* to be the Lorentz frame in which a particular edge-segment of the disk is momentarily (approximately) at rest as the segment passes a nearby fiducial point in the laboratory system S Let the two events with respect to which the increments $(\Delta x, \Delta t)$ in S and $(\Delta x^*, \Delta t^*)$ in S^* are defined be the passages opposite the fiducial point in S of the two adjacent point-markers on the disk-edge that delimit the segment in question Then $\Delta x = 0$ and $\Delta x^* = s_0 = 2\pi r/n$ Also, $\Delta t = T/n$ where $T = 2\pi/\omega$ is the rotation period of the disk, as measured in S (This is merely the assertion that n segments pass the fiducial point in S in n times the time required for one segment to pass it) Using these values of Δx Δx^*, and Δt in equation (1), we obtain

$$\frac{2\pi r}{n} = \frac{-vT}{n\sqrt{1 - \frac{v^2}{c^2}}}$$

Hence,

$$\frac{2\pi r}{T} = r\omega = \frac{-v}{\sqrt{1 - \frac{v^2}{c^2}}} \qquad (2)$$

Squaring, we have

$$r^2\omega^2 = \frac{v^2}{1 - \frac{v^2}{c^2}}$$

or

$$v = (\pm)\frac{r\omega}{\sqrt{1 + \frac{r^2\omega^2}{c^2}}} \qquad (3)$$

Thus, $|v|$ is always less than c, no matter what the value of $r\omega$ may be. The approximation $|v| = r\omega$ is valid only for $r^2\omega^2 \ll c^2$. No physical restriction is placed by special relativity on the value $r\omega$. This conclusion contrasts sharply with the usual[1] supposition that $r\omega < c$ is a requirement of relativity.

More generally, if a 'round-trip speed' $v_{r\,t}$ for the re-entrant motion is introduced via the definition $|v_{r\,t}| = r\omega = 2\pi r/T =$ (distance travelled) / (round-trip time), then the instantaneous speed v, which must be used in the equations of the Lorentz transformation, is related to this round-trip speed by

$$v = \frac{v_{r\,t}}{\sqrt{1 + (v_{r\,t})^2/c^2}} \qquad (4)$$

In classical physics, of course, the round-trip speed (for uniform motion) is not conceptually distinguished from the instantaneous speed, although it is always operationally distinguishable.

Discussion of the numerous surprising implications of this result for all types of re-entrant motion, including the 'twin paradox' (from equation (2), $v_{r\,t} = v/\sqrt{1 - v^2/c^2}$, hence, $v_{r\,t}$ appears anomalously fast by the factor $(1 - v^2/c^2)^{-1/2}$, this just compensates the time-dilatation 'staying young' of the space traveller by the same factor, so that the twins age the same (both 'stay young')), and for the nature of space-time (for example, because of the equal ageing, independently of motion, just mentioned, one may postulate that the proper-time increment $d\tau$ is an exact differential, dt inexact; in this case Fermat's principle, $\delta \int dt = 0$, describes the motion of both material particles and photons; and space-proper-time, rather than Minkowski space, is the appropriate 4-space for physical description), had best be deferred until the validity of the present derivation is confirmed. It may be objected that special relativity should be viewed as incapable of treating any sort of accelerated motion. However, Pauli in his well-known work on special relativity[2] indicates that the subject is founded not on the ignoring of acceleration but on the explicit assumption that acceleration produces no specific effect on relativistic kinematics. Such an assumption has recently received impressive experimental confirmation[3] for accelerations up to $10^{16}g$. Therefore the burden of proof would seem at present to rest on those who believe that special relativity is entirely inapplicable to re-entrant motion.

T. E. Phipps

U.S. Naval Ordnance Test Station,
China Lake, California.

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CRYSTALLOGRAPHY

The C–H ... O Hydrogen Bond in Crystals

Over the past ten years, numerous examples of 'short' intermolecular distances between a carbon atom with at least one hydrogen atom attached to it and an oxygen atom have appeared in papers on the crystal structures of organic molecules. By 'short' it is meant that the intermolecular distance is considerably less than the sum of the van der Waals radii for the atoms or groups concerned. In practically all cases, the significance of these interactions has not been noted.

The most electronegative of the atoms can take part in hydrogen bonding. Fluorine, oxygen, nitrogen, chlorine and bromine all form hydrogen bonds of different strength, the strongest ones being associated with fluorine, the most electronegative element. The hydrogen atom is located between the two electronegative atoms, and the bond formed by these three is thought to be linear or nearly so. The number of compounds containing hydrogen bonds, in which the positions of the hydrogen atoms are accurately known, is comparatively small. It is usually assumed that the deviation from linearity (the angle H–A ... A′ between the direction of the hydrogen atom A–H and the direction of the hydrogen bond A ... A′) is not greater than 15°. Recently, Hamilton[1] has shown in a neutron diffraction study of dimethylglyoxime that this angle is as much as 24°. The angle O–H ... N is 140 ± 3°, a value significantly different from 180°. The hydrogen bond is therefore not necessarily linear.

The C–H group may be activated by other atoms or groups of atoms promoting ionization or partial ionization of the hydrogen atom. Under these conditions, it resembles the O–H and N–H groups, etc., and it may form hydrogen bonds. As an example, chloroform in which activation of the C–H group by the chlorine atoms occurs will hydrogen bond with strong bases like pyridine and triethylamine. In the crystalline state, hydrogen cyanide at −120° and −80° consists of molecules arranged in infinite chains with 'short' carbon–nitrogen distances of 3.18° along the C–H ... N directions[2]. These were assumed to be hydrogen bonds. There was, however, no value for the C–H ... O hydrogen bond, and I could find very few papers where the possibility of the 'short' C ... O distance being such a bond was considered.

A few examples of 'short' C ... O contacts are given in Table 1. These occur mainly in the crystal structures of heterocyclic molecules but sometimes in those of compounds containing atoms of different electronegativity with, frequently, a conjugated double bond system or a series of double bonds. The carbon atom of the C–H group is directly attached to the hetero or the more electronegative atom or group, or is part of a conjugated double bond system containing a hetero atom. The right conditions exist for the activation of the C–H group and the formation of hydrogen bonds. It must now be shown that the

Table 1. Some 'Short' Intermolecular C ... O Distances

Compound	d(C ... O) in Å	Groups involved	Ref.
Caffeine	3.18	CH ... O (keto)	(3)
Theophylline	3.22	CH ... O (keto)	(4)
Glycyl-L-tyrosine hydrochloride	3.07	CH_2 ... O (water)	(5)
Ethylene carbonate	3.11	CH_2 ... O (keto)	(6)
1,3,7,9-Tetramethyluric acid	3.00	CH_3 ... O (keto)	(7)
Acetyl choline bromide	3.00	CH_3 ... O (acetyl)	(8)
Caffeine	3.24	CH_3 ... O (keto)	(3)

Table 2 ANGLES CONNECTED WITH SOME OF THE 'SHORT' INTERMOLECULAR DISTANCES

Compound and hydrogen atom	*d*(H . O) in Å	∠C-H O	∠H-C O	∠H O C
Theophylline (H_1)	2 25	173 3°	4 7°	2 0°
Caffeine (H_1)	2 07	166 7	8 6	4 7
Ethylene carbonate (hydrogen positions calculated)	2 73	102 6	59 4	18 3
1,3,7,9-Tetramethyluric acid (H_1)	2 26	120 8	40 0	19 0
Caffeine (H_2)	2 76	109 1	53 9	17 1

hydrogen atoms are involved in the interactions if these are to be hydrogen bonds

In some of these compounds with 'short' intermolecular C . O distances the positions of the hydrogen atoms are known For these compounds all the relevant angles of the interaction have been calculated and a few examples and some of the angles are given in Table 2 For the CH group the value of *d*(H O), the distance between the hydrogen and oxygen atoms, is about 2 1 Å , which is less than 2 6 Å the sum of the van der Waals radii for these atoms The angles C–H O and H–C O are within the accepted limits for a hydrogen bond These interactions are therefore hydrogen bonds

In the case of the methylene and methyl groups, there is no tendency to form more than one 'short' contact This is in contrast to the NH_3^+ group, which can form three hydrogen bonds The angles for the CH_2 and CH_3 groups are very similar and, for all the examples considered, range from 22°–59° and 102°–142° for angles H–C O and C–H O respectively The hydrogen atom is no longer directed towards the oxygen atom as in the case of the CH group, but the hydrogen bond is not necessarily linear Steric hindrance from the other hydrogen atoms in these groups and the position assumed by the group which is forming only one 'short' contact may prevent a linear arrangement of this bond Until the limits in the angles representing the deviation from linearity of the hydrogen bond are known, it cannot be definitely stated whether these contacts formed by the methylene and methyl groups are true hydrogen bonds Nevertheless, it is felt that these interactions correspond to hydrogen bonds which are typical of these groups

There are many examples of 'short' intramolecular C . O contacts where activation of the C–H group occurs, for example, 2 87 Å in muscarine iodide[9], 3 01 Å in indirubine[10] These probably represent intramolecular C–H O hydrogen bonds

It is noteworthy that many of the compounds cited are molecules of biological importance The hydrogen bond is of great importance in the packing of this kind of molecule and it would seem that the C–H . O hydrogen bond may play a similar part, too

I thank Dr R Sparks for the use of his *Mercury* programme for calculating angles, and the University of London for the award of an Imperial Chemical Industries fellowship

D June Sutor

Birkbeck College Crystallography Laboratory,
21 Torrington Square, London, W C 1

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MINERALOGY

The System Al_2O_3–B_2O_3

Numerous investigations have been made of the thermal behaviour of alumina–boric oxide mixtures In 1887 Mallard[1] identified a compound believed to be $3Al_2O_3$ B_2O_3 Later work by Baumann and Moore[2] and by Scholze[3] indicated the formula to be $9Al_2O_3$ · $2B_2O_3$. Scholze[3] reported an additional compound, $2Al_2O_3$ B_2O_3 A third boroaluminate has been recognized as the rare mineral jeremejevite, Al_2O_3 B_2O_3 (ref 4) Data on the stability of these compounds are conflicting, and no serious effort appears to have been made to incorporate them in a conventional phase diagram We present here a tentative phase diagram for the system Al_2O_3–B_2O_3, based on data from more than 100 runs carried out in the range 600°–1,650° C

Alumina–boric oxide mixtures were prepared from high-purity γ-alumina and crystalline boric oxide, or from the previously prepared boroaluminate compounds, $9Al_2O_3$ $2B_2O_3$ and $2Al_2O_3$ B_2O_3 Samples were sealed in platinum tubes to eliminate volatilization losses, and to ensure a water-free environment They were held for 16–24 hr , and occasionally up to 120 hr , in a platinum 60–rhodium 40 resistance furnace Temperatures were read with a platinum, platinum 90–rhodium 10 thermocouple frequently calibrated against the melting points of gold (1,063° C), diopside (1,391 5° C), and palladium (1,555° C) The fired charges were quenched in air or mercury, and phases were identified with the polarizing microscope and the X-ray diffractometer

Baumann and Moore[2] found $9Al_2O_3$ $2B_2O_3$ to melt incongruently at 1,950° C , whereas Scholze[3] set the melting point at 1,440° C In the present work no fusion was observed in a sealed-capsule sample of $9Al_2O_3$ $2B_2O_3$ held for 6 hr at 1,650° C The 1,950° C value is therefore selected as the more plausible Our tests indicate that $2Al_2O_3$ B_2O_3 melts incongruently to yield $9Al_2O_3$ $2B_2O_3$ and liquid at 1,035° C This is in essential agreement with the value of 1,050 ± 20° C given by Scholze[3] Attempts to synthesize jeremejevite at atmospheric pressure, and in a low-pressure hydrothermal bomb, proved unsuccessful Its reported synthesis by Michel-Levy[5] at about 4,000 atmospheres suggests that it may be a high-pressure phase The work of Golovastikov *et al* [6] indicates that jeremejevite may be a hydrated boroaluminate Our negative results in the attempted Al_2O_3 B_2O_3 synthesis are consistent with either of these two possibilities

Fig 1 incorporates the results of 135 runs on compound stability and liquidus curve determination Small crosses indicate the compositions and temperatures of the liquidus runs The liquidus curve is shown rising continuously from the melting point of B_2O_3 at 470° C The determined curve has been extended only to 1,545° C , when it has been extrapolated as a smooth dashed curve to the melting point of alumina at 2,020° C Conceivably, a two-liquid area might intervene in this extrapolated region However, B_2O_3 is known to behave towards other oxides in much the same way as does SiO_2 towards the same oxides Since SiO_2 shows no liquid immiscibility towards Al_2O_3, it is tentatively assumed that B_2O_3 also lacks an immiscible relation in the liquid region of the Al_2O_3–B_2O_3 system If a eutectic exists in this system it can scarcely differ appreciably, in composition or temperature, from the pure B_2O_3 end-member In fact, the proximity of the liquidus

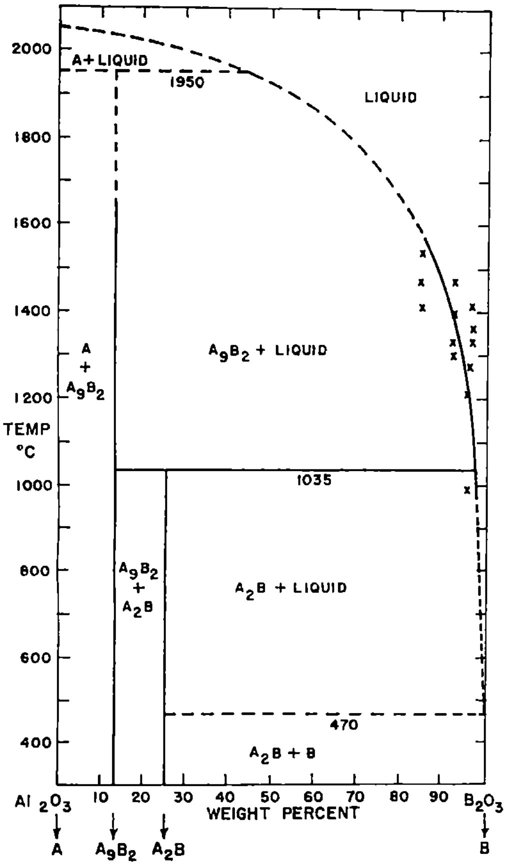

Fig 1

curve to the B_2O_3 extremity strongly favours the monotectic relation shown in Fig 1 Uncertainty as to the stability and composition of jeremejevite, and our own failure to synthesize it, has led to the omission of this phase from our diagram

Our extrapolated experimental liquidus curve indicates a much lower solubility of alumina in B_2O_3 than is suggested by pouring-temperature curve determined by Baumann and Moore[2] On the other hand, the solubility of alumina as determined by Foex[7] at 1,200° C is in good agreement with our liquidus Baumann and Moore[2] had also suggested a possible eutectic horizontal at about 200° C on the basis of the blistering of cones rich in B_2O_3 No evidence in support of such a relationship was encountered in this study

This work was carried out under contract No *AF* 33 (616)–6509, sponsored by the Aeronautical Systems Division, Air Research and Development Command, U S Air Force, Wright-Patterson Air Force Base, Ohio

P J M Gielisse
W R Foster

Department of Mineralogy,
The Ohio State University,
Columbus, Ohio

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CHEMISTRY

Structure of Hydrides of Palladium

The recent determinations of activation energies for slow processes in palladium hydride[1,2] confirm new results obtained in this laboratory

The heat capacities of PdH_x recently completed for x = 0 5 and x = 0 75 are shown in Fig 1 along with points for other concentrations[3,4] Between 35° K and 85° K the heat capacity is obviously independent of the hydrogen concentration up to 0 75 H/Pd ratio

Warm drifts in the calorimeter were again noted Fig 2 presents a logarithmic plot of the warm drift versus the reciprocal temperature for the earlier and new data from the slope of which activation energies were obtained[3]

These results, summarized in Table 1, provide clues leading to a model consistent with modern ligand theory which leads directly to the observed zero point entropy of Pd_2H Neutron diffraction investigations place the hydrogen atoms in the octrahedral positions of the palladium lattice at room temperature[5] and down to 20° K (ref 6) In the proposed model applied to the β-phase, these hydrogens form sheets through hydrogen bonding with neighbouring palladium atoms in the lattice A square planar arrangement of hydrogens about the palladium atoms in these sheets is favoured by resonance leading to the W_{aa} term in the Lacher quasi-chemical treatment The sheets can take right angle bends in any direction, but never intersect[7]

Table 1 Activation Energies for Diffusion in PdH_x

Region of drift	x = 0 125	0 25	0 50	0 75
150° K –200° K	2 9 kcal	2 9 kcal	no drift	no drift
200° K –250° K	6 4 kcal	6 4 kcal	no drift	5 5 kcal

Simple numerology shows that at the composition Pd_2H all palladium atoms at the corners of the face-centred cubes are part of sheets, with hydrogen midway between each palladium atom in the sheets and with no hydrogens outside the sheets Above 0 50 H/Pd ratio cross planes of hydrogen and palladium sheets begin to form This explains the sudden change from metallic to non-metallic conduction around 0 50 H/Pd ratio noted by Schindler *et al* [8]

In the α-phase, hydrogen is randomly distributed between corner palladium atoms which are not part

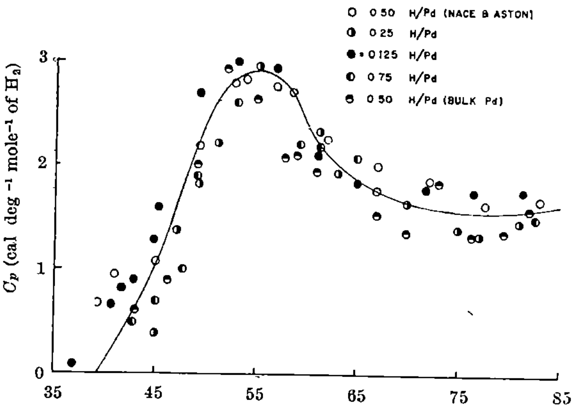

Fig 1 C_p of a mole of hydrogen in block palladium samples of ratio 0 75, 0 50, 0 25 and 0 125 H/Pd and palladium powder sample of 0 50 H/Pd ratio between 35° K and 85° K

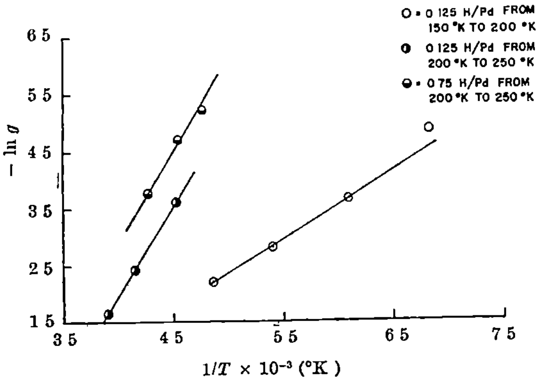

Fig. 2. Plot of reciprocal of T versus ln rate of heat evolution to give activation energies for the three samples

of sheets Thus at low temperatures, due to resonance stabilization of the β-phase, the α-phase has a negligible concentration of hydrogen, as follows from the calculations of Lacher Since resonance favours full occupation of the square planar positions, at low temperatures all vacancies in the sheets will be filled

Below the heat capacity peak at 55° K, PdH_4 molecules librate about an axis through the palladium atom and perpendicular to the fully occupied planes As the temperature increases this libration turns to hindered rotation (ring diffusion) and as rotation starts the potential barrier to rotation decreases (that is, the value of W_{aa} decreases) This is a co-operative type phenomenon and gives the type of heat capacities presented in Fig 1 Since the addition of more hydrogen to the sample merely forms larger sheets the heat capacity effect of the hydrogen is concentration independent

At temperatures where vacancies in the sheets exist, diffusion to neighbouring cells can occur through rotation (ring diffusion) Such a process, leading to filling of vacancies, is the cause of the warm drift starting at 150° K in the 0 125 H/Pd and 0 25 H/Pd compositions On rapid cooling, the system does not reach the equilibrium which favours less vacancies Its slow establishment produces the warm drifts near 150° K with activation energy of 2,900 calories per mole At 0 50 H/Pd ratio and above the sheets of the β-phase are filled and there can be no lack of equilibrium with respect to the filling process By nuclear magnetic resonance methods, valid at equilibrium, Spalthoff[1] measured the activation energy of this neighbouring cell diffusion in a 0 64 H/Pd ratio sample at equilibrium conditions and found an activation energy of 2,400 ± 300 calories

The warm drifts at 200° K and higher are due to long-range diffusion from α- to β-phase or from one sheet to another By rapid cooling the sample cannot establish the true equilibrium which requires less hydrogen in the α-phase The slow return to equilibrium requires the long-range diffusion occurring above 200° K with an activation energy near 6,000 calories The 0 50 H/Pd ratio has no incomplete sheets (no β-phase vacancies) and therefore could not exhibit a warm drift in this region Above 0 50 H/Pd ratio the sheets are cross-linked to form a set of systems of sheets at right angles with no vacancies in any single system Only with the resonance energy thus available can the extra repulsion be overcome

The activation energy for long-range diffusion is the same as the measured activation energy (6–8 kcal) for diffusion of hydrogen through palladium[9], as it should be Rapid cooling produces lack of equilibrium with respect to the size of these units which is slowly restored by the long-range diffusion

At the composition 0 50 H/Pd if certain hydrogens on palladium atoms rotate through $\pi/2$ the sheets suffer a sharp bend by $\pi/2$ Since this rotation can occur at random at equilibrium the situation is equivalent numeralogically to that of ice There are three non-participating face-centred palladium atoms to each corner one Since the species analogous to water is PdH_2 in each corner palladium atom, the basic unit is Pd_4H_2 In keeping with this it was found[10] that the zero point entropy of two Pd_2H units in palladium black is 1 18 ± 0 36 (2 × 0 59 ± 0 18) The theoretical value is 0 82

We thank the National Science Foundation for its financial support in connexion with this work

J G Aston
Paul Mitacek, jun

Cryogenic Laboratory,
College of Chemistry and Physics,
Pennsylvania State University.

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Action of Manganese Dioxide on D-Fructose

Many organic compounds can be selectively oxidized by manganese dioxide at room temperature in non-aqueous media[1,2] High oxidative yields from both primary and secondary αβ-unsaturated alcohols[3] and saturated alcohols[4] have been reported under suitable experimental conditions Little work has been reported on the action of manganese dioxide on carbohydrates Mannitol in aqueous solution has been shown to undergo decarboxylation[5], while recently Bose, Foster, Salim, Stacey and Webber[6] isolated several products from disaccharides treated at 100° for 1 hr with manganese dioxide

The present communication describes the interaction of D-fructose with manganese dioxide at room temperature Since the reaction is heterogeneous, finely ground oxide was used throughout and afterwards all solutions were centrifuged before examination In a typical experiment, D-fructose (1 5 gm) in distilled water (150 ml) was shaken with manganese dioxide (7 5 gm) for 16 5 hr , when $[\alpha]_D$ rose from − 92 5 to − 16 9 No carbon dioxide was evolved during this period The solution was chromatographed in butan-1-ol/acetic acid/water (4 1 5), and spraying with ammoniacal silver nitrate revealed seven spots, together with some streaking, R_F 0 03, 0 09, 0 14, 0 18, 0 23, 0 27 and 0 46 (faint) Potassium permanganate (0 1 N) was decolorized on treatment in the cold, accompanied by precipitation of manganese dioxide The solution gave a positive Schiff reaction

The solution (50 ml) was heated with o-phenylene diamine at 60° for 10 min On cooling the yellow crystals which separated (about 20 mgm) were crystallized from hot water to give 2-(arabino-tetrahydroxybutyl) quinoxaline (I), m p 192°, λ_{max} 237 mμ and 319 mμ (ref 7) Addition of 2 4-dinitrophenylhydrazine solution in 2 *N* hydrochloric acid (200 ml) to a further portion of the solution (80 ml) gave a yellow precipitate in less than 5 min at room temperature Recrystallization from ethanol gave glucose-2 4-dinitrophenylosazone, m p 258° (II) Fructose under similar conditions did not afford these derivatives

Generally, considerable excess of manganese dioxide to substrate (5 to 20 1) is required[2] although Stacey *et al* [6] used equal weights of oxide and disaccharide Optical rotational measurements (Fig 1) indicate that manganese dioxide in excess promotes greater reaction This may be expected owing to the heterogeneous nature of the reaction Exact reproduction in chemical change has not been achieved, possibly due to variation in oxide particle size from one run to another

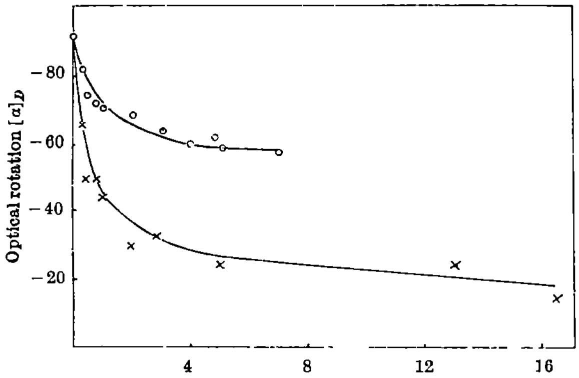

Fig 1 Change in optical rotation of aqueous D-fructose solutions on shaking with manganese dioxide ○, MnO_2/D-fructose, 1 1 (by weight), ×, MnO_2/D-fructose, 5 1 (by weight) 2 dm tube Initial $[\alpha]_D = -92$ 5

None of the solutions showed characteristic absorption spectra between 210 and 400 mμ, indicating the absence of keto or enediol groupings

In another experiment, the residual manganese dioxide from the above 16 5-hr run was resuspended in distilled water (50 ml), centrifuged and dried at 250° The supernatant liquid had zero optical rotation, but gave a positive Schiff test It is therefore possible that some degraded material remained adsorbed on the oxide Fresh fructose solutions could be degraded in similar fashion with this dried manganese dioxide

Manganese dioxide is an efficient, and in some cases selective, reagent for the conversion of primary alcohol groups to aldehydes[4] Such a reaction with the —CH_2OH group at C_1 in D-fructose would lead to 2-oxo-D-arabino-aldohexose The isolation of compounds I and II together with a spot, R_F 0 18, support this view However, chromatographic analysis of even 1, 2 and 3 hr runs indicates that at no time is the mechanism selective The Schiff colour developed immediately with these solutions exhibited a peak at 550 mμ which provided additional evidence for the presence of osone material[8]

Throughout, Hopkin and Williams type manganese dioxide was used (about 90 per cent manganese dioxide together with other oxides of manganese only) Similar patterns, but overall less degradation, were effected using manganese dioxide prepared by pyrolysis of manganese carbonate at 250° (ref 4)

G J Moody

Department of Chemistry and Biology,
Welsh College of Advanced Technology,
Cathays Park,
Cardiff

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Sorption of Dissolved Phosphorus from Sea-water by means of Aluminium Hydroxide

EXPERIMENTS have been conducted in this laboratory on the sorption of dissolved organic matter from sea-water on *in situ* precipitates of ferric and aluminium hydroxides Particular attention was paid to the sorption of dissolved organic phosphorus compounds with the view of facilitating the preservation of total phosphate samples collected on oceanographic cruises It was found that all the dissolved phosphorus in sea-water (both inorganic orthophosphate and organically combined phosphorus) can be removed from solution on a combined precipitate of aluminium and magnesium hydroxides The following procedure is recommended

To 50 ml of sea-water filtered through a 'Millipore *HA*' membrane and dispensed into a centrifuge tube, add 1 ml of 0 3 *N* sodium bicarbonate solution and 0 5 ml of a saturated solution of potassium aluminium sulphate Mix thoroughly and leave for at least 4 hr , then centrifuge in a swing-out head at 300*g* for 5 min Add a further 0 5 ml of potassium aluminium sulphate solution and mix by gentle swirling of the tube, leaving the first precipitate undisturbed After another 4 hr , centrifuge as before Add two drops of 4 *N* ammonium hydroxide to precipitate magnesium hydroxide, mix by swirling, and stand for 2 hr Finally, centrifuge at 600*g* for 5 min , pour off the supernatant, and dry the mouth of the tube with filter paper The precipitate contains all the dissolved phosphorus of the original sample

The total phosphorus content of the precipitate may be estimated by the procedure of Burton and Riley[1] Since there is little or no chloride remaining, it is sufficient to use only 0 4 ml of 70 per cent perchloric acid and 0 2 ml of nitric acid (s g 1 42)

One example of the efficacy of the method is given in Table 1, which shows the recovery of total phos-

Table 1 TOTAL PHOSPHORUS CONTENT OF ALGAL FILTRATE (μGM. PO_4—P/l)

A Direct digestion of filtrate by method of Burton and Riley		*B* Digestion of precipitate by method of Burton and Riley	
63 4		61 4	
61 4		60 8	
60 6	average 62 0	60 4	average 61 6
62 4	*S D* ± 0 88	61 2	*S D* ± 1 04
61 8		61 2	
62 4		63 0	
		63 0	

phorus by the sorption procedure from a filtrate of a mixed marine algal culture. The filtrate was used as an experimental medium in order to test the method at higher phosphorus-levels than were normally encountered in the natural sea-water samples used for the development of the method (10–30 μgm phosphorus/litre). The algal culture was filtered through a 'Millipore *HA*' membrane, and sub-samples of the filtrate taken either for direct digestion by the procedure of Burton and Riley[1] or for treatment by the recommended sorption technique with subsequent digestion of the precipitate. The inorganic orthophosphate content of the filtrate was estimated by a routine acid molybdate–stannous chloride technique[2] and found to be 26 μgm phosphate-phosphorus/l. Therefore more than half the phosphorus in the filtrate was organic.

The technique has the advantage that considerable concentration of low-phosphate water can be obtained, since the precipitate will dissolve in as little as 5 ml of dilute acid. Digestion is simple and rapid, for volumes to be evaporated are small and there is no decrepitation. Reagent blanks are low and there is no 'salt error'. The technique might also be useful for the preliminary isolation of organic phosphorus compounds in sea-water prior to further examination.

Recovery of phosphorus is low and erratic when the precipitation is carried out in wide or shallow vessels, when the precipitate is recovered by filtration or when the precipitate is agitated while in contact with the supernatant. Similarly, some phosphorus remains in the supernatant if the sample is centrifuged too soon. A minimum of 4 hr standing is required for the transition from the initially formed unstable colloidal solution of aluminium oxyhydroxide through the α-hydroxide to the insoluble but stable γ hydroxide. Time of standing is not critical after this initial 4-hr period.

B. Newell

Commonwealth Scientific and Industrial
Research Organization,
Division of Fisheries and Oceanography,
Cronulla, New South Wales

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BIOPHYSICS

Protein in the Nucleus Pulposus of the Intervertebral Disk

The nucleus pulposus of the normal human intervertebral disk consists of collagen fibrils dispersed in a gel of polysaccharide and protein complexes. During ageing collagen fibrils are deposited as a matrix showing increasing orientation with age as seen from X-ray diffraction investigations[1]. In the case of specimens of more than 70 years of age, the normal photograph shows with the diagram of oriented collagen a clearly defined ring of spacing 4.7 Å, which is similar to that associated with a denatured β-protein[1]. The presence of other components in the disk, for example, calcium phosphate, etc., will be discussed in a later publication. It was postulated from consideration of earlier work[1] that the soluble protein molecules normally found in a folded form in the nucleus had unfolded and formed a hydrogen-bonded lattice which gave rise to this spacing.

From this it was thought possible that in certain cases the typical denatured β-protein ring might be found in younger specimens, in consequence an extensive survey was made of disks from all age groups. The results of this survey were based entirely on X-ray diffraction observations (using copper K_α radiation at 40 kVp and 40 m.amp.). The specimens were freeze-dried and exposures were of only 0.5 hr duration in order that the swollen matrix could be preserved. About four samples from each nucleus were examined. The results are summarized in Table 1.

Table 1

Age range (years)	Without β-protein reflexion	With β-protein reflexion	Percentage with β-protein
0–15	15	0	0
16–30	13	3	19
31–45	8	1	11
46–60	2	5	71
61–75	11	15	58
76 and over	0	4	100

Ten per cent below 45 years of age showed the β-protein reflexion, while 65 per cent above 45 years of age showed the β-protein reflexion. About 20 per cent between 16 and 45 years showed the β-protein reflexion, this is of some significance since the age range 0–15 years included a large number of very young disks (less than 3 weeks old). In general the reflexion when it appears in the photograph of the younger disks is weaker and more diffuse, but in aged specimens is sharp and strong. There is a high coefficient of correlation 0.92 between the appearance of the 4.7 Å reflexion and the age of the disk, which suggests that the occurrence of the β-protein ring may be taken as an indication of what has been referred to by other writers[2] as "pathological ageing".

Further preliminary investigations[3] indicate that the incidence of the β-protein reflexion in the nuclei pulposi of prolapsed disks is higher than in normal disks. If further investigations, which are being carried out, confirm this then the prolapsed disks can be regarded as having undergone some form of "pathological ageing". It might be possible, therefore, that the younger disks of Table 1 showing the β-protein ring would have been more susceptible to prolapse than those which did not. On the other hand, the presence of the β-protein phase may be consistent with a greater degree of fibrosis in the nucleus, and if this were so then the disk may be less susceptible to prolapse than one with a lower degree of fibrosis. If, however, some of the increased β-content of the nucleus is associated with the process of prolapse and is not solely inherent in the nucleus before this occurs then it could be that the physical disturbance caused by prolapse may initiate a spontaneous transformation of some of the soluble nuclear protein to an insoluble β-form, a condition somewhat similar to that obtaining in the transformation of soluble to insoluble protein in denaturation (for example soluble → insoluble silk fibroin).

P. R. Blakey
F. Happey

Department of Textile Industries,
Bradford Institute of Technology,
Bradford, 7

A. Naylor
R. L. Turner

Bradford Royal Infirmary

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Measurement of Creep in Keratin Fibres

CREEP characteristics of wool fibres in water have yielded valuable information on the fine structure of keratin[1–4] Creep fluidity at high extensions is also of technological significance[5]

The absence of plastic flow in unmodified fibres (as made evident by the perfect reversibility of extensions greater than 70 per cent) and knowledge of the α–β transformation has led to the use of constant loads throughout creep tests on the assumption that the load per molecular chain remains constant Even under these conditions variations among fibres drawn from the same staple are large with respect to initial extension and rate of creep[6]

We have noted that the occurrence of the well-known stages of the load-extension curve are relatively constant with respect to extension but extremely variable with respect to stress Stress at 30 per cent extension varies between 4 and 7 kgm /mm², two typical examples are set out in Table 1

Table 1 STRESS OF WOOL FIBRFS AT 30 PER CENT EXTENSION (*S*30)

Sample *A* Fibre No	Sample *A* Diameter (μ)	Sample *A* *S* 30	Sample *B* Fibre No	Sample *B* Diameter (μ)	Sample *B* *S* 30
1	22 4	4 60	1	18 2	6 13
2	24 7	4 62	2	21 4	5 56
3	26 0	4 51	3	23 8	5 78
4	21 8	4 75	4	21 0	5 98
5	19 2	5 88	5	20 2	6 31
6	20 3	4 65	6	21 4	6 06
7	22 4	5 36	7	20 1	6 44
8	21 3	4 69	8	19 2	6 02

The application of a constant stress of 6 kgm / mm² would result in an initial extension of 30–50 per cent for sample *A* fibres, whereas sample *B* fibres would give initial extensions of *c* 30 per cent A comparison of these two samples would not involve creep at the same level of extension

It is well known that extension to 30 per cent under specified conditions does not damage wool fibres[7] and by calibrating a fibre in this way it is possible to estimate, with reasonable accuracy, the load required to give any extension Details of an experiment comparing this technique with the constant stress method are given in Table 2

Table 2

Group	Fibre No	*S* 30 kgm mm²	Stress applied kgm mm²	Stress applied as a percentage of *S* 30
1	1	5 54	3 88	70
	2	5 75	4 03	70
	3	6 14	4 30	70
	4	5 57	3 90	70
			mean 4 03	
2	5	5 01	4 03	80 4
	6	5 47	4 03	73 7
	7	5 77	4 03	69 8
	8	4 68	4 03	86 1
			mean 4 03	

Both groups (drawn from the same staple) have the same mean stress applied Group 1 fibres have variable stresses but each stress represents a constant fraction of *S*30, whereas Group 2 fibres have constant stresses bearing no relation to *S*30 The creep curves of these fibres in distilled water at 15 4° C is illustrated in Fig 1

The great reduction in variation of fibres in Group 1 indicates the value of the technique in experiments requiring the comparison of creep properties at a definite extension We have used the method with equal success in the post-yield region for example, by using stresses of 130 per cent *S*30 It appears that investigations of load-extension of fibres to the breaking point will enable accurate prediction of creep properties at any given load in unmodified keratin fibres

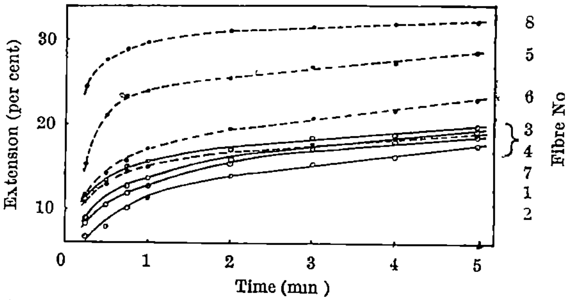

Fig 1 Creep of wool fibres in distilled water at 15 4° C Full lines, fibres under stress equal to 70 per cent *S* 30, broken line, fibres under mean stress of 4 03 kgm /mm.²

E BALASUBRAMANIAM
K J WHITELEY

School of Wool Technology,
University of New South Wales,
Sydney

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BIOCHEMISTRY

Identification of Sugars in Royal Jelly

THE presence of sugars in the nutrients of the honey-bee termed royal jelly has been reported, but the analyses were based on fermentation and reducing power investigations[1,2] or paper chromatography evidence[3,4] The chromatographic evidence indicated the presence of components having the same R_F values as glucose and sucrose in all samples studied, and in certain samples, R_F values corresponding to sucrose, maltose and ribose Because this evidence was not based on an evaluation of physical constants of the crystalline sugars and their derivatives, an investigation was undertaken to isolate and identify precisely the carbohydrate components of this bee larval food

A fresh quantity of royal jelly (100 gm) (kindly supplied by R B Wilson, Inc , 250 Park Avenue, New York, 17) was placed in casing and dialysed in water at 5° C with frequent changing of this solvent, until the dialysate showed a negative Molisch test This required 20 days and about 5 litres of water The dialysate was concentrated (*in vacuo*) at 40° C to a thin syrup and the residual water removed by adding increments of dry ethanol and evaporating the ethanol–water azeotrope under vacuum As the water was removed, the syrup hardened into a plastic solid which, after complete dehydration, was then transferred with 100 ml of ethanol to a Waring blender During the homogenization process which followed, requiring 15 days, a total of 5 litres of dry ethanol, added in 150 ml increments, was used for extracting the sugars from the amorphous product Approximately 15 per cent of the dialytic substance was insoluble in ethanol and may be principally the pteridin compound which has been reported[5]

The alcohol-soluble components, principally sugars, were concentrated (*in vacuo*) at 40° C to a thin syrup and the sugar constituents separated using the method of descending paper partition chromatography[6]. A sample of the syrup (approximately 50 µl.) was placed on the 'starting line' of Whatman No. 3 *MM* chromatography paper (18.25 in. × 22.5 in.) and the sugar components subjected to partition during 33 hr. of irrigation, by the solvent, pyridine/ethyl acetate/water (4:10:3). The paper was dried, and three small strips (1 cm wide) were cut lengthwise from the middle and edges of the paper and sprayed with Tollen's reagent. After heating the strips to hasten the development of 'spots' denoting the exact positions of the separated sugars, the three strips were replaced alongside the rest of the chromatogram and the 'spots' used as indices to deduce the location of the sugars remaining on the unsprayed, main portion of the paper. The sections of paper containing a sugar were cut from the chromatogram and each placed in beakers with water (20 ml.) to elute the sugars from the paper. Nineteen additional fractionations using chromatography paper were effected in this manner.

After combining the related sugar fractions from the twenty chromatograms, the solutions were filtered through glass wool and concentrated (*in vacuo*) to a thin syrup. Three pure sugar solutions were thus obtained from royal jelly and crystalline products were isolated by crystallization (twice) from aqueous methanol (50 per cent v/v).

The sugar corresponding to D-glucose showed a m.p. of 146°–149° C. $[\alpha]_D^{23} = +51.7°$ in water, and an R_F value of 0.509 (in the solvent quoted). An authentic sample of D-glucose showed a m.p. of 147°–149° C., $[\alpha]_D^{23} = +52°$ in water, and an R_F value of 0.509 in the same solvent. The sugar corresponding to D-fructose showed a m.p. of 108°–109° C., $[\alpha]_D^{23} = -86.4°$ in water and an R_F value of 0.547. An authentic sample of D-fructose showed a m.p. of 108°–109° C., $[\alpha]_D^{23} = -92.0°$ in water and an R_F value of 0.546. The sugar corresponding to D-sucrose showed a m.p. of 185°–187° C., $[\alpha]_D^{23} = +65.5°$ in water and an R_F value of 0.358. An authentic sample of D-sucrose showed a m.p. of 186°–187° C., $[\alpha]^{23} = +66.5°$ in water, and a R_F value of 0.358.

To confirm the identity of these sugars the acetate derivatives were prepared following a procedure as described in a recent paper[7], except for a modification of the size of apparatus and proportions of reagents used. Table 1 lists the quantities of reactants used in the present work, the melting points of the acetate derivatives which were isolated, and the melting points of the corresponding reference acetate compounds. The size of apparatus and amounts of wash solutions, etc., used were modified so as to accord with the amounts of reagents used.

Considering the results of these experiments, especially the close agreement between the melting points of the experimental and reference compounds, it is to be concluded that D-glucose, D-fructose, and D-sucrose are components of fresh honey-bee royal jelly. It seems likely that these three sugars in royal jelly are derived from plant nectars, where they are also present as the principal sugars[8], and present in relatively high concentration.

Table 1. TABLE OF VALUES REGARDING THE ACETYLATION OF SUGARS

Reactants	Acetylation of D-Glucose	D-Fructose	D-Sucrose
Weight of sugar (gm.)	0.0178	0.0395	0.0187
Weight of catalyst (gm.)	0.002	0.002	0.002
Volume of acetic anhydride (ml.)	2.00	2.00	3.00
Products			
Melting point of derivative (° C.)	96–97	128–129	72–73
Melting point of derivative lit. value (° C.) (ref. 7)	97	128–129	73.5

It was observed that trace amounts of other substances, possibly sugars, provided 'spots' with Tollen's reagent on developed chromatograms. Although maltose[3] and ribose[4] have been reported to be components of royal jelly, we did not find conclusive evidence of these sugars in the samples with which we worked.

It is to be noted that the royal jelly used for this experiment was obtained from a commercial source and therefore is assumed to be a representative, but only 'average', sample. There may be qualitative and quantitative differences in the carbohydrate components when comparing specific types of larval food, this was found to be the case for lipid components of royal jelly[3].

The quantitative analysis of glucose, fructose, and sucrose in royal jelly has been concluded and soon will be submitted for publication.

I thank Mr. R. Swor for his assistance in carrying out part of this work. This investigation was supported in part by grant *RG*-7233 of the U.S. Public Health Service.

G. M. CHRISTENSEN

University of Minnesota, Duluth,
Duluth, Minnesota

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Biosynthesis of Royal Jelly Acid from Sucrose

THE relatively large amounts of royal jelly acid (10-hydroxydec-2-enoic), about 8–9 per cent in lyophilized royal jelly, invites speculation as to its biosynthesis. Recently, Law and Weaver[1] reported on experiments using isotopically labelled compounds in an effort to demonstrate formation of hydroxy fatty acids in worker bees. Sodium acetate-1-^{14}C and sodium stearate-1-^{14}C were fed to worker bees and also included in an incubation medium with excised mandibular glands. In both cases considerable radioactivity was detected in lipid extracts of the mandibular glands, but the hydroxy acid fraction did not become labelled.

We now report on our experiments involving the feeding of uniformly labelled sucrose. About 1.5 lb. of worker honey bees were deprived of their queen, confined on two frames of honey and pollen and forced to rear queen cells. Sucrose, uniformly labelled with carbon-14 was fed to the bees immediately on confinement. In the first test 10 µc. of sucrose labelled with carbon-14 was diluted in 70 ml. of 1:1 sucrose water syrup and placed in two feeder bottles. The solution was consumed in about 5 hr. In the second and third tests the dosage of labelled sucrose was doubled and fed in the same way.

Five hours after confinement thirteen grafted queen cells were given to the confined bees. Royal jelly was collected at the end of 1, 2 or 3 days and the cells immediately re-grafted and returned to the bees Each group of bees was used for 6–7 days after which time the confined bees became distressed to the point that no further queen rearing was possible Two collections of jelly at 3-day intervals were found to give an optimum yield Radioactivity in the jelly did not decrease up to 7 days after feeding A total of 8 8 gm (fresh weight) of labelled royal jelly was produced

A sample of each collection of royal jelly was dried and checked for radioactivity on a Nuclear-Chicago model *D*47 gas-flow detector The acids from the royal jelly were isolated and separated chromatographically by the method of Brown and Freure[2]. The chromatograms were checked for radioactivity Only the spot corresponding to 10-hydroxydec-2-enoic acid (R_F 0 70) was found to be active The spot was eluted and a total of 80 mgm of radioactive royal jelly acid was recovered from 6 gm of whole royal jelly The recovered acid was shown to be identical with an authentic sample of inactive royal jelly acid by comparison of infra-red absorption spectra

W H Brown
Ethel E Felauer

Department of Chemistry,

M V Smith

Department of Apiculture,
Ontario Agricultural College,
Guelph, Ontario

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Variations in the Blood Saccharoid Fraction in Diabetes

Recent reports have stressed the advantages of using highly specific 'glucose oxidase' methods for the clinical determinations of blood sugars[1–3] Traditional methods, such as those of Folin and Wu measure the total reducing capacity of the blood, and include the so-called saccharoid fraction as well as the true glucose-level The exact nature of this fraction and its significance in pathological conditions, is not yet fully understood

Clinical investigations have suggested that in a variety of conditions affecting blood sugar there is little correlation between blood glucose and the saccharoid fraction, and that this latter fraction is erratic and unpredictable during glucose tolerance tests Saifer and Gerstenfeld[1] believe that this fraction is significantly increased in diabetic patients, and Marks[2] has shown a reduction in the saccharoid fraction following insulin therapy, although the fall is delayed and erratic compared with that of the true glucose fraction Similar clinical studies are being carried out in this department, together with corresponding determinations on normal, alloxan-diabetic and insulin-treated alloxan-diabetic rats Preliminary results of this latter investigation are shown in Table 1 and Fig 1 Diabetic rats had been treated 3–4 months previously with 180 mgm alloxan per kgm body-weight (2 per cent solution, citrate-phosphate buffer, *p*H 4) and had blood sugars greater than 200 mgm per cent (glucose oxidase). Table 1 shows that the true glucose-level increases from a mean of 79 ± 2 61 to a mean of 253 ± 17 8 This represents an increase of 3 2 times. The saccharoid fraction (obtained by subtraction) shows a parallel increase, although the factor is somewhat larger (4 0 times) When diabetic rats are treated with insulin (100 units/kgm) this trend is reversed as shown in Fig 1 It would appear that in the case of the alloxan-diabetic and insulin-treated rat, corresponding changes occur in both the true glucose fraction and the saccharoid fraction It is of interest that in the rat approximately half the value obtained by the Folin–Wu method of estimation is due to substances other than glucose This is higher than the corresponding fraction in the human, where they represent approximately 25 per cent of the Folin–Wu value It appears that there may be a marked and characteristic species difference and it is interesting that Hernandez and Coulson found no evidence of a saccharoid fraction in the alligator[4]

Table 1 Blood Glucose-Levels of Normal and Diabetic Rats measured by Folin–Wu and Glucose Oxidase Methods
The values are the mean of 9 normal and 6 diabetic animals

	Folin–Wu (mgm per cent)	Glucose oxidase (mgm per cent)	Saccharoid fraction (by difference)
Normal rat	136 9 ± 4 7	79 4 ± 2 6	57 4 ± 3 8
Diabetic rat	484 0 ± 48	253 3 ± 17	233 0 ± 30

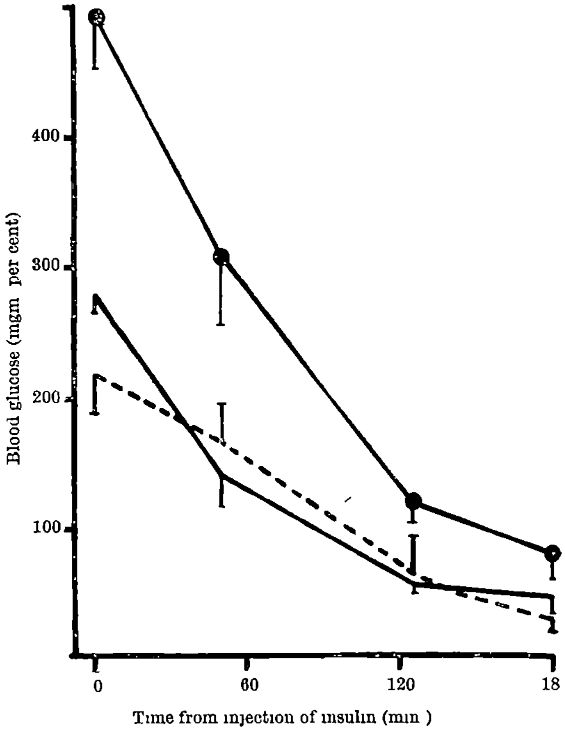

Fig 1 Glucose-levels in blood of alloxan-diabetic rats following the injection of insulin Each point represents the mean of three animals The vertical lines indicate the *S E* ●—●, Folin–Wu, —, glucose oxidase, – – –, 'saccharoid fraction' (difference)

These results emphasize the importance of standardizing methods of glucose estimation, not only clinically but also in experimental animal investigations

C N Graymore
M J Towlson

Department of Pathology,
Institute of Ophthalmology,
Judd Street
London, W C 1

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Crystalline Œstrone isolated from Urine of the Laying Hen

Although œstriol, œstrone and œstradiol have been detected in mixed avian excreta[1] and in extracts of ovaries of laying hens[2] by chromatographic methods, it would appear that only œstradiol-17β has been isolated hitherto from avian material in crystalline form and identified by infra-red spectrophotometry[3]. We have now secured similar evidence for the presence of œstrone in avian urine.

Approximately 30 l. urine (equivalent to 128 bird-days) was obtained from a laying hen furnished with exteriorized urethral orifices by the method of Dixon and Wilkinson[4]. Œstrogen extracts of the acid-hydrolysed urine were prepared by the method of Bauld and Greenway[5], and the lipophilic fraction was chromatographed on paper in a chloroform–formamide system[6]. The presumptive œstrone zones were cut out and eluted with methanol/dichloromethane (1 : 1 v/v). The solvent was removed and the residue dissolved in a mixture of benzene and carbon tetrachloride (1 : 2 by vol.) and purified by passage through a column of Woelm alumina as described by Ittrich[7]. After removal of the solvent the residue was crystallized twice from aqueous ethanol. The final yield was approximately 0·25 mgm.

The sample was dissolved in acetone and the solution evaporated on powdered potassium bromide, which was then ground by hand and pressed into a disk. A sample of reference œstrone (Steroid Laboratories, Montreal) was similarly prepared. Spectrophotometry was performed with a Perkin–Elmer model 21 spectrophotometer fitted with a sodium chloride prism.

The infra-red absorption spectra of the presumptive œstrone from hen's urine and of the reference œstrone are shown in Fig. 1. There were differences in the region of —OH stretching bands, but these were probably a consequence of differences in crystallinity or water absorptivity as between the samples. There was excellent agreement between the two spectra for all points below 2,000 cm.$^{-1}$. Accordingly it can be concluded that the crystalline material isolated from hen's urine was identical with the reference œstrone.

This work was supported by a grant-in-aid of research (No. *BT*-56) from the National Research Council of Canada.

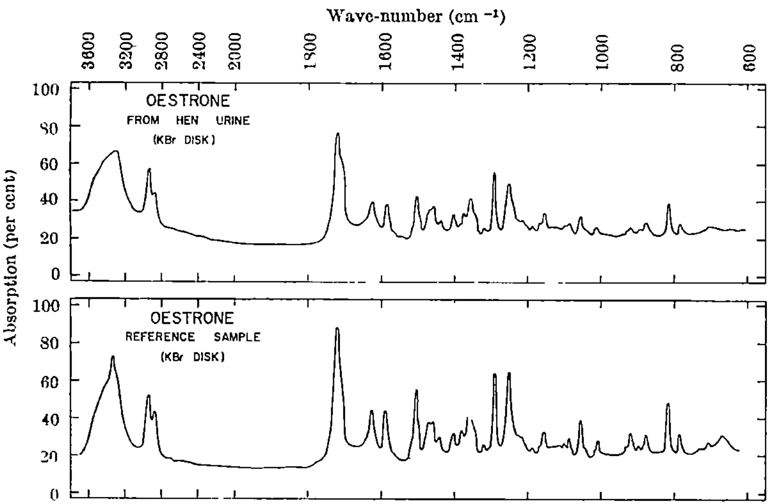

Fig. 1. Upper curve: Infra-red absorption spectrum of crystalline œstrone isolated from urine of laying hen. Lower curve: Infra-red absorption spectrum of reference œstrone

We thank Dr. R. Norman Jones and Mr. R. Lauzon, National Research Council of Canada, Ottawa, for determining the infra-red absorption spectra on which the identification of the crystalline œstrone from hen's urine was based.

L. Ainsworth
R. H. Common

Department of Agricultural Chemistry,
Macdonald College,
McGill University,
P.Q.

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Effects of Administration of Oxytocin and Prolactin on Nucleic Acids and Phosphoprotein Contents of Mammary Glands in Lactating Rats

Benson and Folley[1] have demonstrated from the histological point of view that the injection of oxytocin as well as prolactin into lactating rats after removal of their litters showed a marked retarding effect on the involution of mammary gland tissue, and this finding was confirmed by us[2] and others[3,4]. In previous experiments[2,5], however, we failed to see the effects on the *in vitro* respiratory activity of the mammary gland tissue.

Contents of deoxyribonucleic acid (DNA) of the mammary gland under various conditions have been estimated by many workers[6–9], and are considered as an indicator of the quantity of parenchymal tissue in the gland. Amounts of ribonucleic acid (RNA) and RNA : DNA ratio in the lactating mammary gland appear to be closely related to the rate of milk-protein synthesis taking place in the gland. In addition to these, amounts of phosphoprotein contained in the gland can be regarded as a conventional scale of milk accumulation in the gland[9], since phosphoprotein found in the gland is mainly derived from casein.

It was considered of interest, therefore, to reinvestigate the problem by using the determinations of nucleic acids and phosphoprotein in the gland.

Thirty-nine Wistar strain female rats in first lactation, weighing 170–220 gm., were used. Litters were reduced to six on the first day of lactation, three of each sex being kept whenever possible. They were weaned at the stage of full lactation (on eleventh–thirteenth day of lactation). Hormonal treatment was started from the afternoon of the weaning day and continued for 3 days. Oxytocin ('Syntocinon', Sandoz, Ltd., Basle, 1·0 and 2·0 i.u. in 0·5 ml. of

Table 1 EFFECTS OF ADMINISTRATION OF OXYTOCIN AND PROLACTIN ON THE CONTENTS OF NUCLEIC ACIDS AND PHOSPHOPROTEIN OF MAMMARY GLANDS OF LACTATING RATS AFTER DEPRIVATION OF LITTERS ($\pm S E$)

Experimental group	No of rats	Total posterior mammary gland gm /100 gm body-wt	DNA-P mgm /gland/100 gm body-wt	RNA-P mgm /gland/100 gm body-wt	$\frac{\text{RNA-P}}{\text{DNA-P}}$	Phosphoprotein-P mgm /gland/ 100 gm body-wt
Normal lactating control	6	2 89±0 16	0 92±0 03	3 05±0 17	3 33±0 10	0 54±0 05
Weaning control	7	1 81±0 10	0 38±0 02	0 42±0 03	1 13±0 08	0 45±0 05
Oxytocin* 1 I U × 3/day	4	1 83±0 10	0 48±0 02§	0 54±0 06	1 16±0 07	0 43±0 09
Oxytocin* 2 I U × 3/day	5	1 92±0 13	0 52±0 03‡	0 42±0 02	0 82±0 04‡	0 36±0 03
Prolactin† 5 I U × 2/day	5	3 12±0 36§	0 55±0 09	0 96±0 19	1 76±0 16‡	1 02±0 23
Prolactin† 25 I U × 2/day	5	3 03±0 30§	0 59±0 08	0 94±0 07	1 69±0 21§	1 09±0 24
Prolactin† 50 I U. × 2/day	5	3 73±0 16‡	0 83±0 05‡	1 16±0 10	1 39±0 06§	1 51±0 13‡

* 'Syntocinon', Sandoz, Ltd , Basle '

† 'Luteotrophin', Squibb and Sons, Ltd , New York

‡ The values are significantly different from those of the weaning control group ($P < 0\ 01$)

§ The values are significantly different from those of the weaning control group ($0\ 01 < P < 0\ 05$)

distilled water) was injected intraperitoneally three times daily and prolactin ('Luteotrophin', Squibb and Sons, Ltd , New York, 5, 25 and 50 I U in 0 5 ml of distilled water) was injected intramuscularly twice daily Seven rats, injected with 0 5 ml of distilled water intraperitoneally three times daily for 3 days after the weaning and six rats on the fifteenth day of lactation, were served as the weaning and the normal lactating controls respectively 30–60 min after the last injection, rats were killed by decapitation Six posterior mammary glands were quickly removed from the skin and stored in deep-freeze after weighing

DNA, RNA and phosphoprotein in the mammary gland were extracted and fractionated according to the method of Schneider–Schmidt–Thannhauser[10] with the Delory's treatment[11] Perchloric acid was used as a protein-precipitating and nucleic acid-extracting agent instead of trichloroacetic acid Nakamura's modification[12] of Allen's method[13] was used in the determination of phosphorus contents of nucleic acids and phosphoprotein The results obtained were expressed as DNA–P, RNA–P and phosphoprotein–P respectively in terms of mgm per total posterior mammary gland which was checked by body-weight of the animals (Table 1)

Administration of prolactin effectively retarded the involution of the mammary gland which was caused by the removal of litters in lactating rats Considerably large amounts of DNA–P and RNA–P remained in the glands of rats treated with prolactin even at the dosage-level as low as 10 I U a day, while only small amounts of them were found in the glands of rats which received only water In the largest dose group (100 I U /day of prolactin), structural integrity of the glands which was expressed by the DNA–P contents seemed to keep the level of normal lactation Well-maintained DNA–P contents of the glands in the prolactin-injected group agreed well with the observations of previous workers obtained from the histological point of view[1,2,14–16]

Maintaining the high DNA–P/RNA–P ratio and the relatively large amounts of phosphoprotein by the administration of prolactin seems to suggest that protein synthesis is still continued in the mammary gland under experimental conditions It is of interest to compare these results with those of previous experiments showing the immediate cessation of fat synthesis after the removal of litters despite the same hormonal treatment, although an essential difference between the biochemical significance of the RNA/DNA ratio and that of the respiratory-quotient value as an indicator of synthetic activity of the mammary gland should be taken into consideration It may be suspected that fat synthesis in the mammary gland is damaged more than the protein synthesis in the same gland by the ill-effect of engorgement

Daily administration of oxytocin retarded the depression of DNA–P content significantly, but the RNA–P/DNA–P ratio of oxytocin-treated groups was almost equal to or lower than that of weaning control group

It can be deduced from these results that the effect of oxytocin on the involution of mammary glands is not similar to that of prolactin Oxytocin administered to the lactating rats after the removal of litters affects only the structural integrity of the gland tissue, while prolactin administered in the same way, at least in the doses used, maintains both structural integrity and metabolic activity of the tissue

Amounts of PP–P found in the mammary gland of oxytocin-treated groups were less than those of any other experimental groups These results seem to indicate the accelerated re-absorption of accumulated milk[17–19] which is expelled from the mammary alveoli into the mammary ducts by the milk-ejecting action of oxytocin

We thank Dr R B Edwards (Squibb and Sons, Ltd , New York) for a supply of prolactin preparation ('Luteotrophin', Squibb) and Drs R W Zwicky and H Ogura (Sandoz, Ltd , Tokyo) for oxytocin preparation ('Syntocinon', Sandoz) This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Japan

K ÔTA
A YOKOYAMA
Y SHINDE

Faculty of Agriculture,
Nagoya University,
Japan

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Developmental Changes in the Plasma Protein Pattern of the Mouse

Knowledge of the changes in the pattern of plasma proteins during the development of the mouse is desirable not only from the embryological but also from the genetical point of view, because of the existence of many inbred lines of this versatile laboratory animal.

Smithies's technique of starch-gel electrophoresis[1] with Poulik's discontinuous buffer system[2] was applied to blood plasma from strain *A* mice. Each sample was carried by a small strip of filter paper inserted in a slit in the starch-gel. This procedure apparently immobilizes the γ-globulins[1], so that the observations that follow do not concern this fraction.

Plasma from adult females at various stages of pregnancy gave twelve fractions in the regions under consideration (Fig. 1, 1).

The earliest embryos from which blood could be reliably collected were aged 12 days from conception. Many of the fractions of the adult are already present (Fig. 1, 2) but certain interesting differences can be noted. First, fraction 1 (albumin) is present in the early embryos at a very low concentration.

Secondly, fractions 2 and 3 of the adult are absent; but there is a second fraction slower than both the above, and by far stronger than albumin.

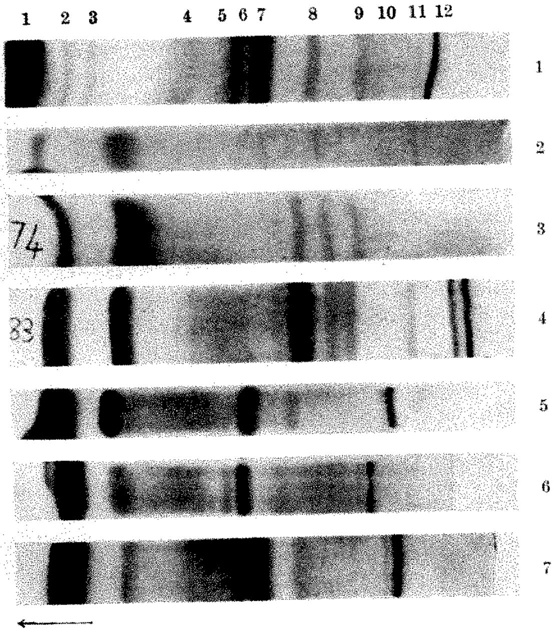

Fig. 1. Starch-gel electrophoresis pattern of plasma proteins of mouse. 1, 15-day pregnant female; 2, 12-day fœtus; 3, 15-day fœtus; 4, 19-day fœtus; 5, new-born; 6, 12-day-old young; 7, 18-day-old young

Thirdly, all other fractions are found in a concentration that is both low and about equal for all, that is, the bands in the transferrin region and the α_2-globulin are not stronger than the others, as in the adult.

The second fraction is still the strongest in the 15-day fœtus (Fig. 1, 3), but by the latter days of intrauterine life (Fig. 1, 4) and in the new born (Fig. 1, 5) albumin equals it in concentration, to exceed it by the twelfth day after birth (Fig. 1, 6).

The single second fraction persists after birth in the young, providing a contrast with the two fractions (2 and 3) of their mothers as tested during pregnancy.

Finally, the transferrin and slow α_2-globulin bands (6, 7, 12) do not reach their typical prominence over other fractions until a few days before birth (Fig. 1, 4).

E. M. Pantelouris
Patricia A. Hale

Department of Zoology,
Queen's University,
Belfast.

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α-Galactosidase Activity of Rumen Bacteria

In a recent search for bacterial α-galactosidases, Carrere, Lambin and Courtois[1] tested many organisms isolated from the intestinal tracts of a wide range of animals including several ruminants. Only one bacterial species, from the guinea pig, containing α-galactosidase was obtained. The authors commented on the apparent rarity of this enzyme in bacteria, although it is fairly widespread in yeasts and plant tissues[2]. Bacteria producing α-galactosidase might be expected in rumen micro-flora, particularly in animals grazing pasture, as small amounts of oligo- and poly-saccharides containing α-linked galactose are present in many pasture plants. In addition the leaves of plants such as red clover (*Trifolium pratense*) contain up to 1 per cent (dry wt. basis) of lipid bound galactose[3], present either as monosaccharide or 6-*O*-α-D-galactopyranosyl-D-galactose glycosidically joined through a β-link to the lipid glycerol[4]. The ability of rumen bacteria to release this α- and β-linked galactose has not been demonstrated. One rumen organism, *Streptococcus bovis*, appears to be a potential producer of α-galactosidase, as an extract from cells of one strain of this species has been shown able to hydrolyse melibiose[5]. The action of cell extracts from mixed rumen bacteria and *S. bovis* on various compounds containing α-linked galactose has, therefore, been investigated.

Mixed rumen bacteria, freed from protozoa and plant debris by repeated centrifuging, were prepared from the rumen contents of a cow feeding on fresh red clover. *S. bovis* cells (strain I)[6] were collected from cultures grown in a medium[6] containing glucose (2 per cent w/v) as sole carbohydrate. Bacterial cell extracts (25 ml. from 1–2 gm. wet wt. of cells) were prepared in acetate buffer (0·05 *M*, *p*H 6·0) and tested for carbohydrase activity by the procedures described previously[5], all incubations being done at 39° C. Total plant lipids were extracted from fresh cut red clover leaves with boiling 80 per cent (v/v) aqueous ethanol and, after removal of the ethanol, freed from sugars by suspending in water and centrifuging at high speed. Galactose was the only sugar detected after hydrolysis of the lipids in *N*-sulphuric acid for 1 hr. at 100° C. Approximately one-third of the plant lipid galactose in clover lipids is present as the disaccharide[3]. Mono- and di-galactosyl glycerol, prepared from the lipids by alkaline hydrolysis and column chromatography, were supplied by Mr. R. O. Weenink, Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington.

Extracts from the mixed rumen bacteria readily hydrolysed sucrose, maltose, cellobiose, lactose, salicin and mono-galactosyl glycerol. Melibiose and methyl α-D-galactoside were also hydrolysed, but at a much slower rate. With raffinose and stachyose the rapid release of fructose, producing melibiose and manninotriose respectively, was followed by a much slower release of galactose. The di-galactosyl glycerol was also hydrolysed ultimately to galactose and glycerol. Although these results confirm the suggestion that rumen bacteria may serve as a source of α-galactosidase the extracts from the mixed organisms contain too many other carbohydrases to be of any value as an α-galactosidase preparation.

The extracts from the *S. bovis* cells gave a strikingly different pattern of hydrolysis. The following compounds were not hydrolysed: isomaltose, gentiobiose, maltose, cellobiose, lactose, nigerose, turanose, sophorose, α-α-trehalose, sucrose, methyl-α-D-glucoside, salicin, arbutin, rutin, amygdalin and mono-galactosyl glycerol. Melibiose and methyl-α-D-galactoside were, however, readily hydrolysed and galactose was rapidly liberated from di-galactosyl glycerol, raffinose, stachyose and verbascose. With these latter compounds mono-galactosyl glycerol and sucrose, in addition to galactose, were detected as end products of hydrolysis. Raffinose and stachyose could be detected during intermediate stages in the hydrolysis of stachyose and verbascose respectively. It is evident that at least one common, easily cultured, species of rumen bacteria is able to produce an α-galactosidase free from other disaccharidases, particularly invertase and β-galactosidase. Previously[5], when the organism was grown in a sucrose medium to produce dextran, the cell extracts contained invertase and isomaltase, as well as the α-galactosidase.

None of the cell extracts from either source liberated galactose from the plant lipid preparations. When, however, mixed rumen bacteria were incubated, under toluene, with the lipids galactose was liberated. This did not occur with *S. bovis* cells. It is possible that lipolysis must free the mono- and di-galactosyl glycerols before they can be hydrolysed and that *S. bovis* does not contain any lipase.

R. W. Bailey

Plant Chemistry Division,
Department of Scientific and Industrial Research,
Palmerston North, New Zealand.

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ε-Lysine Acylase in Bacteria

Although a number of reports have been published on α-amino acylase, little is known about ε-lysine acylase, which hydrolyses ε-*N*-acyllysine. Paik *et al.*[1] first confirmed the activity in rat tissues, and Kameda *et al.*[2] and Wada[3] reported the activity in a micro-organism of *Pseudomonas*. The activities of the enzymes in these reports were very low and the enzymes were not obtained as a pure protein.

We also investigated the distribution of ε-lysine acylase in various micro-organisms in order to obtain a suitable enzyme source for the optical resolution of ε-*N*-benzoyl-DL-lysine, and previously showed that the enzyme is inducibly formed in several moulds, especially in *Aspergillus oryzae*[4]. This mould enzyme, however, showed no optical specificity on the hydrolysis of ε-*N*-acyl-DL-lysine[5].

Investigation has been continued further, and a micro-organism isolated from the soil collected at Nakamura, Kochi Prefecture, has been found to produce very high ε-lysine acylase activity, and purification of this enzyme has been attained. This micro-organism was identified as being *Achromobacter pestifer* by Dr. H. Iizuka, the Institute of Applied Microbiology, University of Tokyo. The type culture of *Achromobacter pestifer* in the Institute, however, shows very low activity, about 1/2,000 of that of the isolated strain. So to make a distinction, the strain isolated by us is named here *Achromobacter pestifer EA*.

In order to obtain the pure enzyme preparation of ε-lysine acylase from the culture of *A. pestifer EA*, the micro-organism was cultured under aerobic condition in a jar-fermenter at 30° C. for 24 hr. The medium used had the following composition: glucose 5 per cent, peptone 1 per cent, potassium biphosphate 0·2 per cent and magnesium sulphate 0·1 per cent, and the *p*H was adjusted to 7·0.

The cells collected by centrifugation were autolysed with the addition of toluene and extracted with *M*/15 sodium sulphate solution. The extract was purified by the following procedures: the first ammonium sulphate fractionation (45–70 per cent saturation), 0·3 per cent rivanol treatment, acetone fractionation (45–60 per cent volume), the second ammonium sulphate fractionation (48–60 per cent saturation), and fractionation with vertical zone electrophoresis. The electrophoresis was carried out on a methanolysed cellulose powder with a veronal buffer of 0·0505 ionic strength, *p*H 8·8, in the apparatus designed by Chiba *et al.*[6], which enables extensive electrophoresis without electro-osmosis. A potential of 300 V. (20 m.amp) was applied for 160 hr. at 6° C. On completion of the run, the column was eluted with the same buffer as described here. Enzyme activity and protein concentration of the respective fractions collected in test-tubes were measured. As shown in Fig. 1, the enzyme activity only occurs at the main peak of the protein concentration curve, and the specific enzyme activities of fractions No. 26–34 are coincidental, that is, 15,000 lysine μmoles/hr./mgm. of protein are obtained through the fractions, when ε-*N*-benzoyl-L-lysine is used as the substrate. Accordingly, it is obvious that these fractions consist of a single enzyme protein, and this is first the isolation of ε-lysine acylase in pure form.

By comparing ε-lysine acylase activities of various enzyme sources, as shown in Table 1, it is apparent that the activity of *A. pestifer EA* is the highest ever reported.

Table 1. ε-LYSINE ACYLASE ACTIVITY OF VARIOUS ENZYME SOURCES

Enzyme sources	Enzyme activity* Crude extract	Purified preparation	Optimum *p*H	Ref.
Rat kidney	0·023	2·59	7·0	(1)
Pseudomonas (*KT*83)	0·32	1,190	6·0	(3)
Aspergillus oryzae No. 10	2·90	31·6	8·2 ~ 8·4	(5)
Achromobacter pestifer EA	240	15,000	5·0	(†)

* Liberated lysine micromoles/hr./mgm. of protein.
† Present communication.

With this pure preparation the enzymatic properties of bacterial ε-lysine acylase were investigated. In the absorption of ultra-violet light the enzyme showed $E^{1\%}_{1\text{cm.}}$ (280 mμ) 12·00 and $E_{280/260}$ 1·84. The *p*H optimum was 5·0 in acetate or veronal buffers. The activation energy was 9,280 cal.

The enzyme was inhibited by heavy metal ions, Ag^+, Hg^+ and Hg^{++}, and by SH-inhibitors such as monoiodoacetic acid, ω-chloroacetophenone and *p*-chloromercuribenzoate. Since the inhibition due to the last-mentioned agent was completely overcome by

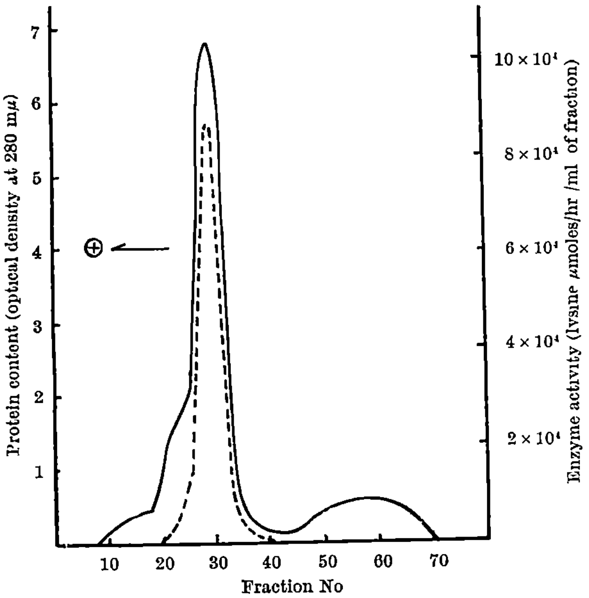

Fig 1 Zone electrophoresis The protein content of each fraction was calculated from the optical density at 280 mμ The enzyme activity of each fraction was measured as follows a mixture of 0 5 ml of $M/10$ acetate buffer (pH 5 0), 0 5 ml of 0 012 M ε-N-benzoyl-L-lysine solution and 0 5 ml of appropriately diluted fraction was incubated at 42° C for 10 min , and the liberated lysine was measured by Work's acidic colorimetric ninhydrin method ——, Protein content, – – –, enzyme activity

the addition of cysteine, the enzyme is considered to be an SH-enzyme

The influence of various di- and tri-valent metal ions on the activity of the enzyme was also investigated But none of the metal ions tested enhanced the activity, as observed in Co^{++} in the case of α-amino acylase With regard to the influence of various metal chelating agents, although the inhibition by ethylenediamine tetraacetic acid and citrate was very slight, addition of oxalate or pyrophosphate inhibited considerably the activity of the enzyme However, the enzyme solution inactivated by the presence of the chelating agents was completely restored to activity after dialysis towards the buffer solution containing no chelating agents

In order to clarify the specificity of the enzyme, various N-acyl derivatives of lysine and its analogues were synthesized, and the susceptibility towards this bacterial ε-lysine acylase was investigated The enzyme shows optical specificity and ε-N-benzoyl-L-lysine is readily hydrolysed, although D-isomer is not hydrolysed The compounds containing no α-amino group, such as ε-N-acylaminocaproic acids and ε-N-benzoylamino-α-halogenocaproic acids, also α-N-acyl and α,ε-N-diacyl derivatives of lysine, are not susceptible to the enzyme The compounds having no free carboxyl group, such as monobenzoylcadaverine, ester and amide derivatives of ε-N-benzoyl-L-lysine, are not hydrolysed by this enzyme

The above results suggest that a free α-amino group and a free carboxyl group are required for the substrate to be hydrolysed by this bacterial enzyme As the homologues of ε-benzoyllysine, δ-N-benzoyl-L-ornithine and ζ-N-benzoyl-DL-homolysine are unsusceptible, the carbon chain of the substrate also shows a significant effect on this enzyme reaction Accordingly, the bacterial ε-lysine acylase has a higher substrate specificity than α-amino acylase, and susceptible substrate is limited to ε-N-acyllysines Among the ε-N-acyllysines, the hydrolytic rate decreases in the order benzoyl > monochloroacetyl > acetyl

As the asymmetric hydrolytic activity of this bacterial enzyme is very high, the enzyme produced by *A pestifer EA* can be used for the optical resolution of ε-N-acyl-DL-lysine

ICHIRO CHIBATA
TSUTOMU ISHIKAWA
TETSUYA TOSA

Osaka Research Laboratory,
Tanabe Seiyaku Co , Ltd ,
Kashima-cho. Higashiyodogawa-ku,
Osaka

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Biosynthesis of Valine and Isoleucine in the Wheat Plant

As valine and isoleucine are essential amino-acids for animal organism, the mechanism of their biosynthesis in higher plants is of importance Experiments carried out with micro-organisms have shown that the two final steps in biosynthesis of valine and isoleucine are based on the dehydration of their α,β-dihydroxy-analogues to α-keto-analogues, which are further metabolized by transamination[1]

Our experiments on the biosynthesis of valine and isoleucine were carried out with ripening wheat ears, green wheat seedlings, seedlings of other plants and homogenates of green seedlings Keto-analogues of valine and isoleucine (α-keto-isovaleric and α-keto-β-methyl-n-valeric acids respectively) were synthesized in the form of sodium salts by the methods described by one of us[2,3] Quantitative analysis of amino-acids was carried out by paper chromatography technique[4 5] Asparagine was determined by acid hydrolysis followed by quantitative chromatography of the resulting aspartic acid[6] The keto-analogue of isoleucine was determined by quantitative paper chromatography of its 2,4-dinitrophenylhydrazone A mixture of *tert* butanol/ethanol/water (50 10 50) was used as solvent[6]

We have shown[7] that the introduction of the keto-analogues of valine and isoleucine (0 05 M solutions) into ripening wheat ears through the transpiration current of the stem results in accumulation (in 10 hr) of considerable quantities of valine and isoleucine respectively The synthesis of valine and isoleucine is accompanied by the decrease in glutamic acid, alanine and to some extent glutamine

Acid ammonium phosphate introduced into wheat ears exerted no stimulating effect on the synthesis of valine and isoleucine from their keto-analogues but played the part of some 'preserving factor' of the amino-acids already mentioned and glutamine On introduction of these keto-analogues, accumulation of γ-aminobutyric acid was observed

The keto-analogue of valine being introduced into green wheat seedlings was readily transformed into valine[8] The synthesis of γ-aminobutyric acid also probably plays some important part in the nitrogen metabolism of plants[9] Here, the synthesis of valine was accompanied by decrease in more sets of different amino-acids than in the case of the synthesis of valine from its keto-analogue in wheat ears (decrease in glutamic acid, serine, glutamine, asparagine, alanine and isoleucine has been observed) The rate of the synthesis of valine from its keto-analogue in the dark was as high as in the light As in the case of the experiments with wheat ears, introduction of acid ammonium phosphate exerted no stimulating effect on the synthesis of valine from its keto-analogue, but it showed 'preserving activity' on the amino-acids and amides already mentioned

The results obtained show that the processes of biosynthesis of valine and isoleucine from their keto-analogues in ripening wheat ears as well as in green wheat seedlings are brought by transamination of these keto-acids with different amino-acids and amides

It has been shown that the keto- and dihydroxy-analogues of isoleucine introduced into green wheat seedlings[6] by vacuum infiltration are readily transformed into isoleucine The synthesis of isoleucine is accompanied by decrease in several amino-acids (glutamic and aspartic acids, alanine, serine and glutamine) present in the seedlings These observations indicate the contribution of transamination reaction to the biosynthesis of isoleucine Acid ammonium phosphate had no stimulative effect on the biosynthesis of isoleucine Infiltration into the plant of the dihydroxy-analogue of isoleucine brought about the synthesis not only of isoleucine, but also of some other amino-acids (phenylalanine, γ-amino-butyric acid threonine) The substrates being introduced into the green plant undergo conversion not only into the compounds of which they are the direct precursors, but also into other different compounds which are products of their metabolic transformation Identical results were obtained by Kretovich and Uspenskaya[4,5], in their work on the biosynthesis in plants of phenylalanine from phenylpyruvate

Table 1 FORMATION OF THE KETO-ANALOGUE OF ISOLEUCINE (α-KETO-β-METHYL-*n*-VALERIC ACID) FROM THE DIHYDROXY-ANALOGUE (α,β-DIHYDROXY-β-METHYL-*n*-VALERIC ACID) IN HOMOGENATES FROM WHEAT SEEDLINGS

No	Final *p*H	Total nitrogen in sample (mgm)	α,β-Dihydroxy-analogue of isoleucine added	α-β-Dihydroxy-analogue of isoleucine + $(NH_4)_2HPO_4$ added
1	7 7	9 1	15 5	14 9
2	7 1	11 9	7 8	10 8
3	7 5	15 5	12 0	13 5

5 0 ml of homogenate from wheat seedlings in 0 067 *M* phosphate buffer incubated with different substrates (final concentration 0 025 *M*) for 5 hr at 30° Keto-analogue of isoleucine, μmoles/5 0 ml (125 μmoles of substrates)

In homogenates from green wheat seedlings the synthesis of isoleucine was weak and was possible only from its keto-analogue Thus the destruction of plant cell structure decreased the rate of transamination processes, in the case when the dihydroxy-analogue of isoleucine was added to the homogenate, considerable quantities of its keto-analogue were formed (Table 1) These results indicate that in homogenates from green wheat seedlings the dihydroxy-analogue of isoleucine is readily transformed into its keto-analogue, which proves the presence of a very active dehydrase of dihydroxy acids, found previously only in micro-organisms[10-12]

From the results obtained, we concluded that the two last steps of biosynthesis of valine and isoleucine in green plants are identical with those in micro-organisms, that is, dihydroxy-analogues are dehydrated to keto-analogues, the latter being further metabolized by transamination

From this point of view, the results of Wixom and Wikman[13] are of interest They found that in a diet for young rats valine can successfully be replaced by its keto-analogue, but not by its dihydroxy-analogue In homogenates from liver of those rats these authors failed to detect dehydrase of dihydroxy acids

As we have shown[7] in homogenates from liver of mice the keto-analogues of valine and isoleucine are readily transaminated with glutamic acid

On the other hand, in different animal organs, particularly in mice liver, there exists reductase of α-keto-β-hydroxy acids, the enzyme catalyses the reduction of α-keto-β-hydroxy-isovaleric and α-keto-β-hydroxy-β-methyl-*n*-valeric acids to dihydroxy-analogues of valine and isoleucine respectively[14]

Our results, as well as those of the aforementioned authors[13 14], suggest that the mechanism of necessity of valine and isoleucine for the animal organism is connected with the absence of the dehydrase of dihydroxy acids

W L KRETOVICH
Z S KAGAN

A N Bach Institute of Biochemistry,
Academy of Science of the U S S R
Moscow

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PHYSIOLOGY

Diurnal Periodicity in Epiphyseal Growth Cartilage

IN order to utilize radiotracers effectively, in an examination of bone cells, it became desirable to determine if the mitotic event was subject to the same diurnal changes observed for many other tissues[1] The young proliferating cartilage epiphyseal plate served as a model since (*a*) the zones in which mitotic activity occur are well defined, (*b*) the tissue homogeneity obviates problems of cell identification which obtain in metaphyseal bone Two 'spot-checks' of serially independent groups of animals were made during a single 24-hr period utilizing the colchicine method

Thirty one-month-old CF_1 mice weighing 13–18 gm were singly housed and maintained under controlled conditions of temperature (78° ± 1° F) and light for a period of 9 days prior to experimentation, the photoperiod has been shown to be critical for the stability of periodic phenomena[2] The room was illuminated from 09 00 until 21 00 alternating with 12 hr of darkness Intentional disturbance was limited to 09 00–09 30 when the animals were attended to minimize the event of extraneous stress Day (02 00–10 00) and night (15 00–23 00) groups, each consisting of 15 mice, males and females, were injected with 0 1 mgm /100 gm body-weight colchicine subcutaneously 7 hr before death

The hind limbs were dissected out and the knee joints were fixed in neutral formalin, decalcified in a buffered sodium formate–formic acid solution, embedded in celloidin or paraffin, sectioned longitudinally at 10μ and the sections were stained with iron hæmatoxylin or hæmatoxylin and eosin The number of c-metaphase figures appearing in all regions of the proliferative zones of the distal femoral and proximal tibial epiphyseal cartilages was counted under an oil immersion lens by an ocular grid at a magnification of × 970 Two hundred fields, or about 4,000 cells, were observed, the number of metaphase arrests was tabulated as an average value for 1,000 cells

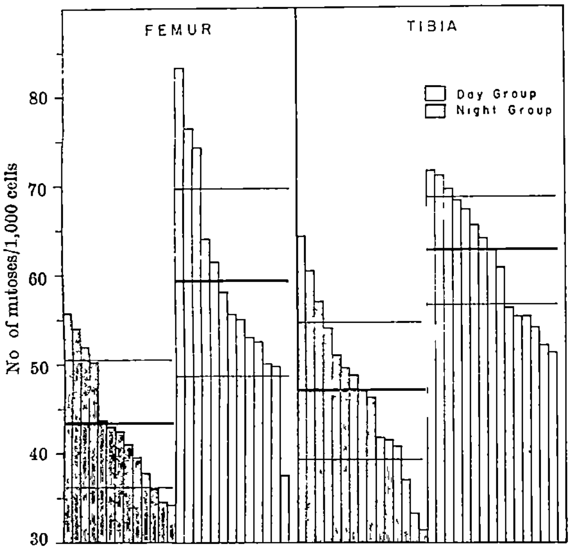

Fig 1 Plot of the individual variation in the day and night groups showing the shift in mitotic rhythm The horizontal lines denote the mean and the range of standard deviations

The number of c-metaphases in the proliferative zones of the knee joint cartilages has been plotted individually in Fig 1 Only 13 values for the distal femoral cartilage in each group were recorded owing to the failure to obtain reliable sections in four instances The broad amplitude of individual scores in both groups is perhaps due in part to the difficulty with which mitotic figures in the long, narrow cells of the most actively proliferating zones of the cartilages may be discerned, and the values overlap somewhat From the mean values obtained, the distal femoral and proximal tibial cartilages grow roughly 26 8 and 23 3 per cent faster during the day than during the night respectively Similarly, the growth of the tibial cartilage exceeds that of the femur by 7 7 and 5 3 per cent at these times Approximately 20 0–23 1 per cent of the individuals consistently lie above the standard deviation, and for a given tissue, the percentages are coincident, the ratio between the number of individuals falling within and below the limits of the standard deviation is, however, more variable Although deletion of the lowest value for the day femurs would eliminate the overlapping standard deviations, a microstatistical analysis of the extreme outliers indicates that these derive from the same population[3]

The preliminary findings indicate that a diurnal periodicity exists for the cells of the young growing epiphyseal cartilages of mice which is similar to that of most tissues other than the adrenal cortex It is, however, impossible to estimate the actual hour of maximum and minimum proliferative activity since colchicine operates by accumulating mitoses in metaphase arrest More exhaustive studies involving intermediate time-periods would be necessary for precise information[1] In view of collateral information that adrenalectomized animals (*a*) fail to evidence marked diurnal rhythms, and (*b*) evidence stimulated chondrogenesis leading to thicker cartilage plates[5], it may be suggested that the day–night differences here reflect the cyclic secretory activity of the adrenal cortex Since serum corticosterone should be minimal at 11 00 under the photoperiod utilized, proliferative activity of the cartilages might be expected to increase towards the onset of the light period with additional increments throughout the first half of the period

This investigation was supported in part by a research fellowship (*DF* 12,985) from the National Institute of Dental Research, U S Public Health Service

DAVID J SIMMONS

Department of Physiology,
University of Chicago

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Effect of Dinitrophenol on Active Relaxation of Unstriated Muscle

SOME unstriated muscles, such as those of guinea pig uterus and frog stomach muscle, relax without the application of any external force when treated with adrenaline[1–3], during such relaxation the oxygen consumption increases[4] Relaxation is prevented if these muscles are previously asphyxiated or treated with cyanide and can be partially restored with glucose Asphyxia or cyanide causes relaxation of loaded muscle On the basis of these experiments it has been suggested that unstriated muscle contains two contractile mechanisms One is responsible for the phasic response and a kind of tone susceptible to asphyxia and relaxes passively, the other is responsible for the asphyxia-resistant tone, relaxes actively and thus is able to maintain contraction or tension without expenditure of energy

Dinitrophenol is known to uncouple respiratory oxidation from adenosine triphosphate formation and it has interesting effects on the relaxation of frog's stomach muscle In unloaded muscle, dinitrophenol (0 2 mM–0 5 mM) completely prevents the relaxation by adrenaline, 10^{-6}–10^{-5}, the control muscles relaxing by 23–40 per cent in 15 min at room temperature, 25 to 30° C (10 experiments) The loaded muscle is at first completely relaxed by dinitrophenol; after 1–2 hr , it may then contract, and the contraction is unaffected by adrenaline, asphyxia or cyanide This asphyxia-resistant contraction by dinitrophenol is favoured by keeping the *p*H of the saline at 6 5 (phosphate buffer) Thus formation of adenosine triphosphate is necessary not only for relaxation, but also for keeping the muscle relaxed

INDERJIT SINGH

Department of Physiology,
Medical College, Agra.

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Toxicity of Endotoxin and Snake Venom in the Hagfish

REACTIONS to parenteral administration of endotoxin in mammals include pyrexia, leukopænia followed by leukocytosis, vasodilation, and irreversible shock[1] Spink[2] reviewed the actions of snake venom and endotoxin, and concluded that their mechanism of action is similar, however, in recent studies Condie *et al* [3] showed that there are few parallels between the biological responses of rabbits to *Escherichia coli* endotoxin and *Agkistrodon piscivorus* venom and that the two toxins are distinct immunochemically

Stetson[4] postulated that the reactions to endotoxin may reflect hypersensitivity in animals the enteric flora of which include Gram-negative organisms Since earlier studies[5] in the California hagfish, ***Eptatretus stoutii***, indicated that this primitive vertebrate is essentially anergic and unable to develop either immediate or delayed hypersensitivity to bacterial or virus antigens, we felt that a comparison of the toxic action of snake venom and endotoxin in this species would be of interest

Freshly obtained California hagfish, weighing 30–80 gm, were kept in well-aerated sea-water, maintained at 10° C, for a preliminary 24-hr observation period, and then divided into groups of two animals per dose of toxin The doses of *Agkistrodon piscivorus* (water moccasin) and *Naja flava* (yellow cobra) venoms and *Escherichia coli* endotoxin were made up to 2 ml volumes in 0 9 per cent saline and injected into the subdermal lateral sinuses in the tail region of the fish, anterior to the caudal heart This approximated an intravenous injection in this animal, which has an intermediate-type open circulation, with return vessels similar to *Amphioxus* Ten-fold variations in dosage were studied (Table 1), and dramatic differences in response to endotoxin and snake venoms were noted

Endotoxin, even in the 100 mgm dose, failed to produce a detectable reaction in the hagfish, and the animals remained coiled up, their normal attitude when undisturbed There were no observable differences between these endotoxin-treated fish and the control animals receiving 2 ml of 0 9 per cent saline By contrast, all doses of the venoms, except 1 mgm of *Agkistrodon piscivorus* venom, were lethal within 5 hr, and the highest doses produced death within 30 min The dying animals uncoiled to a straightened position, and swimming movements stopped The complete suppression of these movements suggests action of the venom on the nervous system, since Campbell[6] has shown that swimming movements of segments continue after the hagfish spinal cord is sectioned The isolated aneural heart of the hagfish has been studied by one of us (D J)[7], and the effects of snake venom on this organ are now being tested and will be reported later

Table 1 RESPONSE TO ENDOTOXIN AND SNAKE VENOMS IN THE HAGFISH

Toxin dose	Deaths* 1 hr	24 hr	48 hr
Agkistrodon piscivorus venom (mgm)			
1	0/2	0/2	0/2
10	0/2	2/2	—
100	2/2	—	—
Naja flava venom (γ)			
50	2/2	—	—
100	2/2	—	—
1,000	2/2	—	—
Escherichia coli endotoxin (mgm)			
1	0/2	0/2	0/2
10	0/2	0/2	0/2
100	0/2	0/2	0/2

* No of deaths within the period specified after administration of the toxin

Table 2 RESPONSE TO ENDOTOXIN AND SNAKE VENOMS IN THE RABBIT (1 KGM)

Toxin dose	Deaths* 1 hr	24 hr	48 hr
Agkistrodon piscivorus venom (mgm)			
1	1/5	1/5	1/5
10	9/10	9/10	9/10
Naja flava venom (γ)			
100	0/10	0/10	0/10
200	0/4	4/4	—
500	0/4	4/4	—
Escherichia coli endotoxin (mgm)			
1	1/20	1/20	1/20
10	2/5	3/5	4/5
100	1/3	1/3	2/3

* No of deaths within the period specified after administration of the toxin

That a 100-mgm dose of endotoxin was without effect in these animals is of interest when compared to the effect in rabbits (Table 2) The dose in the hagfish is equivalent to about 1 5 gm/kgm, and the consistent lethal effect of 1/10, and often of 1/100, of that amount in rabbits is important in dissociating the activities of endotoxin and snake venom

The hagfish may be immune to endotoxin because of unknown natural or tissue inhibitors Alternatively, they may be able to break endotoxins down to less toxic materials However, in view of the extremely high doses of endotoxin used in this study, we believe that these hypotheses are less attractive than the Stetson theory that reactions to endotoxin in mammals are a result of hypersensitivity to the products of gut tract flora This hypothesis could be tested further in germ-free mammals using toxins and dosages comparable with those used in this work

These investigations were aided by grants from the U S Public Health Service, American Heart Association, Minnesota Heart Association and the National Foundation

BEN W PAPERMASTER *
RICHARD M CONDIE †
ROBERT A GOOD ‡

Pediatric Research Laboratories,
Variety Club Heart Hospital,
University of Minnesota, Minneapolis

DAVID JENSEN §

Scripps Institution of Oceanography,
University of California, La Jolla

* U S Public Health Service trainee
† Research Associate Cardiovascular Clinical Research Center, grant No 6314
‡ American Legion Memorial Heart research professor of pediatrics
§ Advanced Research Fellow, American Heart Association

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PHARMACOLOGY

Inhibition of Ascites Tumour Growth by the Trypanocide, Ethidium Bromide, in Combination with Azaserine

Ethidium bromide (3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide) has been used to treat trypanosome infections of cattle in Africa for some time[1,2]. (This chemical was kindly supplied by Dr. M. R. Gurd, Boots Pure Drug Co., Ltd., Nottingham. Its alternative name is 2,7-diamino-9-phenyl-10-ethyl-phenanthridinium bromide.) Recently we have examined the biochemical effects of this compound on Ehrlich ascites tumour cells, and have found that it inhibits completely the incorporation of preformed purines (adenine, guanine and hypoxanthine) into nucleic acids[3]. The possible application of this biochemical effect to cancer chemotherapy was immediately considered. It was believed, however, that ethidium bromide, if used alone, would at best be only moderately carcinostatic because it does not appreciably impair the *de novo* pathway of purine biosynthesis[3]. Because the antibiotic azaserine is well known to inhibit completely the *de novo* synthesis of purines, it was therefore believed that if these two compounds could be used in combination without undue host toxicity, the conditions for a complete concurrent blockade[4] of the purine nucleotide supply for nucleic acid synthesis could be effected. Because both drugs maintain their inhibitory effects for less than 24 hr.[3,5], for more nearly maximal effect it was felt that both should be administered more often than in the daily treatment schedule usually used in tests of carcinostatic efficacy.

Female Swiss mice, 26–28 gm., were inoculated intraperitoneally with approximately 1 × 10^6 Ehrlich ascites carcinoma cells (hypotetraploid line). Morning and evening drug treatments were begun the following day and continued for 6 days. The mice were weighed at the beginning and end of this period. Both drugs were administered in solution in isotonic saline, and control groups received saline injections. The experiments were terminated 50 days after tumour implantation, and the average survival times calculated. All animals alive at 50 days were considered to be 50-day survivors in calculating the average survival-time. The animals received food and water *ad lib.* Averaged results from two experiments are presented in Table 1.

Table 1. Treatment of Ehrlich Ascites Tumours with Combinations of Ethidium Bromide and Azaserine

Dose (mgm./kgm.) Ethidium bromide	Dose (mgm./kgm.) Azaserine	Average survival-time (days)	50-day survivors/total No. of animals	Average weight change (gm.)
0	0	8.9 ± 1.9*	0/14	+ 6.2
0	0.1	17.5 ± 1.8	0/14	+ 2.0
4	0	11.8 ± 3.8	0/14	− 1.1
3	0	12.5 ± 3.4	0/14	+ 2.2
4	0.1	34.8 ± 8.3 (31.8)†	7/14	− 1.6
3	0.1	26.7 ± 9.7 (23.6)†	6/14	+ 1.2

* Average deviation from the mean.

† Numbers in parentheses are the average survival-times of the animals which did not survive 50 days.

Ethidium bromide prolonged survival-time slightly if at all at a dose of 4 mgm./kgm., which was the highest dose which could be given on this treatment schedule without excessive weight loss or death[3]. The combination of azaserine with this dose of ethidium bromide, however, increased survival-time almost 400 per cent, which clearly indicates potentiative action. Of particular interest was the finding that half the mice so treated survived 50 days and were free of gross tumour growth at that time.

To our knowledge, phenanthridinium compounds have not previously been shown to have carcinostatic properties, alone or in combination with other drugs. It is hoped that the carcinostatic effects of ethidium bromide will be examined in more detail, and that closely related compounds will be examined for their biochemical and biological effects.

This work was supported in part by research grant *CY*-2928 from the National Cancer Institute, and in part by training grant 2*G*-26 from the Division of General Medical Sciences of the U.S. Public Health Service, Bethesda, Md.

T. S. Kandaswamy
J. Frank Henderson

Department of Pharmacology,
George Washington University
School of Medicine,
Washington D.C.

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Inhibition of Release of Corticotrophin

Most present concepts of the mechanism of stress activation of the adenohypophysis include the idea of neuro-endocrine 'releasing factors' which liberate a portion of the stored preformed corticotrophin[1]. In contrast to the depletion of corticotrophin demonstrated by Rochefort *et al.*[2] during prolonged stresses we have not been able to demonstrate any depletion after stress of short duration during the period of marked elevation of circulating corticotrophin. In adrenalectomized animals, there is actually an elevation of adenohypophyseal corticotrophin content coincident with the period of peak blood-level of corticotrophin[3]. This has led us to consider that, in the pituitary, stimulation of biosynthesis of corticotrophin is a very important element in the response to acute stresses. For this reason, we felt that it might be of interest to determine whether a blockade of corticotrophin biosynthesis would have any effect on the subsequent ability to release this hormone. We examined the effect of ethionine pre-treatment upon the stress-induced secretion of corticotrophin in rats.

D,L-ethionine (25 mgm./c.c. in saline) was administered subcutaneously in doses of 50 mgm. to groups of 150 gm. female Wistar rats. At various time-intervals after this treatment, the rats were stressed by 1 min. of ether anæsthesia followed by sham unilateral adrenalectomy. At 2.5 min. following the onset of the stress, the rats were decapitated, bled, and the mixed trunk blood assayed for corticotrophin activity by measuring adrenal ascorbic acid depletion according to the method of Hodges and Vernikos[4]. Control groups were pre-treated with saline or with ethionine plus a small molar excess of methionine.

This experiment (Table 1) demonstrates clearly the inhibitory action of ethionine on the acute secretion of corticotrophin after stress. The inhibition is well established in as short a time as 1 hr. of pre-treatment,

Table 1 CORTICOTROPHIN ACTIVITY IN THE BLOOD OF ETHIONINE-TREATED RATS 2 5 MIN AFTER STRESS

(Figures in parentheses are the number of animals in each treatment or assay group Adrenal ascorbic acid of saline-injected assay rats was 546 ± 11 mgm /100 gm adrenal tissue)

Treatment and time prior to stress	Adrenal ascorbic acid depletion activity of blood mgm /100 gm adrenal tissue ± *S E*
Saline 2 ml s c 3 hr (6)	110 ± 11 (6)
Ethionine 50 mgm s c 1 hr (6)	23 ± 16 (6)
Ethionine 50 mgm s c 3 hr (6)	21 ± 17 (6)
Ethionine 50 mgm and Methionine 60 mgm s c 3 hr (6)	109 ± 17 (6)

and is present as long as 3 hr after a single dose of ethionine That the effect is due to the biological antagonism of ethionine for methionine is shown by the complete reversal of the blockade when the normal agonist is administered along with the antagonist The dose and time relationships of this inhibition are subjects of active investigation

The use of amino-acid antagonists offers a new means of investigating the dynamic aspects of corticotrophin synthesis and release Since such marked inhibition of release of corticotrophin is produced by pre-treatment with ethionine, we are encouraged to believe that synthesis of new hormone may be essential for the normal response of the adenohypophysis to mild stresses of short duration

This work was supported by U S Public Health Service grant *A*–5110

BERNARD H MARKS
JOAN VERNIKOS-DANELLIS

Pharmacology Laboratory,
Ohio State University College of Medicine,
Columbus

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HÆMATOLOGY

Distribution of the Serum Group-Specific Components in Israel

THE group-specific components (*Gc*) of human sera, described in 1959 by Hirschfeld[1], constitute a system of α_2-globulins varying in relative electrophoretic mobilities Family studies disclosed that this system is controlled by two co-dominant autosomal alleles, Gc^1 and Gc^2 (refs 2 and 3) Three common phenotypes can be distinguished by immunoelectrophoresis as indicated by the different position and shape of the *Gc*-precipitates a fast-moving component (*Gc* 1–1), and a slow-moving component (*Gc* 2–2) correspond to the homozygous types, the heterozygous type is characterized by the presence of both components forming an extended, two-peaked precipitate (*Gc* 2–1) Population surveys have revealed considerable variations in the distribution of *Gc* allele frequencies in different geographical areas and ethnic groups The frequencies of Gc^2 varied from 0 306 in Indians (Bombay) to 0 023 in a population of Navajo Indians[4,5] In the various Jewish populations in Israel the distribution of several genetic traits has been extensively investigated Differences in the distribution of blood groups[6], and incidence of erythrocyte glucose-6-phosphate dehydrogenase deficiency[7], have been demonstrated The differences demonstrated in the distribution of haptoglobins were not statistically significant Significant differences in the taste sensitivity to phenylthiourea were found only in males The present investigation is concerned with the distribution of the group specific components among various populations in Israel

388 sera were obtained at the Tel-Hashomer Government Hospital from blood donors and patients without serious illness The material consisted of unrelated individuals of different Jewish populations 99 Ashkenasi Jews (European origin), 64 North African Jews (Morocco, Tunisia, Algeria and Libya), 49 Persian Jews, 85 Iraqi Jews, 49 Yemenite Jews and 42 Kurdish Jews 48 sera from unrelated, apparently healthy Arabs were collected in villages in the area of Tel-Hashomer The samples were examined by immunoelectrophoresis according to Scheidegger[8], modified by Hirschfeld[9], using a voltage gradient of approximately 7 V /cm for 120 min The antiserum used in this work was the horse antiserum No 13411 against normal human serum from the Pasteur Institute, Paris

The distribution of the group-specific component in the Israeli populations studied is shown in Table 1 The frequencies for Gc^2 varied from 0 338 in Ashkenazi to 0 190 in Kurdish Jews The frequency of Gc^2 in a small sample of Arabs was found to be within this range, 0 260 The Gc^2 frequency of 0 338 in Ashkenasi Jews is the highest incidence of Gc^2 observed in a population thus far The distribution of *Gc* genotypes in the populations studied was in agreement with the expected distribution based on the Hardy–Weinberg equilibrium Statistical analysis by heterogeneity-χ^2-tests, applying Yates correction for small samples, did not reveal significant differences within the various Jewish populations However, if the material is analysed by comparing the Ashkenasi

Table 1 DISTRIBUTION OF GROUP-SPECIFIC COMPONENTS IN ISRAEL

Population Country of origin	Total		*Gc* 1–1		*Gc* 2–1		*Gc* 2–2		Gc^1	Gc^2	*s′*	χ^2	*P*
Ashkenasi Jews	99	obs	45	45 45%	41	41 41	13	13 13%	0 662	0 338	0 034	0 5581	>0 30
		exp	43 33	43 76%	44 33	44 77%	11 34	11 45%					
Non-Ashkenasi Jews													
North Africa	64	obs	31	48 44%	28	43 75%	5	7 81%	0 703	0 297	0 040	0 1472	>0 70
		exp	31 64	49 44%	26 72	41 75%	5 64	8 81%					
Persia	49	obs	30	61 22%	14	28 57%	5	10 20%	0 755	0 245	0 043	2 5355	>0 10
		exp	27 94	57 02%	18 12	36 98%	2 94	6 00%					
Iraq	85	obs	48	56 47%	33	38 82%	4	4 71%	0 759	0 241	0 033	0 3131	>0 50
		exp	48 94	57 58%	31 11	36 60%	4 95	5 82%					
Yemen	49	obs	30	61 22%	19	38 78%	—	0	0 806	0 194	0 040	2 8343	>0 05
		exp	31 84	64 98%	15 32	31 26%	1 84	3 76%					
Kurdistan	42	obs	27	64 29%	14	33 33%	1	2 38%	0 810	0 190	0 043	0 2748	>0 50
		exp	27 53	65 54%	12 95	30 84%	1 52	3 63%					
Arabs	48	obs	26	54 17%	19	39 58%	3	6 25%	0 740	0 260	0 045	0 0366	>0 80
		exp	26 26	54 70%	18 49	38 52%	3 25	6 78%					

Gc^1 allele frequency of *Gc* 1
Gc^2 allele frequency of *Gc* 2
s′ standard error of gene frequency estimates
χ a goodness of fit of Hardy–Weinberg equilibrium

and non-Ashkenasi Jews, the latter composed of Oriental and North African Jews, a significant difference is obtained ($\chi^2 = 7.0285$, $P < 0.01$). In the sera examined during this work the *Gc* phenotypes corresponded to the three common phenotypes, *Gc* 1–1, 2–1 and 2–2. None of the recently described variations of the *Gc* phenotypes was found[4,5].

This work was supported in part by a grant from the National Foundation (*CRMS*–163) and the National Institutes of Health (*A*–1542 *C*5).

HARTWIG CLEVE
Rockefeller Institute, New York

BRACHA RAMOT
Tel-Hashomer Government Hospital,
Israel

ALEXANDER G. BEARN
Rockefeller Institute,
New York

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Effects of Fibrin Surface on Coagulation of Blood

RECENT reports[1,2] suggest that fibrin surfaces have an anticoagulant effect. One theory[3] concerning blood coagulation proposes a continuous physiological process of intravascular fibrin deposition and spontaneous lysis. If a fibrin surface is anticoagulant and if the vessels are normally lined with a thin fibrin layer, the therapeutic use of fibrinolytic agents in the treatment of thrombosis might result in the undesired effect of increasing blood coagulability. These considerations led us to re-investigate the *in vitro* effects of fibrin on blood coagulation. The results demonstrate that fibrin has no clot-inhibiting effect but rather a clot-promoting action similar to 'foreign effect' of glass[4,5].

In the first experiment 3-ml. aliquots of normal citrated plasma, prepared from blood in contact with plastic (polystyrene) or siliconed surfaces only, were incubated (37° C.) for 30 min. in plastic tubes with (1) nothing, (2) 300 mgm. 'Pyrex' glass powder, (3) 100 mgm. many times washed human fibrin. After incubation the tubes were centrifuged and duplicate 0.2-ml. samples transferred to 12 × 75 polystyrene tubes and recalcified by addition of 0.2 ml. 0.025 *M* calcium chloride. Table 1 demonstrates that pretreatment with glass or fibrin shortens the recalcification time but does not affect the prothrombin or plasma-thrombin times.

Table 1. EFFECTS OF FIBRIN AND GLASS TREATMENT ON THE SUBSEQUENT CLOTTING OF PLASMA IN POLYSTYRENE TUBES

Preliminary treatment	Clotting time in polystyrene tubes (sec.)		
	Recalcification time	Prothrombin time	Thrombin time
None	440	14.2	21.2
Fibrin	155	14.1	21.4
Glass	197	14.1	21.4

The second experiment attempted a reproduction of Copley's observations[2]. 10 ml. 5 per cent solution of bovine fibrinogen (Armour-Fraction I) was clotted in a 600 ml. beaker by addition of 1 ml. bovine thrombin (5 units). The clot was dried overnight at 37° C., the residue scrapped into a fine powder and 30 mgm. placed in each of eight 13 × 100 glass tubes. Tubes 1 and 2 were washed repeatedly with water and saline, then drained. Tubes 3 and 4 were washed with water and the fibrin residue redried. Tubes 5 and 6 contained dry unwashed fibrin, tubes 7 and 8 wet unwashed fibrin. Tubes 9 and 10 were empty glass and 11 and 12 empty silicone-treated glass. Fresh blood (silicone technique) was added in 0.5 ml. amounts to each tube, the contents mixed as well as possible with a brief shaking and clotting times at 37° C. recorded. Table 2 shows that unwashed fibrin had a marked anticoagulant effect which could be readily overcome by the addition of calcium. Washed fibrin, on the other hand, had no effect on blood coagulation in glass tubes.

Table 2. CLOTTING TIME (SEC.) WHOLE BLOOD PLUS FIBRIN

Tube No.	1 and 2	3 and 4	5–8	9 and 10	11 and 12
Clotting time	240	232	> 7,200*	230	540

* After 2 hr., 0.2 ml. 0.025 *M* calcium chloride added; all tubes clotted.

These experiments show that washed fibrin was not anticoagulant and, in fact, might enhance coagulation in much the same fashion as a glass surface. Such clot-promoting effect could be due to the fibrin surface *per se* or to the adsorption of activated Hageman factor or other clot accelerator on the fibrin.

Table 3. Clotting times (sec.) of 1.5 ml. normal whole blood (silicone technique) in polystyrene tubes containing (*a*) 30 mgm. washed human fibrin, (*b*) 30 mgm. of the same fibrin, first suspended in water in a boiling waterbath for 10 min., then dried and reground, (*c*) an equivalent volume of powdered glass, (*d*) nothing

Clotting time (sec.) 37° C.			
Fibrin	Fibrin—100° C.	Glass	Nothing
405	510	330	1,950

In the next experiment fibrin powder was 'inactivated' by heat. Table 3 shows that even after heating at 100° C. fibrin powder has a coagulant effect. The heat-treated fibrin was more granular than native fibrin and proved difficult to pulverize finely.

In the last experiment fibrin powders were prepared from patients' plasmas deficient in Hageman factor, antihæmophilic factor (AHF) and plasma thromboplastin component (PTC) and their effects compared to normal fibrin and glass. As shown in Table 4, all fibrins had an equal clot-promoting effect.

Table 4. Clotting times of 1.5 ml. normal whole blood (silicone technique) in plastic tubes containing the twelve-times washed, dried and pulverized fibrins derived from 10-ml. citrated plasma samples

	Clotting time (sec.) 25° C.					
Fibrins	Hageman	AHF	PTC	Normal	Glass	Nothing
	600	600	600	600	445	2,040

The coagulation time of whole blood is markedly affected by surface contact. Blood in contact with uninjured endothelium clots extremely slowly if at all. Shed blood in contact with skin, glass or other surface clots, the rate being dependent on the nature of the surface. It is difficult, therefore, to define an 'anticoagulant effect' unless surface exposure is constant. In these experiments it is clear that unwashed bovine fraction I is anticoagulant and that this effect is associated with the citrate content of this fraction as the anticoagulant action can be readily corrected by addition of calcium chloride. On the other hand, washed fibrin does not affect coagulation in glass tubes but accelerates the clotting of blood exposed only to plastic or silicone-treated surfaces This appears to be due simply to a foreign surface effect[4,5] and not to contamination with any serum clot accelerator

This investigation was supported by research grants (*H*–2254) from the Division of Research Grants of the National Institutes of Health, U S Public Health Service, and from the Pittsburgh Health Research and Services Foundation (*A*–48)

Gary R Dalin
Jessica H Lewis

Department of Medicine,
University of Pittsburgh,
Pennsylvania

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PATHOLOGY

A Thymus Responsive Phase in Leukæmogenesis in *AKR* Mice

Endocrine tumours can be produced in animals by disturbing the hormonal balance so that an unbalanced, and abnormally strong, proliferative stimulus impinges on the target endocrine organ During the development of these tumours, three phases can be shown to occur Initially the tumour cells are dependent for their survival on the continued presence of the abnormal environmental stimuli (dependent phase) There follows a phase when the cells are no longer dependent for their survival on such stimuli but will grow more rapidly in the presence of such stimuli (responsive phase) Finally the tumour cells proliferate equally rapidly in the presence or absence of specific environmental stimuli (autonomous phase)[1]

In mice of the high-leukæmia strain, *AKR*, deaths from lymphoid leukæmia commence after the age of 6–7 months It was initially demonstrated by McEndy *et al* [2] that thymectomy in 6-week-old *AKR* mice greatly reduced the subsequent incidence of lymphoid leukæmia This effect was shown to be due, in part at least, to a non-cellular influence exerted by the thymus on lymphoid cells, rendering them susceptible to leukæmogenesis[3] Once leukæmia developed in *AKR* mice, the leukæmic cells grew equally well after transplantation to either normal or thymectomized recipients[4] In the terminology used for describing carcinogenesis in endocrine tissues, the state of the lymphoid cells in 6-week-old *AKR* mice could be described as being 'thymus dependent', while fully developed leukæmic cells could be regarded as being in a 'thymus autonomous' state The experiments reported here were undertaken to investigate the state of the lymphoid cells in apparently normal 6-month-old *AKR* mice—that is, in mice late in the pre-leukæmic period

Seven-week-old male ($AKR \times C3H)F_1$ hybrid mice (incidence of spontaneous leukæmia 50 per cent at 1 year) were submitted to either thymectomy or sham thymectomy The following day, pools of spleens were collected from histologically normal 6-months-old male *AKR* mice (3 spleens per pool) These spleens were minced and prepared as cotton-filtered single-cell suspensions in tissue culture 199 medium Each F_1 hybrid mouse was injected intravenously with 10–20 × 10^6 spleen cells in 0 1 ml fluid Approximately equal numbers of thymectomized and sham-operated mice received cells from each spleen pool The mice were observed for the development of leukæmia When moribund, the mice were killed and tissue from each leukæmic animal was transplanted intramuscularly to four 6-week-old male recipients of the following strains *AKR*, *C3H* and ($AKR \times C3H$) F_1, to determine the genotype of the leukæmic cells

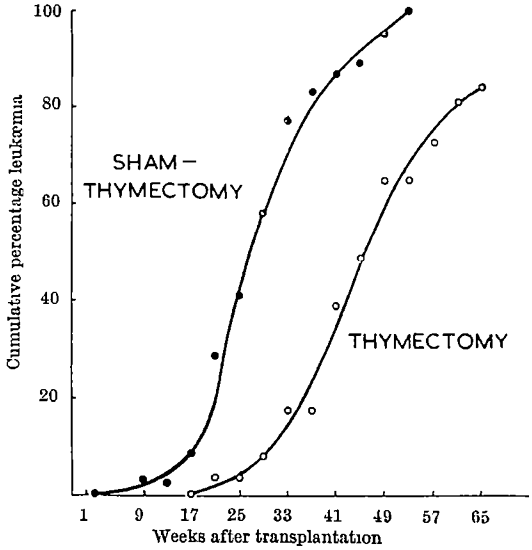

Fig 1 Cumulative development of advanced lymphoid leukæmia in thymectomized and sham-operated ($AKR \times C3H)F_1$ hybrid mice injected intravenously at the age of seven weeks with six-month-old *AKR* spleen cells

Fig 1 shows the cumulative development of advanced leukæmia in 23 thymectomized and 31 sham-operated F_1 hybrid mice injected with the *AKR* spleen cells Leukæmia occurred in all 31 sham-operated mice and appeared earlier than in the thymectomized mice In the sham-operated recipients, the leukæmias were lymphoid in type, and took the form of a thymic lymphoma with, or without, spread to other organs In the thymectomized recipients 20 of the 23 mice developed lymphoid leukæmia, the disease involving the lymph nodes, liver, spleen and kidneys An additional thymectomized mouse, aged 66 weeks developed an atypical reticulum cell sarcoma Transplantation of these lymphoid leukæmias revealed the following distribution of genotypes *Leukæmias in sham-operated mice* , No tested 29/31 No of *AKR* type 17 No of ($AKR \times C3H)F_1$ type 12 *Leukæmias in thymectomized mice*, No tested 18/20 No of *AKR* type 16 No of ($AKR \times C3H)F_1$ type 1 (One lymphoid leukæmia in the thymectomized group failed to grow on transplantation)

Considering only those lymphoid leukæmias shown to have originated from the injected *AKR* cells, the mean latent interval between injection and the development of advanced leukæmia was 29 weeks for the sham-operated mice, and 43 weeks for the thymectomized mice

On intramuscular transplantation to test *AKR* mice, the 16 *AKR* leukæmias arising in thymectomized hosts killed the recipient mice as rapidly (mean latent period to death eight weeks) as did the 17 *AKR* leukæmias arising in sham-operated mice (mean latent period to death seven and a half weeks)

The results indicate that the splenic lymphoid cells from the preleukæmic 6-month-old *AKR* mice did not need the presence of the thymus to progress to frank neoplasia However, they became neoplastic more rapidly if the thymus was present Supporting this conclusion was the observation that the *AKR* leukæmias arising in the sham-operated F_1 recipients appeared to originate in the thymus On transplantation, no difference was observed between the growth-rates of *AKR* leukæmic cells having originated in thymectomized mice and those arising in sham-operated mice

The results suggest that in *AKR* mice late in the preleukæmic period, the lymphoid cells are in a thymus-responsive state, and that in the development of leukæmia in *AKR* mice, the lymphocyte–thymus interrelationships fit the pattern of progressive stages previously demonstrated in the genesis of endocrine tumours (dependency to responsiveness to autonomy) The present results do not provide information on the problem of whether or not thymus-responsive lymphoid cells are, in reality, neoplastic cells It may be argued that there are dormant leukæmic cells present in *AKR* mice late in the preleukæmic period If this is the case, the present evidence would then suggest that the emergence from such a dormant state is stimulated by but not dependent on, the presence of the thymus

DONALD METCALF

Cancer Research Laboratory,
Walter and Eliza Hall Institute,
Melbourne

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Successful Dissection of the Salivary Glands of *Anopheles* Mosquitoes Three Months after Storage at –70° C.

It has been demonstrated that the sporozoites of several species of plasmodia remain viable for several years when stored at temperatures around – 70° C [1]

The technique usually adopted is to dissect the salivary glands and, after confirming the presence of sporozoites, to store them in serum or plasma obtained from the susceptible host, that is, *P vivax* and *P falciparum* in human serum, *P cynomolgi* in rhesus serum, etc To be able to preserve malarial sporozoites for indefinite periods outside the intermediate host is of great value to research workers, but the technique of dissecting the salivary glands following the rupturing of mature oocysts must be carried out before the glands become depleted as the result of repeated feedings by the mosquito, and also the technique of dissecting is time-consuming

With these criticisms in mind it occurred to us to investigate whether the internal tissue of mosquitoes could be dissected after they had been stored for some time at low temperatures We have now demonstrated that both the salivary glands and the mid-guts of mosquitoes stored dry in sealed test-tubes for three months at – 70° C can be dissected as easily as in freshly killed mosquitoes.

Our usual routine technique for dissecting the salivary glands was used[2] The dissections were carried out 3 hr after the mosquitoes had been removed from the deep-freeze chamber

We propose shortly to repeat this experiment, using infected mosquitoes and after storing for several months, to dissect the glands to test the viability of the sporozoites by injecting them intravenously into a susceptible host It may be worthy of mention here that during the practice of malaria therapy over the past 38 years, we have injected by intravenous injection of sporozoites from dissected salivary glands about a thousand patients and that we have never had a failure[3]

P G SHUTE
M E MARYON

Malaria Reference Laboratory,
Horton Hospital,
Epsom, Surrey

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Enzyme Leakage, Antihistamine Drugs and Adrenalectomy in Renal Tubular Necrosis

THE release into the circulation of cellular respiratory enzymes, notably iso-citric and glutamic dehydrogenase, as a result of experimental hepatic necrosis is well documented[1], and the phenomenon has provided a useful means of following the course of such necrosis and of demonstrating the prevention of damage to liver by drugs such as 'Phenergan' (promethazine hydrochloride) and by prior adrenalectomy[2,3]

The investigation reported here represents an attempt to demonstrate similar leakage of enzymes following necrosis of renal tubules produced by heavy metals and to prevent such damage by prior adrenalectomy and by administration of drugs that protect animals against toxic liver necrosis The excretion of lactic dehydrogenase in patients suffering from a variety of renal disorders has already been demonstrated[4]

Kidney damage was produced in male rats of the albino Wistar strain and weighing 150–200 gm by a single intraperitoneal injection of uranyl nitrate (6 mgm /kgm of the salt) This dose caused widespread necrosis of the proximal convoluted tubule[5], demonstrable at 24 hr and maximal at 48 hr after injection Anti-histamine drugs were given by subcutaneous injection 15 min before administration of uranyl nitrate in a dosage of 25 mgm /kgm , repeat injections (12 5 mgm /kgm) being given 6 hr and 24 hr later Bilateral adrenalectomy was performed on 12 rats 12 days before injection of uranyl nitrate, the animals being maintained on 1 per cent sodium chloride in place of drinking water Urine was obtained by placing the rats in a metabolic cage and collecting the urine 2–3 hr later, and iso-citric dehydrogenase activity determined on 0 4 ml samples by the previously published method[1] Samples were stored at – 10° until required

Kidney sections were obtained by killing animals at 15, 24, 48, 72, 96 and 168 hr after injection of uranyl nitrate The material was fixed in neutral buffered formol-saline and the sections stained with hæmatoxylin–eosin

Following the injection of uranyl nitrate, there was at no time a detectable rise in the level of iso-citric dehydrogenase in 1 ml samples of serum However, as can be seen in Table 1, significant amounts of this

Table 1 RENAL TUBULAR NECROSIS AND THE EXCRETION OF ISOCITRIC DEHYDROGENASE AFTER INJECTION OF URANYL NITRATE

Time after injection of uranyl nitrate (hr)	Isocitric dehydrogenase in urine (μmol TPN reduced/hr /ml)	Isocitric dehydrogenase in serum	Renal tubular necrosis
15	0 60	Nil	Nil
24	0 95	Nil	Early
48	1 50	Nil	Severe
72	0 60	Nil	Severe
96	Nil	Nil	Severe
168	Nil	Nil	Severe

enzyme appeared in the urine, the quantity being highest at 48 hr and none being detectable at 96 hr Thus tubular necrosis and enzyme leakage reached their maximal development at about the same time

The presence of enzymes in the urine but not in the blood seems best explained by some kind of preferential excretion from the damaged tubular epithelium into the urinary outflow Origin from urinary cellular sediment was excluded by examining this sediment Recognizable cells or cell nuclei were very scanty, and no differences were observed between samples taken at 48 and at 96 hr Again, the degree of bacterial contamination was similar at both these times

The administration of a number of anti-histamine drugs and also prior adrenalectomy were used in attempts to prevent renal tubular necrosis after injection of uranyl nitrate The drugs used were 'Phenergan' (promethazine hydrochloride), 'Sparine' (promazine hydrochloride) 'Anthisan' (mepyramine maleate) and methdilazine hydrochloride, each compound being given to 6–12 rats In no case was tubular epithelial necrosis lessened, nor was the excretion of iso-citric dehydrogenase diminished The failure of these measures, in contrast to their efficacy in liver damage due to organic poisons[2,3] or viruses[6], may be attributable to the irreversible nature of the toxic effects of heavy metals

R A PAZ*
K R REES
W G SPECTOR

Departments of Morbid Anatomy
and Chemical Pathology,
University College Hospital Medical School
London

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* Research Fellow of the Consejo Nacional de Investigaciones Cientificas of the Argentine Present address Instituto de Investigaciones Medicas, Calle Donato Alvarez 3,000, Buenos Aires, Argentine

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Distribution as a Determinant of Iron Hepatotoxicity

EXAMINATION of clinical material of an appropriate nature indicates not only that iron is hepatotoxic, but suggests the definition of the conditions under which this is so The most critical of these appears to be the parenchymal accumulation of iron to a level at which liver cell death is effected[1] An attempt to verify this concept in laboratory animals has resulted in an affirmation of this point of view

Mice 8 weeks old of the Great Ormond Street strain were injected with iron–dextran ('Imferon') in divided doses to a total of 1,000 mgm /kgm At the end of 3 weeks, approximately 1 week following the final injection of iron–dextran, a group of control and a group of injected animals were killed by cervical dislocation Slices of liver were fixed in buffered 10 per cent formalin and stained with hæmatoxylin and eosin and Perl's reaction for iron Other slices were frozen and examined histochemically and chemically for glucose-6-phosphatase by the techniques of Chiquoine[2] and Swanson[3] respectively This was repeated at the end of 5 months with animals from the same groups, maintained on the routine laboratory chow

A marked difference was present in the hepatic distribution of iron in the two groups of animals In the animals killed shortly after termination of the injections, stainable iron was predominantly located in reticuloendothelial elements In animals killed after relatively long-term survival, much larger quantities of stainable iron were found in the parenchymal cells, with correspondingly less in the reticuloendothelial elements These observations were similar to those originally made by Golberg[4]

Histochemically, glucose-6-phosphatase is markedly diminished in all iron-loaded livers, a finding which will be discussed in detail elsewhere[5] This reduction was more marked in those animals killed 5 months after the last injection, with predominant parenchymal iron storage

Chemical values show a similar accentuation of loss of glucose-6-phosphatase in the long-term iron-loaded animals as opposed to the short-term animals (Fig 1) Age *per se* did not appear to cause a decrease in glucose-6-phosphatase values in control animals

Glucose-6-phosphatase in the liver can be shown to be consistently reduced as a response to hepatic iron loading[5,6] The results of this experiment demonstrate that this response to iron accumulation is proportional to the intensity of parenchymal cellular iron accumulation This indicates that the intrahepatic distribution of storage iron is significant in

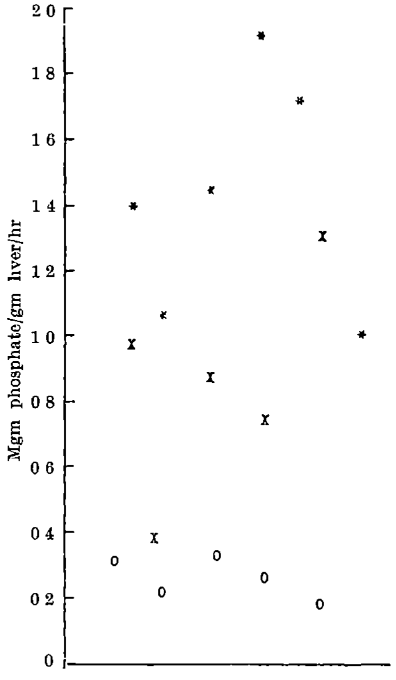

Fig 1 Effect of iron storage and distribution on hepatic glucose-6-phosphatase levels Short-term animals have predominantly reticuloendothelial storage, long-term animals predominantly parenchymal storage *, Controls, ×, short term, O, long term

these terms The reduction of glucose-6-phosphatase does not necessarily reflect a necrogenic property of storage iron, but this property can be inferred from other studies[1,7], and it seems reasonable to expect that if one intracellular biochemical effect parallels the intensity of specifically parenchymal iron stores, others will do likewise These results are thus compatible with the theory of the pathogenesis of certain types of iron storage disease previously proposed[1]

C L WITZLEBEN *

Boston Lying-In Hospital,
Boston, Mass

N J CHAFFEY

Hospital for Sick Children,
London, W C 1

* Fellow of the National Foundation

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BIOLOGY

Protein Synthesis in Chloroplasts during Photosynthesis

SINCE isolated chloroplasts incorporate radioactive amino-acids into their proteins[1-3], it is widely assumed that chloroplasts can synthesize their own protein moieties There is, however, little evidence available to show whether chloroplasts do, in fact, play an active part in the synthesis of proteins in the living leaf cell[4]

I have investigated the incorporation of radioactive products into chloroplast protein after supplying illuminated chloroplasts, isolated from leaves of *Spinacia oleracea*, with $^{14}CO_2$ in the presence of tissue extract containing endogenous amino-acids (for methods see refs 5–7) Among the soluble components formed from $^{14}CO_2$ were alanine and aspartic acid, which were incorporated into chloroplast protein at a rate of 0 1–0 5 per cent of the respective protein-bound amino-acid per hour The incorporation into insoluble protein, sedimentable at 20,000*g*, was up to 10 times higher than that into the soluble supernatant protein The incorporation reaction differed from that usually observed with ribosomes in that ribonuclease and chloramphenicol, even at high concentrations, were not inhibitory Omission of other amino-acids in the reaction mixture did not appreciably decrease the rate of incorporation of ^{14}C-aspartic acid These results may indicate different mechanisms of incorporation of amino-acids into proteins in ribosomes and chloroplasts In view of the low rates of incorporation, however, it cannot be ruled out at present that the reaction in chloroplasts is an artefact spinach chloroplasts contain 15–40 per cent of the total amino-acid activating enzymes of the cell (determined with chloroplasts isolated in an organic solvent after freeze-drying of the leaves (cf refs 8–10) From the observations of Karasek *et al* [11] it can be concluded that an equilibrium exists between the activated amino-acyl adenylate enzyme complex and the free amino-acyl adenylate The latter, however, is known to react unspecifically with protein forming peptide bonds[12,13] This unspecific reaction could possibly explain the low rates of incorporation of amino-acid into the protein of isolated chloroplasts, although it remains uncertain, in that case, why amino-acids are incorporated preferentially into the insoluble protein

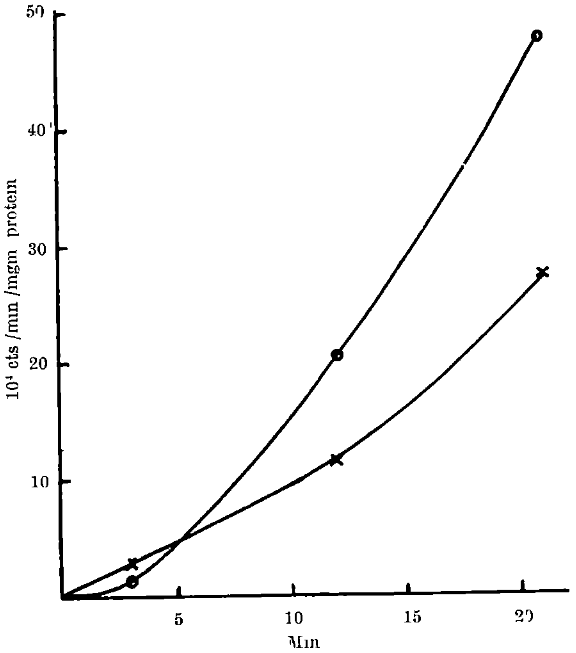

Fig 1 Time-course of incorporation of carbon-14 into soluble proteins of chloroplasts and cytoplasm during photosynthesis of intact leaves in the presence of $^{14}CO_2$ ×, Specific activity of soluble chloroplast proteins, O, specific activity of soluble cytoplasmic proteins

Thus, *in vitro* studies give no clear-cut evidence as to whether, and, if so, to what extent, protein synthesis actually occurs within the chloroplasts Such evidence is obtained from studies *in vivo* After 4-min exposure in the dark to $^{14}CO_2$, mature spinach leaves were illuminated for a certain period of time, then immersed in a petroleum ether/dry ice mixture at about − 80° C, and afterwards freeze dried From the frozen dried material chloroplasts and residue were isolated in petroleum ether/carbon tetrachloride mixtures[14] The specific activities of soluble and insoluble proteins of chloroplasts and residue were determined From these and additional data on the protein distribution within the cell the specific activities of cytoplasmic proteins could be calculated[9,10] Fig 1 shows results of a kinetic experiment In chloroplasts radioactive compounds are incorporated into soluble protein as soon as photosynthesis is initiated Contrary to this, the incorporation of radioactivity into soluble cytoplasmic proteins shows a lag period of at least 2 min It can be inferred that this delay is caused by a lack of radioactive precursors of protein formation which have to diffuse from the chloroplast to the cytoplasm From the earlier appearance of radioactivity in chloroplast proteins than in cytoplasmic proteins it is concluded that protein synthesis actually takes place within the chloroplast, and that photosynthetic products are directly incorporated into protein In chloroplastic as well as in cytoplasmic proteins, especially alanine, aspartic acid, glycine and serine become labelled during the first minutes of photosynthesis The appearance of radioactivity in the amino-acids of hydrolysed chloroplastic protein after 12-min photosynthesis and the intensity of ninhydrin staining of the same amino-acids can be compared qualitatively from Figs 2 and 3

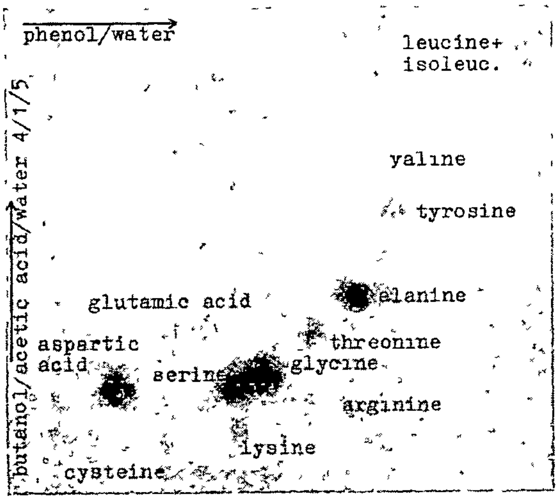

Fig 2 Radioautogram of amino-acids of hydrolysed chloroplast protein, separated by two-dimensional paper chromatography, after 12 min photosynthesis of intact leaves in the presence of $^{14}CO_2$

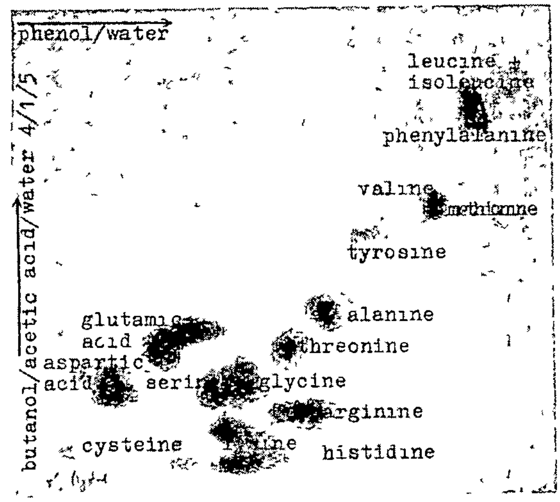

Fig 3 Same amino-acids as in Fig 2, chromatogram stained with ninhydrin

The time-curves of the specific activities of insoluble proteins of chloroplasts and cytoplasm essentially resemble those of the soluble proteins shown in Fig 1, but rates of incorporation are higher. Maximum incorporation-rates observed are about 1–1·3 μgm and 1·9–2·4 μgm amino-acids per mgm. protein in an hour in soluble and insoluble chloroplast proteins respectively. This compares with 1·7–2·2 μgm amino-acids in soluble and 2·4–3 μgm in insoluble cytoplasmic proteins. These values are calculated from the specific activity of the added $^{14}CO_2$ assuming the same specific activity in the carbon of the amino-acids formed therefrom. It must be kept in mind, however, that pools of amino-acids most probably are not saturated with ^{14}C in these short-time experiments. Therefore actual protein synthesis is almost certainly considerably higher than indicated by the foregoing values.

In any event, protein synthesis by chloroplasts contributes considerably to the total protein synthesis of the leaf cell.

This investigation was supported by a grant from the Deutsche Forschungsgemeinschaft.

U. HEBER

Institute of Agricultural Botany,
University of Bonn

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Spore Dispersal in *Pyricularia grisea* (Cooke) Sacc.

Pyricularia grisea is probably best known in relation to 'blast' disease of rice. Some workers have considered this fungus to be the causal organism, but others ascribe the disease to a distinct species *P. oryzae* Cav.[1]. *P. grisea* is associated with 'pitting' disease of Cavendish bananas in Brazil, Trinidad and Australia[2–6]. Pitting is commonest on the finger-stalks and cushions of the large proximal hand of the bunch and, in advanced stages, may result in finger-dropping. Groups of spots or pits may also be scattered over the fruit surface. In attempting to explain the distribution of pits on the bunch, Wardlaw[4] suggested that *P. grisea* "may be widely and abundantly disseminated by wind", however, this was not investigated further. No conidia of *P. grisea* were detected during an earlier survey of the air-spora of Jamaican banana plantations[7], but in recent aerobiological studies appreciable spore counts were recorded and these are the subject of the present communication.

A Hirst spore trap[8] was operated in a banana plantation near Old Harbour from November 1, 1961, until January 26, 1962. Methods of operating the trap and estimating atmospheric spore concentration (expressed here as number of spores per cubic metre of air) were those of Hirst[8,9]. No corrections for variation in trapping efficiency were made so that all spore concentrations quoted are under-estimates[9]. Conidia of *P. grisea* trapped on the slides were obclavate, tapered at the apex, truncate or extended into a short projection at the base, 2-septate, almost hyaline and measured 20–35 × 10–13μ.

The mean diurnal periodicity curve (Fig 1) was derived from the arithmetic means of the estimated numbers of conidia at each even hour, plotted according to Hirst[9]. Rapid conidium liberation started at about midnight and was at a peak near 04.00 hr. E.S.T., low concentrations occurred during daylight. The period of most rapid liberation corresponded with the time when humidity was maximal. The highest 2 hr. estimate recorded was about 530 conidia per m^3 at 04.00 hr. on December 5.

Daily mean conidium concentration was estimated by averaging the 2 hr. counts for the period

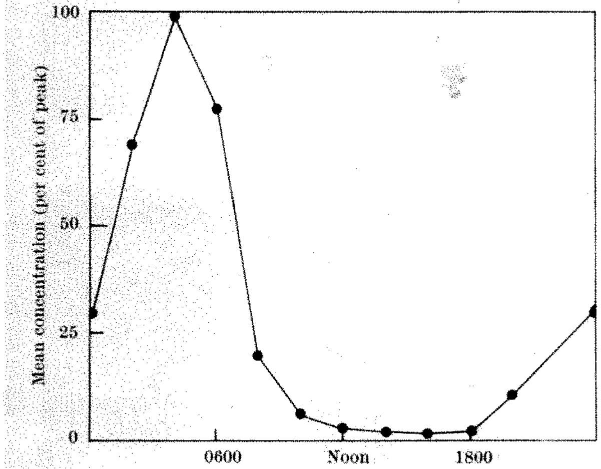

Fig. 1. Mean diurnal periodicity curve for conidia of *Pyricularia grisea* in the air of a Jamaican banana plantation

24.00–24.00 hr. each day. Values are related to total daily rainfall in Fig. 2. In dry spells, concentration rarely exceeded 30 conidia per m.^{3} and on a total of 17 days no conidia were trapped. Rainfall was usually followed by an increased concentration on the following night, the highest value recorded being 136 conidia per m.^{3} on December 5 after 0·24 in. rain during the previous afternoon. Increased concentrations sometimes occurred when there had been no previous rain but when night-time humidity was exceptionally high, for example, during the first half of November.

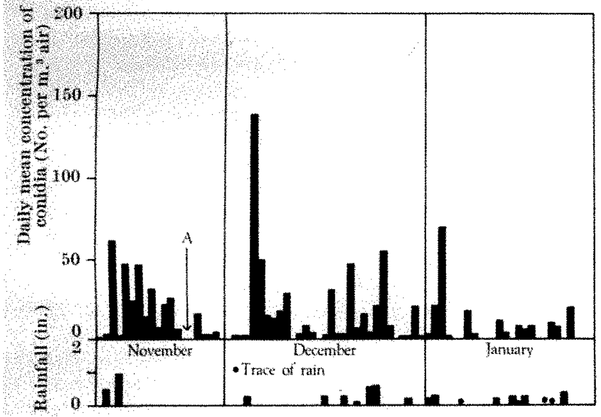

Fig. 2. Daily mean concentration of *Pyricularia grisea* conidia related to rainfall during period November 1, 1961–January 26, 1962. Arrow (*A*) indicates period when trap was out of order (November 14–25)

Colonies of *P. grisea* were found on decaying transition leaves, flower bracts and, more rarely, on old leaf spots caused by *Deightoniella torulosa*, *Cordana musae* or both. However, the fungus appeared to be less common than in Trinidad or Brazil where, according to Wardlaw[4], conidia are formed in great abundance on the Cavendish variety. Laboratory tests showed that high humidity (> 95 per cent relative humidity) was necessary for sporulation, this being at a peak when the spore-bearing substratum was wet. This helps to explain the increased atmospheric spore concentrations following rainfall and, also, the fact that pitting disease is most severe during rainy periods[4].

These findings confirm Wardlaw's[4] suggestion that *P. grisea* conidia are air-borne, although in the plantation sampled conidia of this fungus were rather uncommon and accounted for less than 0·1 per cent of the total air-spora. Periodicity is similar to that in many ascomycetes and basidiomycetes but contrasts with that in several other hyphomycetes, for example, *Deightoniella torulosa*, *Cordana musae* and *Curvularia* spp., which are maximal when vapour pressure rapidly decreases (07.00–09.00 hr.)[7,10,11]. Regular periodicity may be due to the operation of an active discharge mechanism under humid conditions, but this has not been investigated.

D. S. MEREDITH

Banana Board Research Department,
10 South Avenue,
Kingston, Jamaica.

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A Particulate Inoculum for Non-Sporing Fungi

DURING investigations into the soil ecology of *Rhizoctonia repens* Bernard, a non-sporing mycorrhizal fungus of certain orchids, it became necessary to effect a uniform distribution of the fungus throughout the non-sterile experimental soil.

Ludwig, Clark, Julien and Robinson[1] have investigated the suitability of various inocula for the production of a uniform soil infestation and they concluded that a sand–cornmeal inoculum was the best. They attributed its suitability to: (1) the adequate nutrient base; (2) the small particle size of the inoculum, allowing very uniform distribution.

In the case of *R. repens*, sand–cornmeal was tried and found to have two serious defects. First, several additions of water were needed to maintain a suitable moisture content during the long period required for the slow-growing fungus to become thoroughly established in this medium. Secondly, regardless of how long the fungus remained growing in it, residual nutrients supported a prolific growth of 'sugar' fungi when the inoculum was introduced into soil.

These two problems were largely overcome by using exfoliated vermiculite moistened with various nutrient solutions to grow the fungus, then leaching out residual nutrients with several washings of sterile distilled water.

The flakey particles produced by heating micaceous vermiculite crystals in the vicinity of 2,000° F. are capable of taking up large quantities of water, but providing the individual particles are sufficiently large a continuous system of air spaces remains between them at field capacity. From the range of sizes available for building and horticultural purposes, a grade passing A.S. sieves between − 10 and + 18 was selected for the above use. 100 c.c. of this were placed in a 250 ml. conical flask and moistened to approximately field capacity with 35 ml. of potato-dextrose or a synthetic nutrient solution. This was autoclaved and inoculated with the fungus. After 6–8 weeks incubation at 25° C. the contents of the flask were flooded 6 times with 150 ml. lots of sterile distilled water, shaken and decanted. This removed the bulk of the nutrients and separated the vermiculite particles ready for use. The fungal hyphæ were not significantly dislodged from between the crystal lamellæ and remained fully viable.

This medium can be easily varied to satisfy the requirements of other fungi by altering the particle size and nutrient composition and it may perhaps be successfully applied to other experimental situations requiring a discrete hyphal inoculum having negligible nutrient status.

Limitations still inherent in the technique include the fact that vermiculite introduced into soil brings about physical and inorganic chemical changes there. The relatively high cation exchange capacity of the vermiculite gives rise to the latter effect and this same property will also produce changes in the composition of nutrient solutions used to grow the fungus. An alteration in *p*H can result and it may be an advantage to use vermiculite from the Nat Kruit deposit in South Africa, which is unique in possessing a natural *p*H slightly below neutrality.

This work was carried out during the tenure of a Commonwealth Scientific and Industrial Research Organization postgraduate studentship.

G. A. CHILVERS *
School of Agriculture,
University of Sydney.

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* Present address: Botany Department, School of General Studies, Australian National University, Canberra.

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Cobalt and the Fixation of Nitrogen by Root Nodules of *Alnus* and *Casuarina*

THE recent discovery that cobalt is essential for nitrogen fixation in leguminous root nodules[1–5] suggested that there might be a similar requirement for that element in the fixation proceeding in the root nodules of several non-leguminous genera[6] for which molybdenum has recently been shown to be needed[7,8].

Experiments have been carried out with *Alnus glutinosa* and *Casuarina cunninghamiana* using sand and water-culture methods already described[7–9]. The major nutrient reagents were purified by co-precipitation of cobalt with 8-hydroxyquinoline in the presence of iron and aluminium[4,9] followed by co-precipitation with ferrous sulphide at *p*H 8 (ref. 10) and finally by autoclaving the solution in the presence of magnesium hydroxide and calcium phosphate at *p*H 10 (refs. 9 and 11). The cobalt content of the nutrient solutions due to residual contamination of the purified reagents was estimated to be approximately 0·00002 p.p.m.

Alnus seed was sown in purified sand in late May, 1960, the seedlings being transplanted into water culture, then inoculated as previously described[8] and given either 0·01 p.p.m. or no extra cobalt. No combined nitrogen was supplied, apart from an initial addition of 0·8 mgm. ammonium-nitrogen per plant to sustain the plants over the period of nodule formation. Comparable experiments in sand-culture were begun in mid-July 1960. At the end of the rather short first growth season permitted by these starting dates, there were no clear visible differences between treatments, though a harvest of a proportion of the water-culture plants showed a slight superiority in dry weight and nitrogen content on the part of the plants given cobalt.

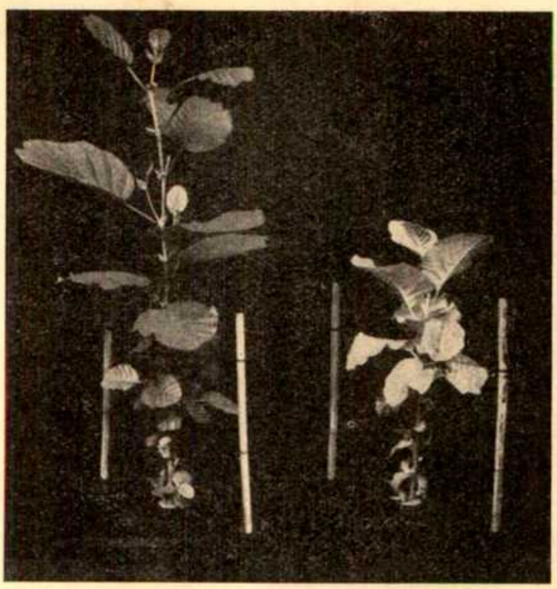

Fig. 1. Nodulated plants of *Alnus* grown in water culture with (left) and without (right) cobalt supplied, photographed by Mr. R. Cowper in late May 1961 (× *c.* ¼)

Plants were retained in the cultures for further observation. In spring, 1961, the sand-culture plants given cobalt emerged from winter dormancy about two weeks before those grown without cobalt, and visible differences in vigour and leaf colour were apparent by mid-June. Differences between treatments were, however, more marked in the water-culture plants, and by mid-May the plants grown without cobalt showed signs of nitrogen deficiency which became progressively more severe as time passed (Fig. 1). Results obtained at the harvest, in late June 1961, of the seven plants in each treatment are given in Table 1 and show a clear effect of cobalt on height, dry weight, percentage nitrogen (especially foliar), and nitrogen fixed. Calculation indicates that when cobalt was provided, 306 mgm. nitrogen was fixed per gm. of nodule dry matter formed, the corresponding figure without cobalt being 131. The addition of cobalt to a deficient plant resulted in a gradual disappearance of symptoms of nitrogen deficiency, complete in two months. Spectrographic analyses for dry material showed that plants given cobalt contained 0·39, 14, 3·7 p.p.m. cobalt in tops, roots and nodules respectively, while those given no cobalt contained 0·11, 0·05 and 0·09 p.p.m. in the same parts.

Casuarina plants similarly developed acute chlorosis characteristic of nitrogen deficiency when grown without cobalt. The results described here clearly

Table 1. EFFECT OF COBALT SUPPLY ON GROWTH AND NITROGEN FIXATION BY *Alnus glutinosa*

Treatment	Mean height of shoot (cm.)	Mean dry weight per per whole plant (gm.)	Mean nodule dry weight per plant (gm.)	Mean percentage nitrogen in leaf dry matter	Mean percentage nitrogen in whole plant dry matter	Mean total nitrogen per plant mgm. *
+ Co	34·4	8·60	0·453	2·34	1·62	139·0
− Co	21·8	3·54	0·245	0·70	0·94	32·7

* Consisting almost entirely of atmospheric nitrogen fixed by the plants; non-nodulated control plants showed a nitrogen content of 0·6 mgm. per plant only.

show that cobalt is required for nitrogen fixation by the root nodules of these non-legumes.

We thank Dr. J. E. Watkin and Mr. P. Ballinger of the N.A.A.S. (Western Province) for the spectrographic determination of cobalt in reagents and plant material.

G. Bond

Department of Botany,
University of Glasgow.

E. J. Hewitt

University of Bristol,
Research Station,
Long Ashton,
Bristol.

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Isolation of Thermophilic Actinomycetes

To judge from reports in the literature, thermophilic Actinomycetes have proved difficult to isolate. For isolations from mouldy hay we have developed a convenient method which promises to be useful in other contexts[1]. The method uses the fact that dry air removes spores of the Streptomycetaceae in preference to bacteria. Instead of suspending the spores in water and plating dilutions in the traditional manner, dry spores are suspended in air and impacted with the Andersen sampler[2] directly on surface-dried medium. In our work, samples of hay are shaken in a small wind tunnel and the air drawn into an Andersen sampler loaded with Petri dishes which had been poured the day before with half-strength nutrient agar (agar content made up to 2 per cent) containing 0·5 mgm./ml. actidione to suppress growth of mould. A slit-sampler could be used as an alternative to the Andersen sampler, and instead of using a wind tunnel, hay or other material can be shaken in a box (pre-sterilized with propylene oxide if required) and a few minutes allowed for larger particles to settle before sampling the air.

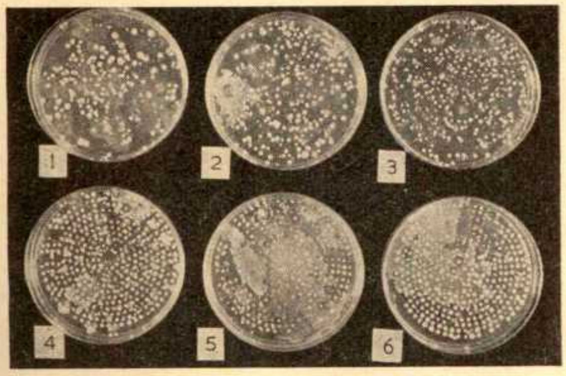

Fig. 1. Petri dishes from stages 1–6 of Andersen sampler exposed to dust from mouldy hay (associated with farmer's lung) for 5 sec. in wind of 4·2 m./sec., incubated at 60° C. for 42 hr. Note antibiotic produced by some colonies of Actinomycetes

After exposure in the Andersen sampler the plates are incubated at either 40° or 60° C. At 60° white sporing colonies develop overnight (Fig. 1), and at 40° many different forms develop more slowly. At either temperature many slow-growing colonies develop on prolonged incubation. During the past two years many thousands of colonies growing at 60° C. have been obtained in culture by this method from hay associated with cases of farmer's lung.

P. H. Gregory
Maureen E. Lacey

Rothamsted Experimental Station,
Harpenden, Herts.

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Collateral Biliary Channels

Following ligation of the common bile duct in the rat normal biliary drainage is restored within 7–12 days after operation.

Investigations previously carried out by Trams and Symeonidis[1] described "recanalization" of the common bile duct occurring 14 days after the occlusion. They suggested that it was brought about as follows: (1) Dilation of the duct above the site of ligation producing a distended sac. (2) Prolapse of the walls of the sac around the ligation. (3) Adherence between the walls of the distal part of the bile duct and the prolapsed walls of the sac. (4) Necrosis of the adherent walls restoring the continuity of the common bile duct.

Our results based on a series of animals killed at intervals ranging between 24 hr. and 3 weeks after operation disproved the mechanisms of recanalization suggested by Trams and Symeonidis[1]. They show conclusively that normal drainage of bile is brought about by the outgrowth of new channels from pre-existing biliary epithelium. These arise from the bile duct immediately distal to the ligation, encircle the ligature and join up with the distended proximal sac (Fig. 1). This process is initiated immediately after ligation and is complete at 7–12 days after operation.

The findings have been confirmed by (*a*) radiological and (*b*) histological observation. The radiological approach was similar to that described previously for the spleen by Braithwaite and Adams[2]. It consisted of injecting a radiopaque medium ('Micropaque', Damancy and Co.) either into the bile duct proximal to the site of ligature (that is, the dilated sac) or alternatively into the bile duct immediately distal to the ligature. This was carried out at varying time-intervals after operation and the passage of medium was observed directly using cineradiography—radiographs were taken at intervals during the course of injection for later examination.

At periods from 7 days after operation the medium passes through the newly formed channels into the distended sac when retrograde injection is performed. When the injection is carried out above the ligation the channels are more difficult to demonstrate.

The outgrowths demonstrated radiologically have been confirmed by histological observation of serial sections of the biliary tract immediately below the

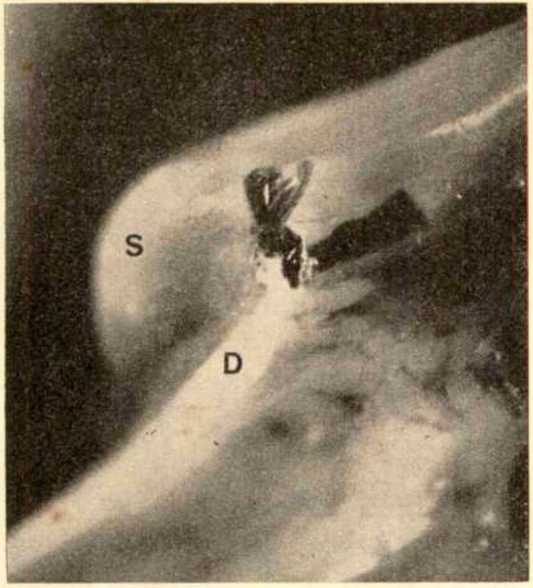

Fig. 1. Collateral biliary outgrowths encircling site of ligation. Specimen injected with 'Micropaque' 7 days after ligation. *D*, Bile duct; *S*, sac. (× 2·7)

site of ligation. Mitotic figures can be clearly seen in material stained with Heidenhain's iron hæmatoxylin.

A comparable series of animals in which the bile duct draining a single liver lobe was ligated shows different results.

Contrary to the views expressed by Kraus and Beltran[3], who postulated that changes occurred similar to those described by Trams and Symeonidis[1] after ligation of the common bile duct, present findings show that their interpretation is incorrect.

Following ligation of the lobar bile duct draining the central lobe of the rat liver, restoration of drainage is brought about by the enlargement of pre-formed biliary canaliculi which course along the walls of the lobar portal veins and their tributaries (Fig. 2). These channels begin to enlarge after operation and form a plexiform system of collateral vessels which is well established after a week. This arrangement is present in similar form at 3 weeks after operation.

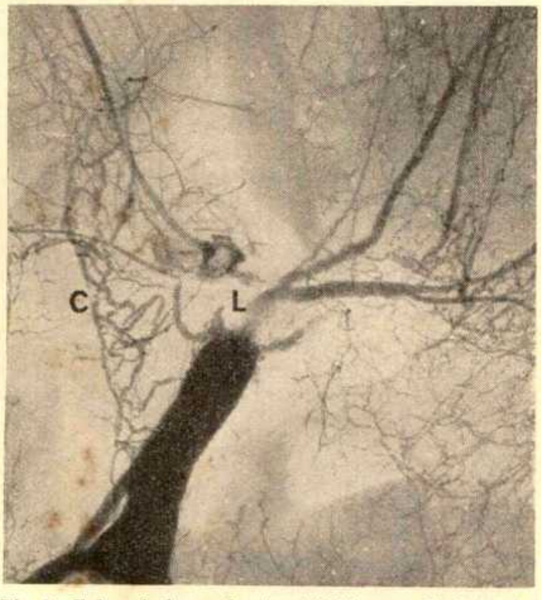

Fig. 2. Enlarged plexus of periportal biliary canaliculi injected with 'Micropaque' 7 days after ligation of central lobar duct. Note site of ligation and course of collateral vessels on wall of portal vein which they outline. *L*, Ligation site; *C*, biliary canaliculi. (× 6)

Periportal biliary canaliculi do not contribute to the collateral drainage when the common bile duct is ligated, since at this site the portal vein on which the biliary canaliculi run is not in close proximity.

The importance of these collateral biliary pathways will be described in detail elsewhere.

J. E. WRIGHT
J. L. BRAITHWAITE

Department of Anatomy,
University of Liverpool.

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A Bivalved Gastropod from the Hawaiian Islands

A SINGLE specimen of a bivalved gastropod (opisthobranchs first described by Kawaguti and Baba[1]) was recently collected alive at Koloa, Kauai, Hawaii. This apparently represents the first living specimen of a species of the sub-family Berthelininae to be described from a Pacific Island.

The specimen was on a sample of *Caulerpa racemosa* var. *turbinata* (J. Ag.) Eubank which was growing around a basalt boulder at the low tide-level. When extended the animal is approximately 6 mm. in length and 4 mm. in diameter. The animal and its shells (Fig. 1) resemble the alga both in form and colour. The convex dorsally carried shells are similar in shape to the inflated fronds of the alga. The body colour is bright emerald green, ornamented with opaque milk-white spots on the oral tentacles and the rhinophores. The head and neck of the animal are microscopically speckled with brown and the dorsal surface of the foot with white. The shell is distinguished from the general body colour by fine radiating lines of red-brown from the mantle, and the adductor muscle shows through as a circular yellow spot on each valve.

The shells are 4·3 mm. in length, 3 mm. in height, and 1·3 mm. in diameter. The valves are smooth, thin, and translucent. The sculpture consists of fine concentric growth lines. The nucleus is on the left valve only, consisting of a translucent white area of 1½ whorls which overlie the dorsum of the valve so that it is on a nearly horizontal plane with the hinge line. It does not extend over the right valve.

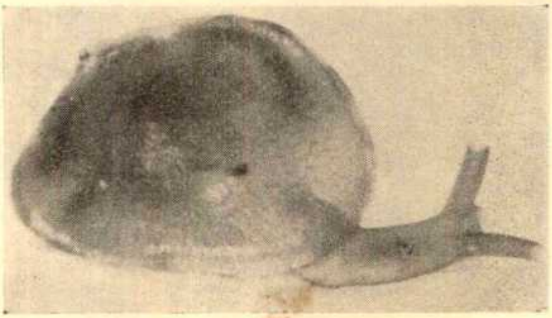

Fig. 1. Living animal of *Berthelinia* sp. from the right side

The specimen has been compared with preserved specimens of *Berthelinia limax*[1] from Japan, *B. typica* (Gatliff and Gabriel) from Australia[2], and *Midorigai australis* Burn[2] from Australia. In the Hawaiian specimen, the shells are shorter from anterior to posterior in relation to height of the valve than are the shells of *B. limax*, and they differ from both *B. typica* and *M. australis* in the blunt, rounded anterior and posterior ends. The nucleus of the Hawaiian specimen differs from those of *B. limax* and *B. typica* in that it is closely applied to the dorsum rather than being elevated and erect; and it differs from that of *M. australis* in that it is smaller in relation to the size of the valve and does not extend over the right valve.

Designation of a specific name will await the collection of additional material.

E. ALISON KAY

General Science Department,
University of Hawaii,
Honolulu,
Hawaii.

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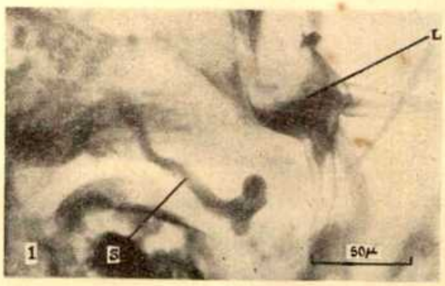
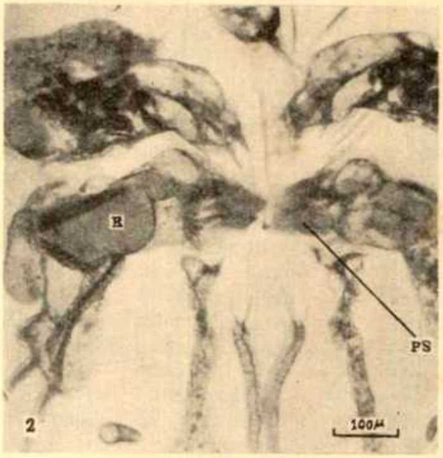
Secretions of the Labral and Trunk Limb Glands in the Cladoceran *Eurycercus lamellatus*

THERE has been much dispute concerning the function of the labral glands of the Branchiopoda. Although Cannon[1-6] has long maintained that they produce a secretion which entangles particulate food prior to its being dealt with by the paired mouthparts, this has not been universally accepted. Among those who dispute this, Nicholson and Yonge[7] expressed the hope that new and independent evidence would be produced by others. Such evidence, which vindicates Cannon's belief, has been obtained from the chydorid cladoceran *Eurycercus lamellatus* (O. F. Müller), in which the labral gland secretions and their origin have been unequivocally demonstrated. Previous evidence was confined to the observation of secretions in the gland cell ducts[1] or, even in the much studied anostracan *Chirocephalus diaphanus* Prévost, to the demonstration of material adjacent to the labrum which was believed to have been produced by the labral glands[5,6]. In *Eurycercus*, secreted matter exuding from the ducts in the labrum has been clearly revealed (Fig. 1 *S*). This material is mixed with the food by special lobes on the first trunk limb (*L*) and by the maxillules.

The importance of entangling substances in the feeding mechanism is emphasized by the discovery of posteriorly originating secretions such as have not previously been reported in the Cladocera. In the Anostraca, secretions produced by superficial, segmentally arranged glands adjacent to the food groove and on the limbs have been described, and divergent views have been expressed regarding their possible function[4,5,8-10]. In *Eurycercus*, secretions are produced by large gland cells located near the base of the fourth trunk limbs. They are stored in voluminous reservoirs within these limbs (Fig. 2 *R*). An exit duct from each reservoir opens on the inner face of each limb. Discharged material (*PS*) is shown near the orifice of the exit ducts in Figs. 2 and 3. This is passed towards the food groove and forwards (Fig. 3 *A*), carrying with it particles filtered by the trunk limbs during the feeding process, an investigation of which is now nearing completion.





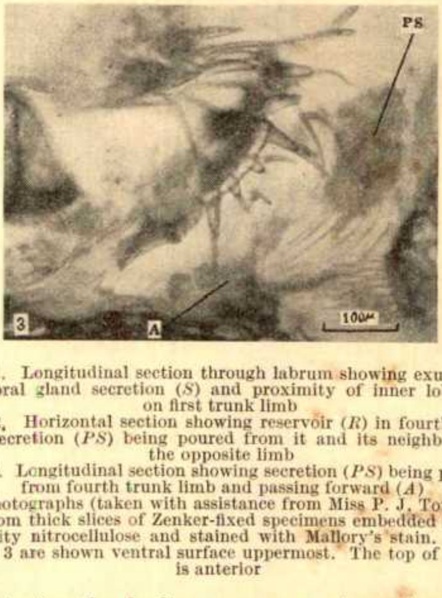


Fig. 1. Longitudinal section through labrum showing exudation of labral gland secretion (*S*) and proximity of inner lobe (*L*) on first trunk limb

Fig. 2. Horizontal section showing reservoir (*R*) in fourth limb and secretion (*PS*) being poured from it and its neighbour in the opposite limb

Fig. 3. Longitudinal section showing secretion (*PS*) being poured from fourth trunk limb and passing forward (*A*)

All photographs (taken with assistance from Miss P. J. Townley) are from thick slices of Zenker-fixed specimens embedded in low viscosity nitrocellulose and stained with Mallory's stain. Figs. 1 and 3 are shown ventral surface uppermost. The top of Fig. 2 is anterior

G. FRYER

Freshwater Biological Association,
The Ferry House,
Ambleside, Westmorland.

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Expansion of the Foot in *Bullia* (Gastropoda)

Sandy-beach gastropods of the family Naticidae and of the genus *Bullia* (family Nassariidae) are capable of a remarkable expansion of the foot. So great is this expansion that it has often been suggested that the turgor of the foot is not due to hæmolymph alone but also to water from the surrounding medium which is supposedly introduced into the foot during expansion and expelled during contraction. Schiemenz[1,2] attempted to show such a mechanism in *Natica* and claimed to have demonstrated the existence of pores opening into an "aquiferous system" which was completely separate from the blood sinuses. Though these findings were accepted by a number of workers, including Gilchrist[3], they have never been corroborated, and most subsequent authors have assumed, as did Pelseneer[4], that the volume of hæmolymph is quite adequate for expansion of the foot in all gastropods. Nevertheless, a small body of opinion has tended to the view that in certain marine gastropods expansion of the foot may be brought about partly by the introduction of sea-water. Morris[5], working on *Uber*, has tried to resolve the controversy by measuring the volume of fluid given off when the animal retracts and comparing it with the volume of water contained in the "free space" and elsewhere when the animal is expanded. Her results are unfortunately inconclusive and her observation that the expelled fluid often contains "blood-corpuscles" adds a complication to the problem.

Bullia, being restricted to southern Africa, has received less attention than the Naticidae already mentioned. Gilchrist[3], in a paper which has been overlooked by subsequent authors, states that the foot of *Bullia digitalis* "is obviously too large for the shell, and it is at once seen that it has been filled in the expanded condition with a comparatively large quantity of water, as, on contraction . . . jets of water are squirted out, sometimes to a distance of several feet. In several cases the quantity of water discharged was found to be sufficient to fill the empty shell after removal of the animal. Sections of the foot showed that there were large spaces between the muscles of the foot, and there was also a slight involution of the epithelium at the openings". Clarke[6] is of the opinion that the expansion is brought about entirely by means of sea-water. In a more recent paper, one of us (A. C. B.)[7] has said that the foot of *Bullia* "is unlike the foot of most gastropods in that much of its interior is taken up by a cavity containing sea-water; this cavity communicates with the exterior by means of a duct opening below the operculum".

We are now in a position to state that the above information with regard to *Bullia* is incorrect. When the living snail retracts its foot, a thin jet of fluid issues with considerable force from what appears to be a pore situated in the mid-line between the posterior cirri; however, serial sections of the foot prove conclusively that no such pore exists. The sections also show that by far the largest space within the foot is the pedal sinus and that this is not in communication with the exterior at any point. The sinus, undoubtedly comprising Gilchrist's "large spaces between the muscles of the foot", contains hæmolymph in the living animal. It is not lined by any epithelium, some of the sections show the presence of hæmocytes, and fluid extracted from the cavity while the animal is alive reacts positively to tests for protein. We must therefore reject any suggestion that the mechanism of pedal expansion is basically different from that in other gastropods.

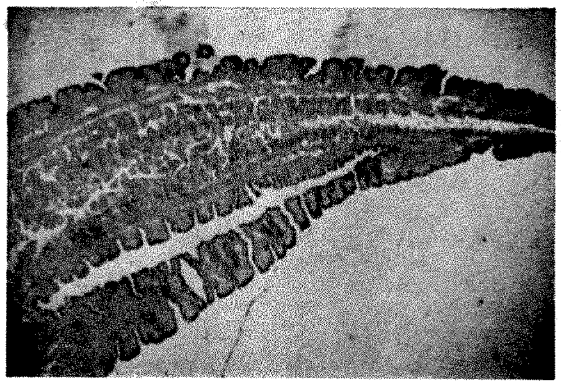

Fig. 1. Longitudinal section of posterior region of foot of *Bullia digitalis* showing 'aquiferous' space with channels to ventral surface. (× c. 30)

There are, however, spaces in the posterior region of the foot which are in direct communication with the exterior and must contain sea-water when the foot is expanded (Fig. 1). Such spaces have not been reported in other gastropods. They are a pair of blind sacs opening to the ventral surface by a series of channels, and it is apparent that they are, in fact, invaginations of the ventral epithelium. A pair of grooves is present on the ventral surface of the foot in this region and these are continued backwards, under the posterior cirri to the posterior margin of the foot. In life the common opening of these grooves gives the appearance of the pore already mentioned. It is apparent that during contraction of the foot water would be expelled from the "aquiferous spaces" and might issue from the posterior of the foot as a jet.

However, while this water may certainly contribute to the jet, it cannot possibly account for the volume of expelled fluid observed by Gilchrist[3] and measured by Brown[7]. The greater part of this volume must come from elsewhere, that is, from the "free space" between the shell and the living tissues, from the mantle cavity, or from the proboscis sheath. Water from these sources does not flow over the dorsal surface of the foot, as no grooves are present to conduct it and carmine particles sprinkled on to its dried surface are not disturbed as the animal retracts. On the ventral surface, however, the lateral margins of the foot are commonly folded over to form temporary channels extending down the length of the foot. A movement of water in these folds can be demonstrated as the animal retracts. As the anterior part of the foot is retracted first it is conceivable that the folds might conduct water from any of the three sources mentioned above, or from all of them.

By introducing a soft piece of plastic catheter through the siphon, it is possible to draw off the water contained in the mantle cavity and to measure its volume, which is somewhat more than half the volume of fluid expelled when the snail retracts. However, when a snail is caused to retract after removal of the mantle water, the volume of the resulting jet is only approximately one-third less than in normal circumstances. We are thus of the opinion that water from the mantle cavity contributes to the jet but that some water remains in the cavity after retraction. The volume of the "free space" does not appear to be great and we have not been able to extract water from it. The proboscis sheath, however, contains a surprising amount of water, the exact volume of which has proved impossible to measure accurately. The introduction of coloured substances into these various spaces with subsequent attempts to recover them from the expelled fluid has proved fruitless as even the smallest particles (of carmine or indian ink) become entangled in copious mucous secretions; coloured fluids have led to equally negative results, for one reason or another.

The foot of *Bullia*, then, achieves expansion in the usual way—by the introduction of hæmolymph. Sea-water accounts for a small fraction of its total volume when expanded, but it is difficult to see what part this water can play in the mechanism of expansion; indeed it may well serve some other function altogether. The water which is expelled as a jet on contraction is probably a defence mechanism and is drawn from several parts of the animal, almost certainly including the foot's "aquiferous spaces" and the mantle cavity, and possibly including the proboscis sheath and the "free space". We are of the opinion that hæmolymph does not contribute to the volume of expelled fluid even during the most rapid contraction as hæmocytes have not been found in it and it reacts negatively to tests for protein.

We thank Dr. L. H. Hyman, of the New York Museum of Natural History, for her advice.

A. C. Brown
L. G. W. Turner

Department of Zoology,
University of Cape Town.

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VETERINARY SCIENCE

Polysaccharide Haptens from Urine of Cattle infected with *Mycoplasma mycoides*

SERUM, plasma, lysed blood cells, urine and pleural fluid or 'lymph' (inflammatory exudate) were obtained from cattle naturally and experimentally infected with *Mycoplasma mycoides*. These materials were examined for antigens of *M. mycoides* by means of the agar-gel double diffusion precipitin test and the quantitative agar-gel precipitin test[1]. They were also examined for antibodies against *M. mycoides* by means of the complement fixation test[2] and the slide agglutination test[3], and for viable *M. mycoides* by growth in broth cultures.

Immune sera were prepared in sheep and cattle by intravenous injections of washed organisms grown in tryptose broth medium which contained no bovine antigens[4]. Sheep developed only a transient pyrexia during immunization. Cattle were initially inoculated with an avianized vaccine and reacted severely to subcutaneous challenge[5] prior to final intravenous immunization.

Urine samples from both naturally and experimentally infected cattle contained no antibodies and no viable organisms. Precipitating antigens, however, were consistently demonstrated and those in the urine of natural and experimental cases appeared to be identical. At least 2 precipitating antigens present in other body fluids were absent from the urine, but those in the urine were present in all the other body fluids.

Fractionation of pooled urine from experimentally infected cattle by precipitation with 3 and 6 volumes of cold isopropyl alcohol and deproteinization with a chloroform–butanol mixture[6] produced 4 precipitating antigens in the 2 fractions. Each fraction contained 3 antigens, 2 antigens being common to both fractions.

Both the fractions were Molisch-positive, biuret-negative, complement-fixing, relatively non-toxic in mice and rabbits, pyrogenic in rabbits and both were haptens as they failed to produce antibodies in rabbits and mice.

Work is in progress to try to elucidate the part, if any, that these haptens play in pathogenesis and immunity. It is also intended to investigate the remaining antigens in the other body fluids.

R. N. Gourlay

East African Veterinary Research Organization,
Muguga,
P.O. Box 32,
Kikuyu, Kenya.

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MICROBIOLOGY

A Calcium-sensitive Strain of *Rhizobium trifolii*

CONTRARY to the findings of Norris[1], it has been possible to show regularly, in detailed growth investigations, a favourable response to calcium by strains of *R. trifolii* and *R. meliloti*. This has expressed itself as faster and more sustained growth in the exponential phase and in a greater stationary population. Calcium is also needed for the normal rod-shaped cell, compared with the markedly swollen and vacuolated condition which is a characteristic result of calcium deficiency[2].

Recently, however, a variant form of *R. trifolii*, strain *SU* 298/531, has been encountered which is markedly sensitive to calcium when grown in the same defined medium as otherwise permits the favourable response to be demonstrated. The calcium-sensitive variant is recognizable as a different colony form but shows serological behaviour similar to the parent strain as well as retaining its ability to nodulate species of *Trifolium*. Typical growth data with single colony re-isolates of the parent and variant colony types are given in Fig. 1.

The parent type shows a normal sustained favourable response to calcium. The sensitive variant, on the other hand, though responding early to calcium, soon ceases growing in the presence of that ion and is surpassed by the culture growing in its absence.

R. trifolii, *SU* 298, and the sub-strains derived from it, are the lysogenic cultures described by Marshall[3]. The early cessation of growth seen here in the presence of calcium could well reflect a disturbance of the normal 'symbiosis' between prophage and

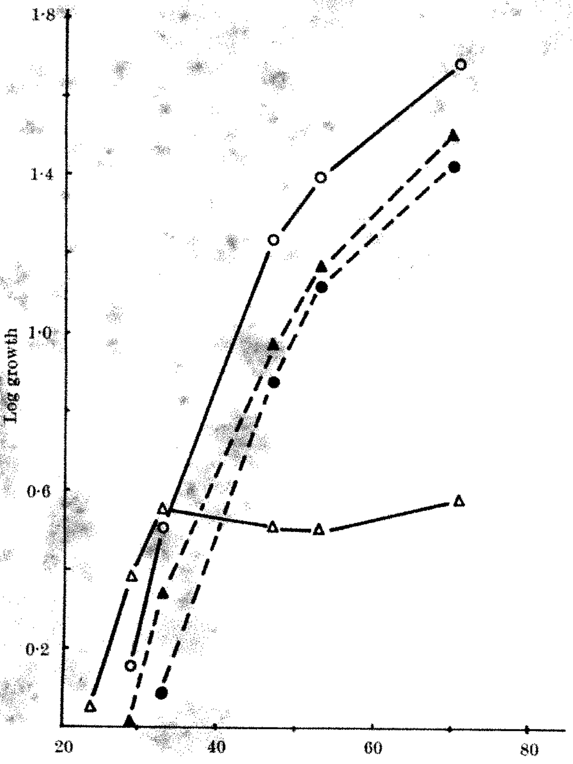

Fig. 1. Response of parent and sensitive strains to calcium. ○, Parent strain + calcium; ▲, sensitive strain − calcium; ●, parent strain − calcium; △, sensitive strain + calcium

bacterium, such that a considerable part of the population autolyses and keeps the turbidity low. The detailed nature of the phenomenon has by no means been defined. The occurrence of sporadic departures from the condition we have described indicates this, but results to date show that this calcium sensitivity is dependent on pH (pH 6 > pH 6·5 > pH 7·0), and is avoided by the addition of glutamate to the medium and by the associated growth of parent-type cells.

J. M. VINCENT
CLAIRE H. JANCEY

Microbiology Laboratory,
School of Agriculture,
University of Sydney.

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Calcium Requirement for Growth of *Staphylococcus pyogenes*

WHETHER calcium is an element essential for bacteria is uncertain. Gorini and Audrain[1] concluded that *Bacillus megatherium* needed calcium only when proteolytic enzymes were used to obtain amino-acids; the results of Shooter and Wyatt[2] with *Staphylococcus pyogenes* were explained similarly. *Diplococcus pneumoniae* also requires calcium[3]. There is no evidence that calcium is required intracellularly although Haines[4] showed that it was required for the formation but not for the activation of bacterial proteases. We have attempted to show the uptake of calcium by a *Staphylococcus* by use of the radioactive isotope calcium-47.

A 0·5 per cent glucose, 1·5 per cent tryptone broth was depleted of cations, except Na^+, by passing it through ion-exchange resins (in preparation). The Ca^{2+} remaining in the medium was not detectable by chemical methods or flame photometry; previous results[2] led us to conclude that the residual Ca^{2+} was about 1 μM. Additions of 'Specpure' salts (Johnson, Matthey, Ltd., London), including calcium chloride, were made; all tubes contained calcium-47 (Radiochemical Centre, Amersham) supplied as calcium chloride in isotonic saline. After dilution with distilled water, the total concentration of radioactive isotope and carrier was 0·25 μM Ca^{2+}. About 23,000 bacteria were added per 10-ml. medium (in preparation); the cultures were incubated at 37° C., 5-ml. samples were taken, centrifuged at 1,000g for 20 min. at 15° C.; 2 ml. of supernatant was withdrawn and diluted to 10 ml. These solutions were counted in a liquid Geiger–Müller counter, type M6, which gave about 600 counts/min. for a concentration of calcium-47 of 1 μc./l. Counting times were such as to give a statistical accuracy of 1·5 per cent or better.

In the first experiment, sixteen combinations of cations were tested and the growth with each combination is shown in Table 1. There was no significant difference between the activities of the supernatants after growth and those of the uninoculated controls (Table 2). In the experimental supernatants all except the two extreme counts/min. of 500 and 570 fell within the range of the controls. The range of activities showed no dependence on the combinations of cations added or on the total Ca^{2+} added.

Table 1. GROWTH OF *Staphylococcus pyogenes* WITH THE COMBINATION OF CATIONS USED FOR MEASURING CA^{2+} UPTAKE

mM cations present					Growth with Ca^{2+} μM added		
K^+	Rb^+	Mg^{2+}	Fe^{2+}	Arginine	0	50	100
0·1	—	1	—	—	347	375	377
0·1	—	1	0·1	—	355	383	(488)
0·1	—	1	0·1	2·5	417	453	(532)
0·1	—	1	—	2·5	423	446	(502)
0·1	—	1	0·4	2·5	417	—	—
0·1	—	0·05	—	—	—	—	332
—	0·2	1	0·1	2·5	—	—	128
0·011	—	1	0·1	2·5	—	142	—
0·011	0·1	1	0·1	2·5	—	265	—
0·011	—	1	—	—	120	—	—
0·011	0·1	1	—	—	234	—	—

Growth expressed as 'Spekker' readings × 1,000 after 24 hr. incubation. Results in parentheses relate to experiments without ^{47}Ca present.

Table 2. COMPARATIVE COUNTING-RATES (PROPORTIONAL TO ^{47}CA CONTENT) OF SUPERNATANTS AFTER GROWTH AND CONTROL MEDIA

	Supernatants after growth	Controls
Mean ± *S.D.*	535·5 ± 14·3	537 ± 8
No. of results	(16)	(9)
Range	500–570	519–547

Analysis of variance: standard error of the difference = 4·4.

The possibility that Ca^{2+} might be needed only at an early stage in growth and later released was also examined. In a subsequent experiment, tubes containing 0·1 mM K^+ and 1 mM Mg^{2+} were incubated for 10, 15, 18 and 24 hr.; Spekker readings of growth were 0·073, 0·198, 0·291 and 0·345 respectively. As before, there was no significant difference between the activities of the supernatants from these tubes and those of the controls.

These results suggest that although addition of Ca^{2+} can increase growth by more than 30 per cent, no accumulation of Ca^{2+} can be detected even in very small concentrations of Ca^{2+}. The following possibilities may be considered:

(1) That Ca^{2+} is required extracellularly.

(2) That Ca^{2+} is required intracellularly in trace quantities only. With the method used here, an

uptake greater than 4 per cent or 0·4 µmole Ca^{2+}/gm. dry wt. cells might have been detected. This would represent an accumulation greater than 125 times the supernatant concentration, with no extra added calcium and no accumulation with 100 μM Ca^{2+} added. This is based on a total cell volume of 2·67 ml./gm. dry wt. (the cell pad volume 3·61 ml./gm. (ref. 5) less 26 per cent interspace volume for close-packed spheres[6]). As the counts of supernatants and controls agree closely, it is probable that uptake was much less and the accumulation without added Ca^{2+} was as low as ten-fold.

(3) That calcium is not needed as such, but in media containing chelating agents, for example, amino-acids, displaces another cation that is required.

There is some support for the third possibility, for experiments with two different media and three strains of *Staphylococci* have shown that Fe^{2+} is as effective as Ca^{2+} in promoting growth. However, for equal effects on growth, equimolar concentrations of them are needed[7], and as the relative affinity of chelates for Fe^{2+} is very much greater than for Ca^{2+}, this makes displacement by Ca^{2+} doubtful. It is more probable that Fe^{2+} and Ca^{2+} are required for enzymes on alternative pathways.

H. V. Wyatt
G. W. Reed
A. H. Smith

Departments of Bacteriology
and Medical Physics,
School of Medicine, Leeds.

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Fixed Pathogenic *L* Forms of *Agrobacterium tumefaciens*

Unstable *L* forms of *Agrobacterium tumefaciens* (strain *B*.38) induced by glycine have been described by Rubio-Huertos and Desjardins[1]. In the present work two strains, *A.T.B.* and *A.T.* isolated from grapes, were grown in agar plates with 4 per cent glycine, where they readily formed typical *L* colonies. These were transferred simultaneously at intervals of 2–3 days to new plates of agar–glycine and glucose–agar without glycine. On the fiftieth transfer no reversion to the normal bacillary form was observed even after 32 further transfers in medium without glycine. The fixed colonies, formed by spherically dense elements, have the same macroscopical appearance as the normal colonies of *A. tumefaciens* grown in the same medium and have lost the typical features of the *L* colonies.

The fixed spherical forms were observed using both light- and electron-microscopes (Fig. 1); they appear to have a definite plastic membrane similar to the *L* forms, more dense cytoplasm and a slime layer; they are also of a more regular size, 0·5–0·8µ, unlike the unstable *L* colonies formed by elements of very different sizes.

Inoculations with the fixed forms of small plants of *Phaseolus vulgaris* gave a positive pathogenic response, and the same type of spherical forms were recovered from the tumours.

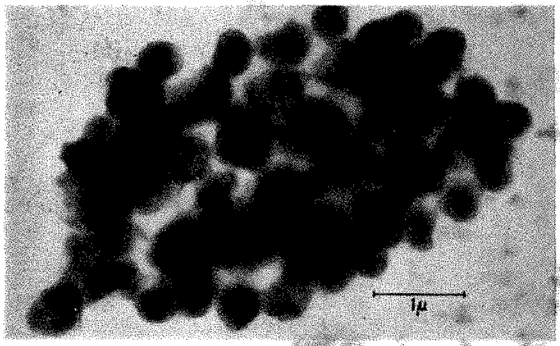

Fig. 1. Electron micrograph of fixed spherical elements of *A. tumefaciens*

The crown-gall tumours induced by spherical forms are of the same size and similar macroscopic appearance as those induced by the normal bacillary parental strains of *A. tumefaciens*.

The pathogeneity of unstable *L* forms could not be proved here because only normal bacillary forms were recovered from the tumours induced by inoculation with these unstable forms. The pathogeneity of *L* forms has been reported in animal pathogen bacteria (Lavillaureix[2], Rubio-Huertos and González[3]), but the pathogeneity of the fixed spherical forms of *A. tumefaciens* is the first case, so far as we know, for plant pathogens.

Comparative tests for sugar fermentation, and other physiological essays revealed that there is no difference between the fixed spherical forms and the normal parental strains.

The pathogenic spherical elements forming the fixed colonies fulfil all the requirements stated by Klieneberger-Nobel[4] for *L* forms except in the macroscopical morphology of their colonies. Thus it is difficult to say if they are real *L* forms or an induced spherical mutant of the normal parental strains.

Miguel Rubio-Huertos
Ramona Beltrá Martinez de Velasco
Instituto "Jaime Ferrán" de Microbiología.
Joaquín Costa, 32, Madrid.

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PSYCHOLOGY

Auditory and Visual Interaction in Man

Some characteristics of auditory and visual interaction were studied in 36 normal young adult subjects, using a method based on recognition of repetitions. This method extends and complements procedures developed by Averback and Sperling[1], Broadbent[2], and Shepard and Teghtsoonian[3].

The stimuli used were supra-threshold ½ sec. auditory and visual presentations of randomly selected numbers from 1 to 7 and 10 to 17. The subjects were presented with 32 short groups of 9 sequential displays, one display every 2 sec. Each display consisted of the simultaneous auditory and visual presentation of two different numbers. Four of the last eight displays of each group contained either a visually or an aurally presented number which was a repetition of a previous presentation within the same group. Repetitions followed two displays after initial presentation, but the subjects were not aware of this. Half the repetitions were aural and half were visual presentations; half of both types of repetition were of numbers initially presented aurally and half visually. The subjects were instructed to press a micro-switch when they noted any repetition of any number already seen or heard.

The principal results were: (1) Auditory and visual probing repetitions were equally well recognized, each accounting for half the total information retrieved from immediate memory storage. (2) While recognition of repetitions of both auditory and visual input declines as more information is added to short-term storage, only 59·5 per cent of visual input was recognized when repeated in contrast to 76·2 per cent of aural input. This difference is significant by the *t*-test ($P < 0{\cdot}01$). (3) Auditory information was retrieved equally well by both auditory and visual probe repetition ($P > 0{\cdot}05$ by the *t*-test). Visual repetition retrieved a significant 4 per cent more of the total visual information retrieved than did auditory repetition ($P < 0{\cdot}01$ by the *t*-test).

The considerable degree of auditory-visual interaction revealed by these results may be due in part to the particular procedure of this experiment which reflects interaction only in immediate memory. It may also be due to the use of numbers as stimuli; these factors might account for results differing from those of Cole, Chorover and Ettlinger[4]. Furthermore, these stimuli were supra-threshold, as demonstrated by 98·6 per cent correct responses by these subjects in a control test in which they had to indicate only if any two numbers simultaneously seen and heard were identical.

I thank Dr. Murray Jarvick (U.S. Public Health Service grant *M*-1225) and Dr. Josiah Macy, jun. (who with Miss Maria Ungson provided the statistical analysis under National Institutes of Health grant *B*-3491).

HERMAN BUSCHKE *

Department of Neurology,
Albert Einstein College of Medicine,
New York 61, New York.

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* Senior post-doctoral Fellow in Neurology (U.S. Public Health Service grant 2*M*-6418).

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Short-term Retention of more than one Aspect of a Series of Stimuli

IT has been found that subjects recalling sequences of digits presented in pairs simultaneously to two sense organs (the two ears, or eye and ear)[1,2] show preferences between strategies of recall which have been attributed by Broadbent[3] to the existence of at least two selectively acting storage systems.

A model for this process envisages the organism as selecting between 'channels' of input. It is not clear whether such 'channelling' of stimuli entails identifications more complex than grouping by source, or to what extent the model may be extended to other processes of selective attention. A situation where subjects are required to recall more than one aspect of a series of stimuli is therefore a test case.

Fifty-two cards measuring 3½ in. × 2¾ in. were each stencilled with a single letter of the alphabet in 72-point Bodoni bold capitals. The letters made up two alphabets, one stencilled in red and the other in black. By shuffling the cards, and dealing them face upwards in time to a metronome at intervals of 0·75 sec., so that each card covered its predecessor, subjects were presented with sequences of 5 letters in random order, each letter being randomly either red or black.

Subjects were required to recall two stimulus aspects independently, reporting the colours in the order of presentation (for example, 'red–black–red–red–black'), and the letters also in the order in which they were presented (for example, '*A–B–C–D–E*'). Instructions were varied so that subjects either reported the letters first and the colours second, or vice versa. Two conditions of instructions were compared: in Condition 1 subjects were told the required order of recall before the items were presented. In Condition 2 subjects were told the required order of recall after the items were presented.

Here Broadbent's model for selection between sources predicts that where the order of recall is known in advance, recall of the first group of items reported (letters or colours in this case) will be better, and recall of the second group of items will be worse than for their equivalents in a condition in which order of recall is not known until after presentation.

Eighteen postmen aged 17–29 years were given 100 trials on each of these conditions. The two orders of recall ('colours first' or 'letters first') occurred equally often in series randomized by transposition from tables of random numbers. Nine subjects started with Condition 1 and nine with Condition 2. All departures from presented sequences were classed as errors.

Results are presented in Table 1.

Table 1. COMPARISON OF KNOWN AND UNKNOWN ORDER OF RECALL FOR GROUPED STIMULUS ASPECTS—MEAN CORRECT RECALL

	Condition 1. Order of recall known in advance (per cent)	Condition 2. Order of recall not known in advance (per cent)
Colours first	87	79
Colours second	72	76
Letters first	83	70
Letters second	49	62

A Wilcoxon test[4] confirms that both colours and letters, when reported first, are significantly better recalled in the known than in the unknown order of recall condition ($P < 0{\cdot}01$ two-tailed). Conversely, both aspects, when reported second, are significantly better recalled in the unknown order of recall condition ($P < 0{\cdot}01$ two-tailed).

These results show an effect similar to that demonstrated by Broadbent[2] for selection between two stimulus sources. They may only be accounted for by a theory of independent storage systems if the definition of what constitutes a 'channel'[1-3] for selection and identification of stimuli is extended to cover a considerably wider field of behaviour than that to which it was first applied.

P. M. RABBITT

Medical Research Council,
Applied Psychology Research Unit,
15 Chaucer Road, Cambridge.

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FORTHCOMING EVENTS

Monday, July 9—Thursday, July 12

British Institution of Radio Engineers, Institute of Physics and the Physical Society, and the University of Birmingham (in the Electrical Engineering Department, The University, Birmingham)—Symposium on "Sonar Systems"

Thursday, July 12—Friday, July 13

Biochemical Society (at the Department of Biochemistry, University of Cambridge) at 9 30 a m daily—International Meeting

Thursday, July 12 at 2 15 p m —Colloquium on "Control of Protein Biosynthesis—the Role of Nucleic Acid"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Tutorial Research Fellow or Student in Chemistry—The Secretary, Bedford College (University of London), Regent's Park, London, N W 1 (July 12)

Staff Tutor in Physics in the Department of Extra-Mural Studies—The Registrar and Secretary, The University, Bristol (July 14)

Assistant Lecturer in Physiology—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W 8 (July 16).

Assistant Lecturer (with interests in ecology, cytology or virology) in Botany—The Registrar, The University, Hull (July 16)

Research Assistant (with a special interest in geomorphology or historical geography) in the Department of Geography—The Secretary, University College, Gower Street, London, W C 1 (July 16)

Sub-Librarian (with a good honours degree, and preferably relevant experience and professional qualifications) in the Faculty of Engineering—The Registrar, The University, Liverpool, quoting Ref CV/122 (July 16)

Lecturer (with a good honours degree in physics or mathematics and some experience in research) in Astronomy—The Secretary, The University, Edinburgh (July 18)

Assistant Lecturers in Philosophy and Chemistry—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (July 20)

Research Demonstrator in the Department of Physics to undertake teaching duties under supervision (mainly in practical classes), participate in the research of the department, and to study for a higher degree—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (July 20)

Assistant in the Department of Zoology and the Gatty Marine Laboratory, St Salvator's College (candidates with specialist interest in entomology should *not* apply)—The Secretary, The University, College Gate, St Andrews (July 21)

Lecturer or Assistant Lecturer in Bacteriology—The Registrar, The University, Manchester, 13 (July 21)

Research Assistant in Veterinary Preventive Medicine to work at the Veterinary Field Station on a programme of research dealing with respiratory diseases in poultry—The Registrar, The University, Liverpool, quoting Ref CV/118 (July 21)

Assistant Experimental Officers (with a degree in chemistry and/or botany, A R I C , or H N C.) for analytical work with crops, soils and fertilizers and for work on crops grown in glasshouse experiments—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (July 23)

Assistant Lecturer in Applied Mathematics—The Secretary, Birkbeck College (University of London), Malet Street, London, W C 1 (July 23)

Lecturer in the Department of Zoology—The Registrar, University College of Wales, Aberystwyth (July 23)

Lecturer or Assistant Lecturer (preferably with experience in the field of nuclear magnetic resonance) in Inorganic Chemistry—The Registrar, The University, Leicester (July 24)

Physical Chemist (with a first- or second-class honours degree in physical chemistry or general chemistry with the emphasis on the physical side) to work on physico-chemical aspects of soil-plant-nutrient relationships—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (July 25)

Lecturer or Assistant Lecturer in Mathematics at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (July 31)

Senior Lecturer or Lecturer in Mathematics in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, July 31)

University Lecturer (with qualifications in numerical analysis or computer programming) in the Mathematical Laboratory—The Director, Mathematical Laboratory, The University, Cambridge (July 31)

Fellow/Lecturer in Anthropology—The Registrar and Secretary, The University, 38 North Bailey, Durham (August 1)

Senior Lecturer or Lecturer in Psychology at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town (August 10)

Assistant Lecturer (with an honours degree in archæology or equivalent, and a good general knowledge of European and British prehistory with emphasis on the periods before the Neolithic) in the Department of Archæology—The Secretary, The University, Edinburgh (August 11)

Chair of Eye Health in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, August 31)

Lecturer in Veterinary Preventive Medicine—The Registrar, The University, Liverpool, quoting Ref CV/117 (August 31)

Senior Lecturer in Zoology at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town (August 31)

University Assistant Lecturer (with a degree in psychology, some post-graduate experience, and some knowledge of statistical methods) in the Department of Education—The Secretary, Cambridge University Department of Education, 17 Brookside, Cambridge (September 1)

Head (with appropriate academic qualifications and experience) of the Department of Science and Food Technology—The Principal, Salford Technical College, Peel Park, Salford, 5

Lecturer (with a university degree or equivalent qualifications) in Metallurgy, to teach foundry technology and refractory materials to final year degree and diploma in technology students—The Clerk to the Governing Body, Battersea College of Technology, London, S W 11

Reader in Medical Parasitology at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, W C 1 (September 18)

Senior Lecturer (well qualified academically with adequate industrial and/or research experience) in Aerodynamics—Head of the Department of Aeronautics and Space Technology, Northampton College of Advanced Technology, St John Street, London, E C 1

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

The British Journal Photographic Annual, 1962 Edited by Arthur J Dalladay Pp 576+32 plates (London Henry Greenwood and Co Ltd , New York Amphoto, 33 West 60th Street, 1962) 8*s* , 2 50 dollars (board covers) , 10*s* 3 dollars (cloth bound) [174

Ambassade de France Service de Presse et d'Information Algeria After the Evian Agreements Pp 13 (London Ambassade de France, 1962) [174

London and Home Counties Regional Advisory Council for Technological Education Thirteenth Annual Report, 1960–61 Pp 39 (London London and Home Counties Regional Advisory Council for Technological Education, 1962) [174

Department of Technical Co-operation Technical Co-operation a Progress Report (Cmnd 1698) Pp 18 (London H M Stationery Office, 1962) 1*s* 3*d* net [184

British Broadcasting Corporation B B C Engineering Monograph No 41 The Design of a Group of Plug-in Television Studio Amplifiers By K J Austin Pp 19 (London British Broadcasting Corporation, 1962) 5*s* [184

Department of Scientific and Industrial Research National Physical Laboratory Mathematical Tables, Vol 5 Chebyshev Series for Mathematical Functions By C W Clenshaw Pp iv+36 (London H M Stationery Office, 1962) 12*s* 6*d* net [194

Bulletin of the British Museum (Natural History) Entomology Vol 12, No 1 The British Pseudococcidae (Homoptera Coccoidea) By D J Williams Pp 1–79 28*s* Vol 12, No 2 New African Caddis-Flies (Order Trichoptera) By D E Kimmins Pp 81–121 15*s* Geology Vol 5, No 9 The Morphology of *Botryopteris antiqua* Kidston By Henry Smith Holden Pp 359–380+plates 56–60 15*s* (London British Museum (Natural History), 1962) [194

Philosophical Transactions of the Royal Society of London Series B Biological Sciences No 719, Vol 245, (19 April 1962) On the Stalks of Certain Peritrichs By J T Randall, assisted by J M Hopkins Pp 59–79+plates 14–41 (London The Royal Society, 1962) 50*s* [194

Royal Radar Establishment *R R E Newsletter and Research Review*, No 1, (March 1962) Pp i+54 (Malvern Royal Radar Research Establishment, 1962) [274

Steel Castings Development Committee Data Sheet 6 The Properties of Heat Resisting Steel Castings Pp 8 Data Sheet 7 The Properties of Corrosion Resisting Steel Castings Pp 9 (Sheffield The Steel Castings Development Committee, 13 Broomgrove Road, 1962) [274

Government of Northern Ireland Ministry of Agriculture Leaflet No 109 The Cultivation of Black Currants Pp 11 (Belfast Ministry of Agriculture, 1962) [274

National Institute of Oceanography Collected Reprints, Vol 9 Reprints Nos 324–361 (Wormley, Godalming National Institute of Oceanography, 1961) [274

Department of Scientific and Industrial Research National Physical Laboratory Units and Standards of Measurement Employed at the National Physical Laboratory 1 Length, Mass, Time-Interval and Frequency Angle, Volume, Density and Specific Gravity, Gravity, Force, Pressure and Hardness Third edition Pp ii+18 1*s* 6*d* net. 4 Temperature Pp ii+18 1*s* 6*d* net (London H M Stationery Office, 1962) [274

Flora of Tropical East Africa Edited by Dr C E Hubbard and E Milne-Redhead Buxaceae By Dr B Verdcourt Pp 3 1*s* 6*d* Fumeriaceae By G Ll Lucas Pp 5 2*s* Papaveraceae By G Ll Lucas Pp 3 1*s* 6*d* Taccaceae By Susan Carter Pp 3 1*s* 6*d* Theaceae By Dr B Verdcourt Pp 8 2*s* (London Crown Agents for Oversea Governments and Administrations, 1962) [274

Science and Society Eight Oxford Lectures Pp 94+2 plates (London Conservative Political Centre, 1962) 3*s* [274

The Association of the British Pharmaceutical Industry Annual Report and Year Book, 1961–62 Pp 40 (London The Association of the British Pharmaceutical Industry, 1962) [274

Belling and Lee, Ltd Some Mechanical Aspects of Design By N D Bryce and G F Redgrave Pp. 24 (Enfield, Mddx Belling and Lee, Ltd , 1962) [274

Water Conservation—England and Wales Pp 14 (Cmnd 1693) (London H M Stationery Office, 1962) 1s 3d net [274

Petroleum Information Bureau U K Petroleum Industry Statistics —Consumption and Refinery Production, 1960 and 1961 Pp 9 (London Petroleum Information Bureau, 1962) [15

Commonwealth Mycological Institute, Kew Phytopathological Paper No 4 Fusarial Wilt (Panama Disease) of Bananas and other Musa Species By Dr E H Stover Pp vi+117 (Kew, Surrey Commonwealth Mycological Institute, 1962) 20s net [15

Ministry of Health and General Register Office Report on Hospital In-Patient Enquiry for the year 1960 Part 1 Preliminary Tables Pp 16 (London H M Stationery Office, 1962) 2s net [15

Universities Council for Adult Education Report on the year 1960–61 Pp 24 (Liverpool Universities Council for Adult Education, 1962) [15

Other Countries

Board of Grain Commissioners for Canada, Winnipeg Grain Research Laboratory—1961 Report Pp v+82 (Ottawa Queen's Printer, 1962) [174

Annals of the New York Academy of Sciences Vol 96, Article 4 Mathematical Theories of Biological Phenomena By N Rashevsky and 13 other authors Pp 895–1116 Vol 98, Article 1 The Biology of the Trachoma Agent By Francis B Gordon and 47 other authors Pp 1–382 (New York New York Academy of Sciences, 1962) [174

Indian Central Jute Committee Agricultural Research Bulletin No 1 A Review of Agricultural Investigations on Jute in India By Dr J S Patel and R L M Ghose Pp viii+51 (Calcutta Indian Central Jute Committee, 1957) Rs 3/50 , 5s 3d [174

Commonwealth of Australia Department of Supply Australian Defence Scientific Service Annual Report of Defence Standards Laboratories for the year ended 30th June, 1961 Pp 49 (Maribyrnong, Victoria Defence Standards Laboratories, 1962) [174

United States Department of the Interior Fish and Wildlife Service Fishery Bulletin No 188 Distribution and Abundance of Skipjack in the Hawaii Fishery, 1952–53 By Herbert H Shippen Pp iv+281–300 20 cents Fishery Bulletin No 189 Abundance and Age of Kvichak River Red Salmon Smolts By O E Kerns, Jr Pp iv+301–320 20 cents (Washington, D C Government Printing Office, 1961) [174

National Science Foundation NSF–62–4 Current Projects on Economic and Social Implications of Science and Technology, 1961. Pp vii+116 (Washington, D C. Government Printing Office, 1962) 40 cents [174

Forest Research in India, 1958–59, Part 1 The Forest Research Institute Pp ii+92 (Delhi Manager of Publications, 1961) Rs 2 60 nP , 4s 3d [174

Commonwealth of Australia Department of Supply Aeronautical Research Laboratories Annual Report, 1960–61 Pp 38 (Melbourne Department of Supply, Aeronautical Research Laboratories 1962) [174

National Science Foundation Surveys of Science Resources Series NSF 62–3 Funds for Research and Development in Industry 1959—Performance and Financing Pp xi+109. (Washington, D C Government Printing Office, 1962) 60 cents [194

Report on the Fisheries of Nigeria, 1961 By A R Longhurst Pp 53 (Lagos Federal Fisheries Service, Ministry of Economic Development, 1962) [194

Comité International des Poids et Mesures Procès-Verbaux des Séances, 2e Série, Tome 28, (49e Session, 1960) Pp 161 (Paris Gauthier-Villars et Cie , 1962) [274

Australia Commonwealth Scientific and Industrial Research Organization Soil Publication No 19 Soil Studies in Central Australia Alice Springs–Hermannsburg–Rodinga Areas By E A Jackson Pp 81+1 plate—1 map (Melbourne Commonwealth Scientific and Industrial Research Organization, 1962) [274

Indian Forest Records, (New Series) Entomology Vol 10, No 4 Control of the Mound-Building Termite (*Odontotermes obesus*) in India with Chlorinated Hydrocarbons etc By Dr M L Roonwal and Dr P N Chatterjee Pp 67–78+4 plates (Delhi Manager of Publications, 1961) Rs 1 70nP , 2s 6d [274

Canada Department of Mines and Technical Surveys Geological Survey of Canada Geological Maps Index Sheet 1 Newfoundland Index Sheet 2 Newfoundland Index Sheet 11 Nova Scotia–Newfoundland Index Sheet 12 Quebec–Newfoundland Index Sheet 20 Nova Scotia Index Sheet 21 New Brunswick–Nova Scotia (Ottawa Director, Geological Survey of Canada, 1960) [274

Food and Agriculture Organization of the United Nations The Rufiji Basin, Tanganyika Report to the Government of Tanganyika on the Preliminary Reconnaissance Survey of the Rufiji Basin Vol 7 Soils of the Main Irrigable Areas By B Anderson With note on the Physical Properties of the Soils at Four Localities in the Rufiji Basin By H C Pereira Pp iv+125+19 maps (Rome Food and Agriculture Organization of the United Nations) [274

Canada Department of Mines and Technical Surveys Annual Report, Calendar Year 1960 Pp vii+61+12 plates (Ottawa Queen's Printer, 1961) 1 dollar [274

Companhia de Diamantes de Angola (DIAMANG) Servicos Culturais Dundo–Lunda–Angola Museu do Dundo Publicacoes Culturais No 54 Subsidios para o Estudo da Biologia na Lunda—Estudos Diversos (XXI) Pp 191 (Lisboa Companhia de Diamantes de Angola, 1962) [274

Canada Department of Mines and Technical Surveys Geological Survey of Canada Bulletin 81 Palaeontology, Stratigraphy, and Structure of the Jurassic Rocks in Salmo Map-Area, British Columbia By Hans Frebold and H W Little Pp ix+31+plates 1–5 1 dollar Paper 61–29 Upper Devonian Formations, Southern Part of Northwest Territories, Northeastern British Columbia, and Northwestern Alberta By Helen R Belyea and D J McLaren Pp iv+74 50 cents (Ottawa Queen's Printer, 1962) [274

Unesco The Problems of the Arid Zone Proceedings of the Paris Symposium (Arid Zone Research, Vol 18) Pp xii+481 (Paris United Nations Educational, Scientific and Cultural Organization , London H M Stationery Office, 1962) 47 25 NF , 69s , 13 50 dollars [15

Norsk Polarinstitutt Skrifter Nr 123 On the Snow Accumulation in Dronning Maud Land By Tornjørn Lunde (Den Norske Antarktisekspedisjonen, 1956–60 Scientific Results, No 1) Pp iii+48+4 plates (Oslo Norsk Polarinstitutt, 1961 Distributed by Oslo University Press, 1961) 9kr [15

Radcliffe Observatory, Pretoria Radcliffe Observatory Memorandum I Magellanic Cloud Coordinates—Coordinates of Harvard Variables, CPD Stars near Small Cloud, and HDE Stars in Large Cloud, (Tables 1 and 2) Pp 20 (Pretoria Radcliffe Observatory, 1961) [15

United States Department of the Interior Geological Survey Professional Paper 353 Beryl Resources of New Hampshire By James J Page and David M Larrabee Pp iv+49+16 plates (Washington, D C Government Printing Office, 1962) [15

Food and Agriculture Organization of the United Nations FAO Agricultural Development Paper No 70 Meat Handling in Underdeveloped Countries—Slaughter and Preservation By I Mann Pp xi+200 (Rome Food and Agriculture Organization of the United Nations , London H M Stationery Office, 1960 10s , 2 dollars [15

Museum of Comparative Zoology at Harvard College Breviora No 150, (January 5, 1962) The Genus *Bethylus* in North America (Hymenoptera Bethylidae) By Howard E Evans Pp 12 No 151, (January 12, 1962) A New Phyllocarid Crustacean from the Upper Devonian of Ohio By W D Ian Rolfe , Pp 7-(1 plate) No 152, (January 15, 1962) New Australian Dacetine Ants of the Genera *Mesostruma* Brown and *Codiomyrmex* Wheeler (Hymenoptera–Formicidae) By Robert W Taylor Pp 10 No 153, (February 15, 1962) *Anolis scriptus* Garman 1887, and earlier name for *Anolis leucophaeus* Garman 1888 By A Stanley Rand Pp 5 No 154 (April 4, 1962) Notes on Hispaniolan Herpetology 5 The Natural History of Three Sympatric Species of *Anolis* By A S Rand Pp 15 (Cambridge, Mass Museum of Comparative Zoology at Harvard College, 1962) [15

Bulletin of the American Museum of Natural History Vol 123, Article 6 New and Little-Known False Scorpions, principally from Caves, Belonging to the Families Chthoniidae and Neobisiidae (Arachnida, Chelonethida) By Joseph C Chemberlin Pp 299–352 (New York American Museum of Natural History, 1962) 1 dollar [15

Rose Polytechnic Institute, Terre Haute, Indiana Bulletin XLVII, No 3, (April, 1962) 1962–63 Catalog Pp 300+12 plates (Terre Haute, Indiana Rose Polytechnic Institute, 1962) [15

Commonwealth of Australia Australian Atomic Energy Commission Ninth Annual Report for the year ended 30th June, 1961 Pp 84. (Coogee, N S W Australian Atomic Energy Commission 1962) [15

Institute of Applied Science of Victoria Report of Activities for the year ended 30th June, 1961 Pp 24 (Melbourne Institute of Applied Science of Victoria, 1962) [15

Skrifter fra Danmarks Fiskeri- og Havundersøgelser, Nr 22 Fiskeriundersogelser i 1961 ved Danmark, Faerøerne og Grønland Ved E Bertelsen og Paul M Hansen Pp 105 (København I Kommission Hos C A Reitzel, J Jørgensen & Co Bogtrykkeri, 1962) 4 00 kr [15

Sitzungsberichte der Sächsischen Akademie der Wissenschaften zu Leipzig Mathematisch-naturwissenschaftliche Klasse, Band 105, Heft 1 An der Grenze Zwischen Hochpannungstechnik und Physik Von Fritz Obenaus Pp 19 (Berlin Akademie-Verlag, 1961) 2 40 D M [15

Canada Department of Mines and Technical Surveys Geological Survey of Canada Bulletin 79 Paleaomagnetism of the Monteregian Hills, Southeastern Quebec By A Larochelle Pp x–44 (4 plates) (Ottawa Queen's Printer, 1962) 75 cents [15

Uganda Electricity Board Fourteenth Annual Report and Accounts for year ended 31st December, 1961 Pp 48 (Kampala Uganda Electricity Board, 1962) 5s [15

United States Department of the Interior Geological Survey Bulletin 1028–S Geology of Unalaska Island and Adjacent Insular Shelf, Aleutian Islands, Alaska By H Drewes, G D Fraser, G L Snyder and H F Barnett, Jr Pp vii+583–676+plates 75–78 Bulletin 1142–H Future Petroleum Producing Capacity of the United States By A D Zapp Pp iii+36 Professional Paper 335 Characteristic Lower Cretaceous Megafossils from Northern Alaska By Ralph W Imlay Pp iv+74+20 plates 2 50 dollars Professional Paper 349–A Geology of the Richardson Cove and Jones Cove Quadrangles, Tennessee By Warren Hamilton Pp iv+55+1 plate Professional Paper 367 Landslides Along the Columbia River Valley, Northeastern Washington By Fred O Jones, Daniel R Embody and Warren L Peterson Pp v+98 Professional Paper 374–C Jurassic (Bathonian or Early Callovian) Ammonites from Alaska and Montana By Ralph W Imlay Pp iii+32+8 plates Professional Paper 374–D Late Jurassic Ammonites from the Western Sierra Nevada, California By Ralph W Imlay Pp iii+30+6 plates Professional Paper 384–A The Serpentine-Group Minerals By George T Faust and Joseph J Fahey Pp v+92 (Washington, D C Government Printing Office, 1961 and 1962) [15

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO , LTD.
ST MARTIN'S STREET, LONDON, W C 2
Telephone Number Whitehall 8831 Telegrams Phusis Lesquare London

Annual subscription £10, payable in advance,
postage paid to any part of the world

Advertisements only should be addressed to
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Telephone Number Holborn 4743

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BRITISH TECHNICAL CO-OPERATION OVERSEAS

THE White Paper on future policy in recruitment for service overseas which the Department of Technical Co-operation has recently issued is of interest for two main reasons First, for its exposition of the principles which the Department is using and proposes to use in future for the recruitment of staff for scientific research and other professional service overseas Secondly, for its indication of the scale of such recruitment, of which the surveys of scientific and technical manpower have hitherto given no indication In 1961 some 581 appointments to the British Overseas Dependent Territories were made through the Department compared with 816 made in 1960 through the Colonial Office, 1,083 in 1959, 1,335 in 1958, and 1,367 in 1957 During the same period, 188 appointments were made under the Colombo Plan to India, Pakistan, Ceylon and Malaya, the annual total varying from 28 in 1959 to 49 in 1957, appointments made from Britain under the Plan to Burma, Laos, Indonesia, Cambodia, Vietnam, Nepal and Thailand totalled 69 Under the Special Commonwealth Africa Assistance Plan coming into operation in 1960 and earlier bilateral arrangements, 107 appointments have been made to Ghana, Nigeria and Sierra Leone Some 7 appointments have been made to Liberia, Guinea, Ivory Coast and Togoland under the Foundation for Mutual Assistance in Africa South of the Sahara, while not counting re-appointments, 57 long-term and 56 short-term appointments have been made to Turkey, Iran and Pakistan under the Central Treaty Organization (Cento)

In addition to this total of 5,659 appointments since 1957, Britain has supplied a further 14 short-term (non-Cento) appointments to Middle Eastern countries and 132 appointments to the Middle East and North Africa as well as 97 to United Nations and other international technical assistance assignments These figures show that the British contribution is not negligible either in relation to technical assistance as such or in relation to Britain's resources of scientific and technical manpower, if in scientific research, in particular, the total is unlikely ever to be large, it is important that conditions should be such as to ensure a flow of candidates of appropriate qualifications The White Paper rightly examines these conditions and the action being taken in respect of particular categories of recruitment, and the information it collects should be widely circulated and noted by the professional organizations concerned

It is recognized, for example, that if the needs of existing and projected universities in Africa and elsewhere are to be met, arrangements for the supply of staff for comparatively short periods will be important, and in co-operation with the British Council, the Department has set up a new body, under the chairmanship of Sir Charles Morris, to encourage and facilitate the supply of staff on a short-term basis through home universities Funds provided under the Commonwealth Teachers Act will be available, where necessary, to initiate projected schemes Again, in April last the Council for Technical Education and Training for Courses was established, with Mr F J Pedler as chairman, to give advice and expert assistance to the Government with the view of promoting technical and commercial education and training for developing countries This would include the provision of advice and information and promotion of contacts between those concerned with such education and training, the promotion and, where appropriate, undertaking of recruitment of staff for service overseas in this field and facilitating the training and education in the United Kingdom of trainees, teachers and others from developing countries The Department of Technical Co-operation will look to this new Council as its main channel of recruiting technical education staff from Britain for Government and other institutions overseas at technical institute level and above

The importance of the work of both these two new bodies is well brought out in the admirable article on the university and technical assistance which Dr R S Morison contributed to the spring issue of *Daedalus* Although he addressed his appeal in the first instance to the Massachusetts Institute of Technology, he made it clear that the part a great university can play in this field goes far beyond co-operating in the secondment of staff, and includes a fresh look at the organization of research itself and the functions and location of the research institute The Department of Technical Co-operation appears to be well aware of the problems that arise here, and in an important passage it examines some of those that are involved in the secondment overseas of staff from home-based establishments for scientific research in tropical countries, either for cadre posts on the establishment of an overseas Government or research organization, or for limited periods to investigate a particular problem

On a small but effective scale, the White Paper points out, pools of research workers have been established for some years in Britain from which experts can be drawn to tackle problems which arise in the dependent territories in certain specialized fields These pools are based on institutions in Britain, and during the periods spent in such institutions members of the pools are under the direction of the director of the institute concerned While financial provision for staffing and operating the pools has been made from Colonial Development and Welfare Funds, the financial responsibility on assignment to a dependent territory normally rests with the employing Government There are five such pools—entomologists, plant pathologists, soil scientists, stored products research workers and pesticides chemists—and they are based respectively on the Commonwealth Institute of Entomology, the

Commonwealth Mycological Institute, Rothamsted Experimental Station, the Pest Infestation Laboratory and the Tropical Products Institute, and there is also an additional post at the Fisheries Laboratory, Lowestoft, to permit the acceptance and study at the Laboratory of overseas problems.

Over a broader field, the Overseas Research Council has examined the problem of the recruitment and retention of expatriate research workers overseas and has concurred in a scheme, now officially accepted, advanced by the Medical Research Council. In this, provision will be made first to encourage a number of senior men overseas to continue in their work by providing them with a home base when their posting overseas would normally expire, secondly, to encourage senior specialized staff recruited to a home base cadre to work abroad, and thirdly, to encourage young research workers to take posts overseas while holding an appointment with the Council. At the instance of the Overseas Research Council, the Agricultural Research Council, too, has examined the possibility of tackling similar problems in agricultural research in the emerging countries of the Commonwealth and it has been agreed that the Agricultural Research Council may make conditional offers of future employment to selected scientists who would, if their overseas service terminated, be particularly suitable to fill vacancies under the Council.

The recent Commission on the Most Suitable Structure for the Management, Direction and Financing of Research on an East African Basis, over which Prof. A. C. Frazer presided, recognized that it would be necessary to recruit expatriate staff of high quality to maintain the interterritorial research services in East Africa for the next fifteen years*. Furthermore, for much longer it might be necessary to recruit a proportion of staff from outside East Africa to provide the wide range of specialized knowledge and experience needed. The Commission suggested that the United Kingdom Research Councils could encourage recruitment by offering a form of dormant contract which would assure, for example, two years employment in the United Kingdom at the end of the East African contract if the recruit did not wish to continue in overseas services. A system of dormant contracts is also suggested to ensure the retention of the services of experienced expatriate research workers in the East African Interterritorial Research Organizations, and also to assist the recruitment and retention of sufficient expatriate technicians to meet the needs of research and to expedite an East African technical training scheme.

These and other recommendations of the Frazer Report are still under consideration by the Governments of Kenya, Tanganyika, Uganda and Britain, and each of these Governments hopes to deal with the recommendations at an early date. Besides dealing with this question of staff they cover the organization of research and the maintenance of the interterritorial research organizations which are considered in a world as well as a territorial context. Some simplification in structure is suggested through the replacement of the existing interterritorial research councils by two new ones, the East African Medical Research Council and the East African Natural Resources Research Council, while formation of an East African Industrial Research Council should be considered. The crucial difficulty is finance. While the value of the Tropical Pesticides Research Institute at Arusha is fully recognized and its continuation recommended, the territories have stated that they cannot afford to provide support for this institute.

Even more serious is the position of fisheries research. Since the Frazer Report was issued the decision was taken last March to close the East African Marine Fisheries Research Organization at Zanzibar in face of the Commission's recommendation that the work should be continued for at least another three years and only a month before the station was to play a major part in the international expedition in the Indian Ocean. Fishery research stations at Freetown and Singapore were also closed when the territories concerned became responsible for their own affairs, although capital costs of laboratories and boats at all three stations have been met entirely by Britain out of Colonial Development and Welfare Funds. At Zanzibar ten years of valuable work had been completed, and it is clear that where the objectives are long-range—even when the results have had invaluable bearing on nutrition and the raising of standards of living—scientific and technical considerations alone are insufficient to ensure the maintenance of the work. Financial stringency is only too likely to lead to political considerations and the desire for rapid results to cause action detrimental to the long-term interests of the community.

Consideration of the position of fisheries research alone fully endorses much that Dr. Morison has argued as to the importance of the university in technical assistance, and it also demonstrates the vital importance of the work which is being undertaken by the Department of Technical Co-operation. That has recently been described in a progress report issued by the new Department*, but at the same time the experience suggests that it is not altogether sound policy to give aid only at the request of the country concerned. Fisheries research, if not also pesticides research, suggests that the emergent territories are not invariably aware of their needs, and that there are problems in which it should be possible to offer aid tactfully without being either arbitrary or dictatorial. In these problems, too, the organization of support on an international basis is not always simple or effective.

This is not to deny that the overseas countries themselves should say what help they most need, and it applies essentially to one only of the twelve main heads of technical assistance set out in the White Paper as showing broadly what the nation's efforts should be most closely geared to supply—the development of scientific research. It is worth noting that in this White Paper reference is again made to the contribution of the Overseas Research Council in matters of general policy, and it would seem that the Department of Technical Co-operation will not neglect that Council, which, from the White Paper, is fully alive to the importance of scientific research in overseas countries and is ready to assist with specialist advice, advisory visits, conferences to exchange information, training scientists from overseas in universities and research institutions in Britain, recruiting scientific staff from Britain, and support for existing research services overseas. The other main heads listed are: training of public servants, supply of university and secondary school teachers and staff for teacher training colleges, technical education and training, staff and training for economic and financial planning, geodetic and topographical surveys, geological surveys, and help in developing mineral resources, supply of staff, advice and training to develop agricultural and other resources, advice, staff and training for industrial development, engineering surveys and assistance in telecommunications, supply of qualified medical and nursing staff, and medical and nursing training, management training, and individual service overseas through voluntary societies.

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* East Africa High Commission. Report of the Commission on the Most Suitable Structure for the Management, Direction and Financing of Research on an East African Basis. Under the chairmanship of Prof. A. C. Frazer. Pp. xii+76. (Nairobi: Government Printer, 1961.) 5s.

* Department of Technical Co-operation. *Technical Co-operation: a Progress Report by the New Department.* Pp. 18. (Cmnd. 1698.) (London: H.M.S.O., 1962.) 1s. 3d. net.

The Department is seeking to secure that the technical assistance provided by Britain as a whole is properly focused on the needs of those it is seeking to help, and to remove some of the obstacles which hinder the free flow of such assistance. It cannot, however, hope to succeed in its task without the understanding and continued support and enthusiasm of many men and women in Britain, and of firms, voluntary societies, local authorities, professional, scientific and educational institutions and a wide range of organizations. These two White Papers provide an instalment of the publicity on which informed criticism and public support must be based. They deserve the attention of scientists and technologists and their professional institutions generally as well as of the universities, technical colleges and other research centres.

SCIENTISTS IN AMERICA

American Men of Science

A Biographical Directory. Edited by Jaques Cattell. The Physical and Biological Sciences. Tenth edition. A–E. Pp. ix+1–1126. F–K. Pp. 1127–2284. L–R. Pp. 2285–3510. S–Z. Pp. 3511–4586. (Temple, Arizona: The Jaques Cattell Press, Inc., Annex 15, Arizona State University, 1960 and 1961.)

THE tenth edition of this monumental work is now well on its way as a published reference book of prime importance.

But its title is a misnomer, yet, *American Men of Science: a Biographical Directory* is now so thoroughly entrenched among to-day's scientific literature, both because of the esteem in which it is held and owing to its traditional background (it was first published in 1906), that one cannot expect, nor would one wish to see, any basic change in the title-page. Nevertheless, it is not mere querulous criticism to direct attention to the misleading title because for the benefit of the most recent, and future recruits to the scientific fold, it should be emphasized that quite a distinct percentage of entries in this great 'Who's Who of Science' refers to people who are not Americans. There are, for example, biographies of men of science from other countries who, though now working and perhaps permanently settled in what was once known as 'The New World', still and perhaps always will for various reasons, prefer to retain their original nationality. Scientists in Canada are also included. Moreover, the editors have, quite wisely, not confined themselves to the continent of America so it is as well that the user should be up to date with his political geography, for here the fiftieth State is included—and some elegant scientific work is now being promoted in the University and various industrial (mainly agricultural) laboratories and fields in Hawaii.

Moreover, there are some distinguished American women of science, they also are included.

We consider it desirable to point out these features in order to emphasize the comprehensive nature of this great publication. The editors have clearly ignored the mischievous editorial adage 'When in doubt, leave out', and have spread their net very widely indeed, not restricting themselves as some new inquirer might naturally assume, to the United States of America. The area thus covered is wide indeed, but the editors have also delved deeply, for entries are devoted not solely to practical scientific workers in field and laboratory. Naturally, all the universities and more important colleges are covered and so also are Federal and State institutions (such as the renowned National Institutes of Health at Bethesda), the Armed Forces, museums and industry. Most of the personnel included in such organizations are an obvious choice for inclusion, but *American Men of Science* goes even deeper and includes others who are working for the advancement of science (most probably not at the laboratory bench) such as the various Foundations (for example, the Rockefeller and National Science Foundations). So also are people concerned with scientific publications. There are even some entries from high schools.

In the first edition of this valuable work of reference there were 4,000 biographies, the tenth edition, when completed, will contain 120,000.

The ninth edition was divided into three volumes: (1) Physical Sciences, (2) Biological Sciences, (3) Social and Behavioral Sciences. It was a wise decision partially to modify this classification in the new edition, for so much has science advanced, and so much do the multitude of scientific disciplines now overlap, that to-day there are many distinguished scientists who, quite justifiably, could be placed in two, perhaps even all three, of the former, ninth-edition volumes.

The tenth edition is to be divided into five volumes. The first four now published, cover the physical and biological sciences—such terms being very widely interpreted. It is hoped to publish the fifth and final volume later this year; this will comprise the social and behavioural sciences. It is conceivable that in due course the editors will decide to jettison even these two categories (social and behavioural). We note, for example in the present physical and biological sciences volume, A–E, the name of a psychiatrist whose main field of interest is stated to be psychiatry and religion. When this book is completed and the fifth volume appears, we can well imagine an inquirer referring to the fifth volume for this entry So perhaps the editors, when preparing the eleventh edition of *American Men of Science,* will seriously consider no divisions at all, but to produce the work in a convenient number of volumes with all entries going straight from A to Z In this case, once the scientist's name is known, the inquirer can immediately select the right volume before he opens its pages

Each entry gives in capital type the name, present professional position and special field of scientific interest or activity Professional (and in many cases private) addresses are presented in smaller type so is the rest of the biography—place and date of birth, whether married, number of children (if any), membership of relevant committees, societies and academies (both at home and abroad), university degrees and any other distinctions, and finally the special scientific interests of the person being noted

American Men of Science is now well past its half-century of publication It was initiated by James McKeen Cattell, and first published as a manuscript reference list for the Carnegie Institution of Washington The founder of this great work was also at one time publisher and editor of the world-renowned American weekly journal, *Science* Eventually, *Science* was handed over to the American Association for the Advancement of Science, and is at present the responsibility of our friends, the secretary of the Association, Dael Wolfle (as publisher), and Dr Graham DuShane (as editor) We are sorry to learn that Graham DuShane is about to resign, but wish him well in the distinguished position he is about to occupy We would also like to take this opportunity of extending a welcoming hand to an equally distinguished scientist, Dr Philip Abelson, who is soon to succeed Graham DuShane in the editorial chair of *Science* (see also p 122 of this issue)

The founder of *American Men of Science,* Dr James McKeen Cattell, devoted more than thirty-five years to it, after which he was succeeded by his son, Jaques Cattell In 1958, Jaques transferred the publication of this work to the campus of Arizona State University at Tempe There, with the assistance of a great and expanding University, he began the production of this tenth edition It must have been a grievous loss to all concerned with this project when, in 1960, Jaques Cattell died However, his nephew, Garrison Cattell, continued the work as vice-president of the Jaques Cattell Press and with Mrs Dorothy Hancock as editor Then, last year, the Bowker Co of New York took over the Jaques Cattell Press This Company had already been publishing the ninth edition under Jaques's editorship, and now promises that it intends "to maintain and continue the work in Tempe and proceed with the plans made by Mr Cattell, maintaining the same close liaison with professional associations and holding the same high standards which have characterized this 'saga of American science' for more than half a century" This makes good reading, for though the "professional associations" concerned are senior in character, and, in this project, come under the auspices of the U S National Academy of Sciences—National Research Council and the American Association for the Advancement of Science, *American Men of Science* was conceived and reared, and has obviously ever since been cherished, by the family Cattell, to whom the entire world of science will always be indebted

BACTERIAL ENERGETICS

The Bacteria

A Treatise on Structure and Function Edited by I C Gunsalus and Roger Y Stanier Vol .2 Metabolism Pp xv + 572 (New York Academic Press Inc , London Academic Press, Inc (London) Ltd , 1961) 15 dollars

UNLIKE the first volume this one includes only one contribution from laboratories outside the United States But surely this sentence from the preface is not even American "Thus with the main emphasis shifted to other aspects of cellular behaviour, one may expect for energy metabolism that gaps in knowledge will eliminate gradually, and some hypotheses altered, but the bases for reasoning will, in the main, be sustained" One may wonder if by titling Volume 2 *Metabolism* and Volume 3 *Biosynthesis* we are to suppose that biosynthetic processes are non-metabolic, and if so, why do both volumes contain a chapter on photosynthesis In fact it is early pointed out that the aim of the volume is to provide an informed concept of the energy-liberating systems encountered in bacteria The large number of references to metabolic processes in yeasts is easily excused in view of the great volume of related and readily applicable information which has been obtained from the study of yeast systems

The book is divided overall into chapters dealing with fermentations, oxidations and 'light-involving' systems The first chapter is a very good general survey of "energy-yielding metabolism in bacteria", while those that follow deal in some detail with the fermentation of carbohydrates and nitrogenous compounds, cyclic mechanisms, dissimilation of high-molecular-weight compounds, microbial electron transport systems, aerobic and anaerobic cytochrome systems, cytochrome-independent systems, bacterial photosynthesis and luminescence The chapter on photosynthesis surveys the evidence obtained from experiments with chromatophore extracts, on this a general formulation of bacterial photosynthesis has been based and a comparison with plant photosynthesis made The discussion of carbon dioxide fixation has been left to Volume 3 The chapter on bacterial luminescence is of special interest for a number of reasons Comparatively little is heard about this fascinating property, and this chapter is engagingly and well written (except for "Pfluger demonstrated that the bacteria from fish was filterable") Bioluminescence is in many ways the reverse of photosynthesis and the light-energy emitted provides a visible indication of the metabolic activity of the cells which enables the effects of added metabolites, narcotics, etc , to be measured

The wealth of well-documented information in this volume illustrates once more the great utility o micro-organisms as objects of biological investigation Their very high metabolic rate coupled wit the advances made in radioisotopic methods hav enabled the whole tempo of biochemical discover to be increased and this volume is an excellent surve of the present state of these discoveries in regar to the energy supply of bacteria Unfortunately, th type of logic used in making conclusions about bio chemical activity in complex living systems does no permit one to state that the entire contents ar immutably true Some of the ideas expressed wi no doubt be modified, but it is because of publication of this kind that a realization of the whole truth wi be more speedily obtained.

A useful addition would have been a separate glossary for the numerous abbreviations used in the text. Policy on how they were to be introduced was undecided; in two chapters they are collected in a footnote on the first page, while in the rest they pop in and out of brackets. There are more than 1,700 references, and excellent author- and subject-indexes enhance its value to the practising biochemist while for the serious microbiologist in any field it provides the perfect reference book into which he will find himself continually delving in search of the fundamental explanation of his observations.

L. B. QUESNEL

NON-LINEAR CONTROL SYSTEMS

Stability in Nonlinear Control Systems

By Alexander M. Letov. Translated from the Russian by J. George Adashko. Pp. 316. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1961.) 68*s*. net.

THE Russian school of mathematical engineers is noted for the detailed and painstaking approach which it has made to many of the problems of control theory. This approach, and the use made of it in the U.S.S.R., differs markedly from the semi-empirical method which is customary in Britain and was, until quite recently, superior even to the American attitude to design.

In recent years translation of Russian papers and books have acted as a spur to Western engineering education and each new one is anxiously scanned to see if, to put it crudely, we have missed something!

One of the more significant of the recent translations for engineers is Popov's monumental *Dynamics of Automatic Control Systems*. This starts with the most elementary assumptions, both of previous knowledge of control systems and of the mathematical methods needed to discuss them, and is well suited to the needs of engineers trained in the British tradition.

Letov's book, dealing as it does with non-linear problems, is of a quite different kind. Naturally, a discussion of such material could scarcely be elementary, although some of the presentations given by Lefschetz and particularly by Cartwright, have at least the merit of being 'interesting enough' to encourage the reader to make the necessary effort. But the austere axiomatic and analytical approach of Letov makes the text one of the most difficult that I have encountered. To be fair it must be said that the formal mathematics needed to understand the book is quite limited; the difficulty lies, however, in the author's failure to relate his equations to the physical realities beloved of the engineer. There is scarcely a single 'number' in the book and no graph or diagram which is thought worthy of a scale or even a caption, a state of affairs which makes puzzling the remark, on the dust-jacket, that "—it does not hesitate to go into abundant details of computation . . ."

These criticisms should make it clear to readers of this review that the book is of far more use to mathematicians than to engineers. There are comprehensive discussions of Lyapunov's direct method for investigating stability, Hurwitz's theorem and Lure's transformations. The reduction of the equations of control systems to various Canonical forms is illustrated by reference to the problem of the 'Isodrome' regulator and, after discussing the stability of unsteady motion, the author examines the effects of velocity feedback and thus leads finally to an examination of the theory of the stability of the bicycle. Lest this everyday conclusion should raise hope of practical results it should be mentioned that the book ends with an analytical expression from which the reader is invited to decide the point for himself!

A. D. BOOTH

ANIMALS OF BRITAIN

Animals of Britain

No. 1: Badgers. By Dr. Ernest G. Neal. No. 2: Horseshoe Bats. By John Hooper. No. 3: Hedgehogs. By Maxwell Knight. No. 4: Water Voles. By Stéphanie Roden Ryder. No. 5: Grey Squirrels. By Monica Shorten. No. 6: Red Squirrels. By Monica Shorten. No. 7: Grey Seals. By H. R. Hewer. No. 8: Otters. By E. G. Neal.

Each number Pp. 24. (London: Sunday Times Publications, Ltd., 1962.) 3*s*. 6*d*.

THE Sunday Times Publications, Ltd., is producing a series of booklets called *Animals of Britain*. Altogether there will be about twenty-six booklets, each dealing with one animal (with the exception of bats, when eleven different species will be divided between four booklets). So far, eight booklets have been published; another eight will appear in the autumn, and the remainder at intervals thereafter. The series is edited by Dr. L. Harrison Matthews.

Those already available are *Badgers*, by E. G. Neal; *Horseshoe Bats*, by John Hooper; *Hedgehogs*, by Maxwell Knight; *Water Voles*, by S. R. Ryder; *Grey Squirrels*, by Monica Shorten; *Red Squirrels*, also by Monica Shorten; *Grey Seals*, by H. R. Hewer; and *Otters*, by E. G. Neal. It can be seen that many well-known naturalists appear among the authors.

Each booklet contains twenty-four pages. These are filled with a large number of black-and-white photographs, some informative diagrams and a detailed, though uncomplicated, text. Many of the photographs are unusual shots, including those of very young creatures and some, as yet, unborn. The diagrams are of such things as the layout of a badger's sett, the distribution of an animal, or comparisons. Except in Booklet No. 2, *Horseshoe Bats*, the text is subdivided under such headings as general appearance, distribution, habitat, food and feeding habits, the senses, breeding cycle, enemies, peculiarities, and general habits. This makes the text very easy to follow. In both *Hedgehogs* and *Red Squirrels* there is a section on legends. *Badgers*, *Grey Squirrels* and *Water Voles* contain sections suggesting how best to watch these animals. Hedgehogs as pets is another short section. Each booklet is concluded with a short list of books for further reading, and a full-page diagram of the skeleton of the animal.

The front cover of each booklet is attractive, with a colour photograph. The price is very reasonable, considering the information contained and the good presentation. Many adults will be interested to have all the details put before them in such a straightforward manner, and it will be an excellent and reliable series for schools to have in their libraries. Young naturalists are sure to be waiting in eager anticipation for the rest of the series to be published.

B. J. G. MAITLAND

Diagnostic Microbiology

A Textbook for the Isolation and Identification of Pathogenic Microorganisms By Prof W Robert Bailey and Elvyn G Scott Pp 327 (Saint Louis The C V Mosby Company, London Henry Kimpton, 1962) 48*s* 6*d*

THIS work is presented in ten parts, the first of which, a classification of the plant kingdom, is irrelevant and should be relegated to a work on taxonomy The second part, entitled "Laboratory Methods", consists of four chapters dealing superficially with the cultivation, microscopical study, staining and isolation of micro-organisms Three short chapters on the collection of specimens, their transport to the laboratory, and their handling there, comprise part three The fourth part, containing ten chapters, is concerned with the cultural isolation of pathogenic bacteria and *Candida albicans* from the natural cavities and ducts of the human body, from the blood and contaminated blood bank specimens, and from cerebrospinal fluid, wounds, morbid exudates, and autopsy material

Methods for the identification of these pathogens are discussed in the fourteen chapters embodied in the fifth part, which is succeeded by a brief sketch of laboratory procedures for the diagnosis of viral and rickettsial diseases There follows a somewhat more informative sketch of methods for the laboratory diagnosis of the systemic mycoses Part eight deals with antibiotic sensitivity tests and its successor with miscellaneous serological procedures The final part is divided into three chapters which deal with the composition and preparation of sixty-eight media, the formulæ and use of ten stains and two mounting fluids, and the composition and use of ten reagents respectively

Apart from a number of line drawings—of which the one depicting *Candida albicans* could be redrawn with advantage—in the mycoses section, the work is not illustrated The text is explicitly written and presented in clear type, but the work in general suffers from the attempt to cover too wide and varied a field in too small a compass, with the inevitable result that too little information is provided for graduate bacteriologists and it is too diffuse for students reading for degrees in medicine and dentistry As an introductory work it is useful for students taking a course in biology

J F D SHREWSBURY

Annual Review of Physical Chemistry

Vol 12 Edited by H Eyring in association with C J Christensen and H S Johnston Pp vii + 514 (Palo Alto, Calif Annual Reviews, Inc , 1961) 7 dollars

THIS volume give a brief summary of papers published in 1960 in many branches of physical chemistry Like the previous volumes in this series, it is valuable to research workers since it provides a concise and yet almost complete review of work done in a particular field All the volumes in the series also provide a useful starting point from which to review special topics The author-index and extensive subject-index are particularly useful in this respect

The editors have again selected a competent group of contributors who are largely from the United States The articles are essentially reviews rather than critical accounts of the subject, since the latter would require a book of considerably larger scope and size In keeping with the policy outlined in the preface of Volume 1 of the series, a new set of authors is selected again to obtain somewhat different points of view on subjects bordering on physical chemistry and to change the emphasis of the importance of any aspects of the subject according to the writer's point of view

The subjects selected for review vary from year to year according to current interest In comparing this with the 1960 volume, a new section on marine geochemistry by E D Goldberg is included The large aqueous chemical system comprising the world's oceans seems to be attracting the interest of many physical chemists at the present time A clear account is given of some of the chemical reactions which occur and the parameters which influence the course of such reactions Elemental and isotopic abundances are discussed, and in certain cases they appear to have remained constant for 2 5 × 10^8 years The change in present-day interests is also demonstrated by the omission of sections on chromatography and photosynthesis

The material of Volume 12 is well presented in clear print, as in previous volumes of this series

A B CALLEAR

British Transistor, Diode and Semiconductor Devices Data Annual, 1962–63

Edited by G W A Dummer and J Mackenzie Robertson Pp lv + 935 (London and New York Pergamon Press, 1962) 140*s* , 25 dollars

THIS volume collects together the data sheets on semiconductor devices issued by British manufacturers and by overseas firms with manufacturing facilities in the United Kingdom The advantages of such a volume over collections of manufacturers' data sheets are two-fold all the data are to be found compactly in one place, and the excellent indexing provided allows the device desired to be identified rapidly Devices are classified throughout in three groups transistors, diodes and (other) semiconductor devices Reference to devices can be made from either a list of application classifications or by manufacturers' type number Under the heading "Applications", transistors are divided into 17 classes, while diodes are listed according to order of peak inverse voltage and are also divided into eight application categories, other semiconductor devices are listed according to their nature—photoconductive / photovoltaic cells, silicon-controlled rectifiers, etc

Indexing by name of manufacturers has been made particularly useful by including a brief description of each device, including its physical nature, field of application, significant aspects of performance (for example, frequency range, voltage or power rating), and any feature of special note

The volume also includes a useful short introductory chapter giving a brief description of typical construction of semiconductor devices and their relevant properties, illustrated by diagrams showing physical configurations

The data sheets themselves are reproductions of those issued by the manufacturers concerned Their juxtaposition directs attention to considerable variation in the selection and presentation of data by different firms To have reduced the material to a consistent form and notation, however, would have been a well-nigh impossible task

A R BOOTHROYD

ON MAKING MUSIC

SUBSTANCE OF A FRIDAY EVENING DISCOURSE AT THE ROYAL INSTITUTION, LONDON, ON MAY 18

By Dr H LOWERY

Principal, South-West Essex Technical College and School of Art, London, E 17

Have you a Musical Ear?

THE vast growth in acoustical engineering which has arisen through the reproduction and recording of sounds has led to extensive research in the properties of the ear and the conditions of hearing Psychologists and physicists have both made outstanding contributions and have almost revolutionized the approach to the traditional acoustics Though space will not allow discussion of the extensive and complicated subject of what constitutes musical ability, some simple facts of hearing may be mentioned As is well known, the range of hearing is approximately 20–20,000 vibrations (or cycles) per second though most adults do not reach this upper limit Some individuals may have tonal gaps in this range, to deal with which 'acoustical spectacles' may have to be provided to cope with the deafness

This is a bare fact indicating that the aural instrument, perhaps aided, responds serviceably in the simplest possible way Music, however makes much more exacting demands It expects the ear to be capable of discriminating between very slight differences in pitch, and in intensity or loudness, from which we readily appreciate that the simple sensation of hearing is by itself not sufficient A mental judgment is involved Hence the popular term 'musical ear' is obviously only a half-truth

We have to realize that in music we are concerned not with individual notes and chords but with their relations with one another Thus notes are played together to form 'chords', or in sequence to form 'melody' It is important to remember that notes in music are never treated in isolation Even if a given note or chord is sounded by itself, the mind automatically relates it to aural images of previously heard sounds

The effect of any one chord depends on the chords which preceded it The study of the sequence or resolution of chords is, in actual fact, a very important part of musical theory called 'harmony', and though this study may reveal fashions based on temporary preferences for certain particular chordal sequences, nevertheless harmony as a subject is an aspect of musical education which cannot be neglected

Considerations of the above facts soon make it evident that the scientific and musical approaches to sound are quite different The physicist is content to study the means of production and reproduction of single sounds If he discusses the effect of sounds in combination he is still interested in the effect as a single phenomenon When combinations of sounds, namely, chords, are compared with one another and the effect of their sequence studied, we enter on the province of the musician

Pianoforte Tone

This difference in point of view is particularly well marked in considering the production of tones on the pianoforte

Some years ago certain American physicists (Hart, Fuller and Lusby) examined the tones produced by the pianoforte string using a cathode ray oscillograph It appeared that it did not matter whether the pianoforte key was depressed by dropping a brick on it, or pushing it down with the end of an umbrella, or allowing a tiny tot to try his hand, the general shape of the wave curve in each case seemed to be the same

There were folks who somewhat hastily jumped to the conclusion that this experiment had debunked all pianoforte teaching Pianists have long emphasized that there exists an almost infinite variety of 'touches' ranging from *legatissimo*, involving the closest binding of notes in succession, to *staccatissimo*, by which they imply the quickest release which can be made to the pianoforte key It is through the unique use of these touches by the master pianists that their personalities become manifest

In discussing this matter, physicists have emphasized that the only thing the pianist can control is the speed of depression of the piano key which in turn means that he is also controlling the speed at which the hammer strikes the strings with consequent changes of loudness All this happens before the key has reached the bottom of its fall To the pianist, this is not new information Piano teachers deprecate pressing the key into its bed, for they know that once the hammer has struck the string no further variation in tone is possible

It should be emphasized again that the physicist is satisfied when he can explain the mode of production of a single note whereas the musician is concerned with the effect that follows from the relatedness of notes to each other It is obvious from very elementary considerations that if the piano string is struck very forcibly, a loud tone is produced with a considerable overtone content, whereas if only feebly struck the higher overtones will scarcely be heard so that a difference in quality between the loud tone and the feeble one will be produced This difference between successive tones on the piano may be so slight as to elude the cathode ray oscillograph but yet may readily be picked up by the ear, which is itself an excessively sensitive instrument It is these subtle variations which the trained pianist is able to make through his fine control over the key action that constitute the life-blood of pianoforte playing They are vital and are charged with emotion in the effects they produce They enable him to impart dynamics to the music besides rendering the music meaningful through skilful phrasing True, the scientist may rightly pride himself on his objectiveness and regard himself as 'untemperamental' But the musician must be subjective in his outlook Tonal perception involves mental judgment This is subjective Music is an activity of the mind

Vibration of Strings

String instruments of the violin family play an important part in musical performance The laws of vibrations of strings are, of course, well known, particularly that which expresses the fact that the pitch of the note produced is inversely proportional to the length of the vibrating segment In the violin, for example, the player presses or stops the string against the finger-board at various points and so is able to alter the length of the vibrating segment to give the note he requires

Moreover, a musical sound is accompanied by overtones the frequencies of which are derived from the series of natural numbers By lightly touching a vibrating string at various points it is possible to isolate certain of these overtones and so make them more obvious

It is apt to direct attention to two simple but none the less fundamental experiments In the first we recall a fact of great importance in practical music, namely, that if two sources of sound are in unison and one of them is set in vibration, the other picks up these vibrations and responds 'sympathetically'

The second experiment was originally described in the *Transactions of the Royal Society*, in 1677, by Dr John Wallis, Cambridge mathematician, and a friend of Newton ("On the Trembling of Consonant Strings—a New Musical Discovery") The actual work was done independently by two young enthusiasts for the new experimental philosophy, namely, Mr William Noble of Merton College, and Mr Thomas Pigot of Wadham College, Oxford They made a string vibrate sympathetically in aliquot parts by sounding an adjoining string of different pitch Their early experiments were done on a lute They then constructed a special piece of apparatus consisting of strings stretched over a flat board and demonstrated nodes and anti-nodes during vibration with the use of paper riders This seems to have been the earliest use of the sonometer

In the technique of violin playing a special department is devoted to this production of overtones through lightly touching the strings at various points The tones produced in this way are known as harmonics

An amusing incident may be mentioned here involving the great Dr William Whewell, a former Master of Trinity College, Cambridge, and author of the first *History of the Inductive Sciences* One of his pupils was the Rev R H Haweis, who afterwards became rector of St George's, Hanover Square, London Haweis was a creditable performer on the violin and played solos regularly at Cambridge concerts At one of these he disported himself in a show piece by de Beriot, throwing off harmonics with the greatest of ease This led Whewell to remark with characteristic frankness "Mr Haweis, I have never held your scholarly attainments in high regard but how you produce those harmonic notes without resort to a foot-rule to sub-divide the violin strings leads me to take a new estimate of your capabilities"

The violin playing of Paganini has become legendary He undoubtedly made significant contributions to the technique of playing the instrument and evolved modes of production of violin tones which were new and startlingly effective

On December 9, 1831, Paganini performed in the ballroom of the Old Ship Hotel, Brighton—still commemorated by a plaque in the room This concert aroused particular interest because of the alleged excessive charge for tickets to hear "a mere foreigner" play the fiddle at a time when the country had so many needy poor Nevertheless, the concert was packed, and, as was usually the case when Paganini played, the audience was whipped into a frenzy

Mr Walker, an enthusiastic amateur, after hearing the concert, wrote to his friend, Mr Pringle, in Shrewsbury, to say that in spite of all criticisms the concert would have been worth ten times the charge to see this wizard play the violin in a way "that defied the very laws of nature" Mr Pringle waited two years to confirm his friend's opinion On August 15, 1833, Paganini gave a concert in the Golden Lion Hotel, Shrewsbury (commemorated by a plaque), whereon Mr Pringle wrote to Mr Walker "My Friend, you indeed spoke but moderately when you opined that Signor Paganini defied the very laws of nature This *maestro* is not a wizard defying the laws of nature, indeed, he is the very god that makes the laws of nature'

Here we have two testimonies that Paganini did something unexpected What is the explanation? It derives from the law which states that the pitches of the notes from vibrating strings are inversely proportional to the lengths of the vibrating segments The violinist normally obtains his high-pitched notes by shortening the vibrating segments obtained from pressing on the finger-board at points nearer the bridge But Paganini made extensive use of harmonics and touched the string at positions farther away from the bridge Hence he seemed to be defying the inverse law by doing the opposite of what was expected

Old Italian violins are greatly prized by players to-day, particularly those by Stradivarius, so much so that most modern violin makers have concentrated on copying the old masters An air of mystery seems to surround the subject Many have sought, without success, a so-called Stradivarius secret It has, for example, been attributed to the kind of varnish used or to the choice of wood from a certain district in the Alps with a favourable sunny aspect, or from the thicknesses of the plates The number of fine old instruments available is far short of world needs hence it is important that modern makers should be encouraged Time will, of course, add its own contribution to their work

I have recently had discussions with a present-day maker, Mr Ernest Cowell, formerly of Norwich and now of Basildon, Essex, who has done some original thinking on the making of violins He does not copy old instruments, but is able to take a piece of wood and mark it out from acoustical principles even to the final placing of the bridge and sound-post It is possible that Stradivarius himself proceeded on similar lines Mr Cowell, like Savart, the French scientist, who investigated the subject a hundred years ago, considers that if there is a Strad secret it probably lies in the form of the instrument As Mr Cowell says, Strad was "a better mathematician than he has been credited for"

Brass Instruments

It is interesting to note that musical education is now so far advanced in schools and colleges as virtually to have brought into being a new industry for the manufacture of brass and woodwind instruments In the forefront stands the English firm of Messrs Boosey and Hawkes, which exports to markets all over the world, their Edgware factory being the largest in Europe Besides practising high levels of traditional hand craftsmanship, it has also been successful in developing new methods of large-scale production involving thousands of instruments a year

If we examine a brass instrument such as a cornet, or trombone, or tuba, we realize that it has to have a tube-length comparable with that of the pipes for notes of similar pitch on the organ Thus, for a note three octaves below middle *C* the length required is about 16 ft For convenience to the player the size is made manageable by coiling the pipe Clearly this creates an immense difficulty in manufacture Messrs Boosey and Hawkes have developed a method of curving pipes into suitable forms by a hydraulic press served from a compressed air line at 80 lb /in ² The straight pipe is first bent roughly by hand around a suitable curve, which has the effect of collapsing it It is then inserted into a hydraulic former in which it is inflated to the correct shape

In spite of the use of mass-production methods, the final toning of both brass and woodwind instruments is checked and adjusted by trained musicians.

THE LARGE HOMOPOLAR GENERATOR AT CANBERRA

INITIAL TESTS

By J W BLAMEY, P O CARDEN, Dr L U HIBBARD, Dr E K INALL, R A MARSHALL
and Sir MARK OLIPHANT, K B.E , F R S

Research School of Physical Sciences, Australian National University, Canberra

THIS laboratory has been engaged for some time in the design and construction of a large homopolar generator[1] The generator was intended to provide a pulse of current, of roughly sinusoidal shape, into the orbital magnetic field coils of an ironless proton-synchrotron for 10 GeV , the peak current being 1 6 million amp , and the initial potential difference 800 V , while the stored energy at 900 r p m , in the two rotors, each weighing 40 tons, is 5×10^8 joules The current is collected from the rotors by continuous sheet jets of liquid metal (sodium–potassium alloy) impinging radially around the periphery of each steel disk at a radius of 69½ in , and vertically along the magnetic field (16,000 gauss) at a radius of 24 in The generator is filled with pure nitrogen at about atmospheric pressure

Fig 1 shows diagrammatically the arrangement used in these initial tests, which were designed to test the ability of the jets and machine to withstand the effects produced by drawing the full current The bottom rotor is clamped solidly in position The lower disk of the upper rotor is driven as a Faraday disk by a current from a rectifier, flowing radially between a set of four equally spaced jets of sodium–potassium, 2 mm in diameter, on a circle of radius 24 in , to four similar jets making contact with the periphery A driving current of 1,200 amp gave adequate acceleration to the limited rotational speeds used in the tests The pulse of current was drawn from the upper disk only, and was conveyed to an electrolytic load by a system of aluminium conductors The load was an arrangement of interleaved steel plates, spaced about ¾ in apart, which could be immersed in a tank of sodium hydroxide solution when this was raised hydraulically from below

The admission of sodium–potassium alloy to the current-collecting jets on the top disk, and the variation in the resistance of the electrolytic load, were programmed to give the required pulse shape Fig. 2 shows how the flow of alloy through a jet varied with time in relation to a current pulse The jets were established before the circuit was closed by entry of the caustic soda solution between the plates of the electrolytic load

A series of pulses of up to 10^6 amp , with a half-second width at half current, has been delivered to the load at potential differences up to 66 V The electrical energy agrees with the change in mechanical energy, within the errors of observation, so that the internal losses are small

It was intended to observe several pulses with peak current of about 10^6 amp before proceeding to the current of $1 6 \times 10^6$ amp for which the generator has been designed However on the second such pulse, the unbalanced electromagnetic forces between the plate assemblies of the electrolytic load, due to uneven separations or current distribution, pushed these into contact, so that the current rose sharply to a peak of $1 8 \times 10^6$ amp as shown in Fig 2 The jets, running at less than full flow, appeared to have carried this current satisfactorily, and measured deflexions of the magnet and the jet structure were

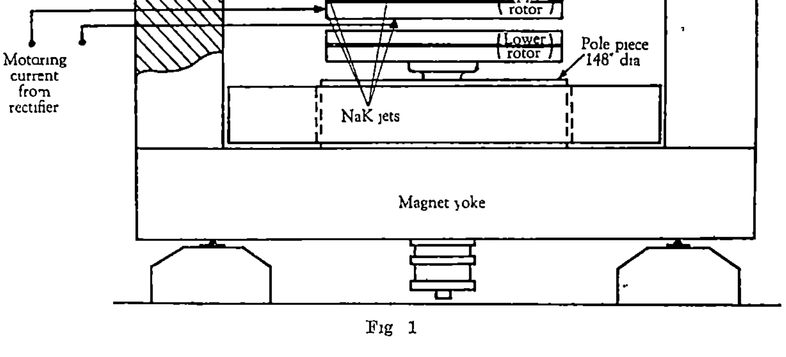

Fig 1

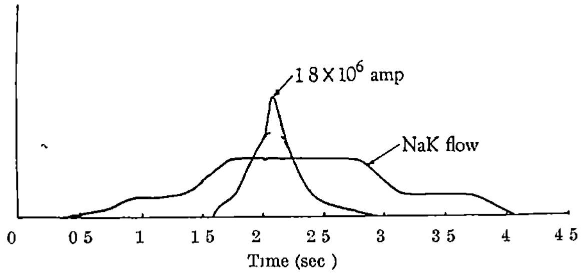

Fig 2

within the tolerances allowed The current reacts on the magnet with a torque of 2 000 ft -tons

These results are very encouraging, indicating that the design analysis is not seriously in error, and that the various assumptions made about the behaviour of the current-carrying jets are justified It is now reasonable to believe that the complete generator will behave as planned A stored energy of 5 × 10^8 joules will then be available for a wide variety of physical experiments, including the production of large magnetic fields over considerable volumes, such as are desirable for work in plasma physics

There remain many questions to which answers must be obtained before all aspects of operation are understood and the generator is working reliably at full power Solutions to many of these problems have been worked out Continuation of the present series of tests will yield information about the validity of these solutions, and will inevitably indicate other problems which have not yet been envisaged However, progress should now be rapid

The tests have provided some interesting observations of the behaviour of liquid conductors moving in a magnetic field These are in general accord with what would be expected from simple magnetohydrodynamic considerations The liquid metal exhibited a high degree of stability and tended to remain continuous, rather than to break up into streamers and drops Further work is planned on these phenomena

We thank Mr D B Shenton of the Atomic Energy Research Establishment, Harwell, who was the first research engineer on the project and to whom much of the basic constructional design is due, Dr D Robertson, who has given assistance and advice, and Mr H Johnson, who was an active member of the team for some years

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FUNCTION, DEVELOPMENT AND EVOLUTION OF THE NUCLEOLUS

By Dr J L. SIRLIN* and Dr J JACOB†
Institute of Animal Genetics, Edinburgh 9

OUR present work on the nucleolus suggests certain conclusions about its function, development and evolution which may serve as guides to future research This work has involved the examination of autoradiographs after treatment of the cells with inhibitors of nucleic acid metabolism, and also investigations with the electron microscope Both these techniques have a high topographical resolution, but, in different degrees, allow of only less-precise biochemical interpretations

Nucleoli are already known to contain transfer RNA[1,2], messenger RNA[3], and a nondescript high-molecular weight RNA[4] It may be assumed on general principles that the messenger RNA originates in the chromosomes In our opinion the presence of DNA in the fully grown nucleolus has not been rigorously demonstrated, except for that present in the nucleolar organizer

Function of the Nucleolus

The system used for autoradiography is the mature chironomid salivary gland cell[1] The nucleolus has a central organizer and RNA is synthesized in close proximity to, but not actually in, this organizer, it then flows centrifugally, the clearance time being about 2 hr

Actinomycin blocks primer DNA but not primer RNA in the RNA polymerase system and in living cells[5], and as used in our system it would presumably not totally block primer RNA The effect is on the DNA and not on the enzyme (Kornberg's DNA polymerase system is much less affected) If our system is treated with actinomycin and the cells then offered labelled nucleosides, three classes of inhibited nuclei are found[6] (1) nuclei in which uptake into RNA is partially inhibited in the chromosomes and completely inhibited in the nucleolus, (2) completely inhibited but healthy nuclei, (3) completely inhibited nuclei with damaged chromosomes and destroyed nucleoli The complete inhibition of nucleolar RNA turnover seen in class 1 indicates that this turnover must ultimately be primed by DNA, and since the nucleolus contains no DNA of its own (except for the organizer, in which, however, RNA does not turn over[1]) this must be chromosomal DNA The effect of actinomycin therefore indicates that the nucleolus requires a contribution of DNA primed RNA derived from the chromosomes Once this RNA has reached the nucleolus we refer to it as the 'extrinsic' nucleolar RNA

TRB (4,5,6-trichloro-β-D-ribofuranosyl-benzimidazole) is a general RNA inhibitor[7] When TRB acts on our cells at the same time that tracer is offered it has no effect When it acts prior to the offer of tracer it results in three classes of inhibited nuclei[6] (1) nuclei completely inhibited, (2) nuclei with completely inhibited uptake in chromosomal RNA and with uptake in nucleolar RNA strictly confined to the vicinity of the organizer (a phenomenon which we call blockage), (3) nuclei with blocked nucleolar RNA but little or no inhibition of chromosomal RNA The blockage of nucleolar RNA is overcome during chase in the absence of TRB, the nucleolar RNA clearing out at a slowed-down rate while the chromosomal RNA does so at a normal rate A similar blockage is caused in isolated thymus nuclei by the related compound DRB[8]

* Agricultural Research Council Unit of Animal Genetics
† British Empire Cancer Campaign

The class 2 nuclei following TRB indicate that some turnover of nucleolar RNA continues in the vicinity of the organizer, even after all chromosomal RNA synthesis is inhibited, and the supply of extrinsic nucleolar RNA shut off. There must therefore be an innate RNA turnover in the nucleolus which produces 'intrinsic' nucleolar RNA in addition to the extrinsic RNA mentioned above. This same conclusion had been previously reached on kinetic grounds[1], and it is known that there is a net RNA synthesis in the nucleolus[9]. Since even the intrinsic turnover is totally abolished by actinomycin it must ultimately depend on a DNA primer. We take it that this primer is the chromosomal DNA and that it produces the extrinsic nucleolar RNA which then, after arriving at the nucleolus, acts as primer for the intrinsic nucleolar RNA synthesis. The nucleolar RNA produced in this way might be either an exact self-primed replication of the extrinsic RNA or the synthesis of a somewhat modified RNA primed by extrinsic RNA.

The extrinsic RNA contributed to the nucleolus from the chromosomes is almost certainly messenger RNA, the presence of which has been demonstrated in the nucleolus[3]. Further support for the demonstration that messenger RNA occurs in the nucleolus comes from the nucleolar inhibition caused by 5-fluorouracil[10] and azaguanine[11], since both these analogues enter into RNA and adulterate messenger[12] as well as other RNA's.

Direct evidence of a free messenger RNA of chromosomal origin can be seen in class 3 of TRB-nuclei[6]. In these the nucleolar RNA is blocked but uptake continues into the chromosomes and there is also present in the nuclear sap a quantity of labelled RNA corresponding approximately to the level of activity remaining in the chromosomes, this free RNA is of high specific activity, that is, is newly made. There is other evidence[10] suggesting that messenger RNA normally passes from the chromosomes to the nucleolus through the nuclear sap, we have attempted to trace with autoradiography any RNA flow there might be along the nucleolar chromosome towards the nucleolus, and if this occurs it should be detectable in chase experiments, but we have been unable to find evidence of it (although there is a flow of particles along the chromosome for distances of a few bands during the early stages of nucleolus formation (see next section)).

One can conclude from these observations that there is an intrinsic nucleolar RNA synthesis which is primed by extrinsic RNA (probably messenger RNA) and which results in the production of an increased quantity of RNA either identical with, or slightly modified from, the extrinsic supply. The most general statement about the function of the nucleolus, therefore, is that it acts as an amplifier of the synthesis of chromosomal RNA.

Development of the Nucleolus

The nucleolus is initially a chromosomal product (cf. ref. 9). In most interphase nuclei prenucleolar bodies arise from the chromosomes and eventually become organized into a single nucleolus. Many nuclei which contain a single nucleolus often also contain small accessory nucleoli. Having regard to all the many different forms of nucleolus found in different cells it seems most reasonable to consider the single well-defined large nucleoli of certain cells as being the organized form which may be taken by a more generally occurring nucleolar substance[9].

The formation of this nucleolar substance has been followed by electron microscopy in the mature sciarid nucleus[13], in which nucleolar organizers are lacking. In the salivary chromosomes numerous small 'elementary nucleoli' form at certain bands (not at interbands). These gradually develop into small nucleoli, which behave metabolically[14] like the larger nucleoli of chironomids, except that RNA is synthesized in their periphery and flows centripetally, whereas in the chironomid it is synthesized centrally and flows centrifugally. The first sign seen of the formation of these nucleolar elements is the appearance of ribonucleoprotein particles of about 100 Å clustered around points in the chromosome fibrils at the bands. These clusters of particles grow by apposition of new particles, fuse into larger masses, and gradually move to the periphery of the band. When a band (pre-puff ?) contains larger granules of about 400 Å diameter these are also added to the nucleolus at that band, if the band does not contain these granules, the nucleolus does not have them either. There are often thick layers of particles on the chromosome surface and these become added to the nucleolar masses as they are extruded, and sometimes connect together several of them coming from nearby bands. Finally, the nucleolar masses lose contact with the chromosomes and become small free nucleoli. During their growth the original chromosomal fibrils, around which the particles were initially clustered, become gradually no longer recognizable.

In the chironomid nucleus and other nuclei containing a single nucleolus, the initial stages of nucleolar formation are probably the same as in the sciarid just described, but they are followed by a later stage at which the elementary nucleoli are brought together into a single body by the nucleolus organizer. This organizer is a gene, and it is clear that it is to be regarded as an evolutionary acquisition by certain forms. When nuclei which normally possess organizers lose them by deletion, they revert to the primitive condition of the sciarid, showing many scattered small nucleoli, both in polytene nuclei and in interphase nuclei (such a condition is eventually but not immediately lethal). The primitive condition therefore seems to be that each gene makes its own elementary nucleolus if it needs it, as many of them seem to do. Something similar still happens in evolved nuclei in the case of differentiating puffs in polytene nuclei and in interphase nuclei which produce accessory nucleoli.

Evolution of Nucleolar Function

We came to the conclusion above that the extrinsic nucleolar RNA which acts as a primer for the intrinsic nucleolar RNA synthesis is messenger RNA derived from the chromosomes. This has been proposed before[15], but we have been able to provide firmer biochemical evidence in support of it.

As stated above, we have directly observed free messenger in the nuclear sap. We can ask if all messenger normally passes through the nucleolus. Observation shows that it need not necessarily do so, since when the nucleolus is blocked with TRB the messenger can be seen not only in the nuclear sap but also in the cytoplasm, where it first accumulates in the neighbourhood of the nuclear envelope, this passage to cytoplasm independently of the nucleolus was also inferred kinetically[16] in normal cells. It is perhaps possible to regard this messenger as having been formed in residual elementary nucleoli within the chromosomes But if we adopt this view, the constituent particles in the elementary nucleoli would in fact amount to no more than the genes themselves plus the messenger RNA which they are manufacturing, and there would be no reason to suppose that the messenger had passed through any specifically nucleolar phase

We may then proceed to ask whether there is *some* messenger which has to pass through the nucleolus, even though much of it need not do so This question cannot be so simply answered from mere observation, although the effect of the nucleolar inhibition by azaguanine on cytoplasmic protein[11] might be held to suggest that the inhibition of the nucleolus leads to a loss of essential cytoplasmic messenger It must also be remembered that some messenger may be used internally for syntheses in the nucleolus itself In its general form, however, the question at issue raises the whole problem of whether the function of the nucleolus is merely that of amplification, or whether it brings about necessary modifications of the chromosomal RNA, that is to say, is the nucleolus in principle redundant or indispensable ? The idea that it is redundant is an old one, recently formalized[9] Its main advantage is that it does not attribute an essential (that is, specificity conferring) function to a structure which in effect lacks DNA The alternative would be to suppose that the nucleolar RNA has a genetic continuity which enables it to function like DNA, but the evidence[9] is rather against this continuity Moreover, intrinsic nucleolar turnover size is large compared with that of single genes, and may be very considerable in differentiating cells (referring here to what was called 'independent nucleolar RNA" in ref 9), and therefore appears suitable to meet the demands for amplification of particular messengers A lack of this amplification may be the reason why lower organisms without nucleoli never attain the multicellular complexity seen in more evolved organisms with nucleoli Such an amplification process would give the nucleolus a rate-limiting function[17]

The alternative hypothesis to amplification, that is, modification of messenger at the nucleolus, still deserves consideration, although we have no positive evidence to support it There is the possibility that the modification involves degradation The fact that messenger can be converted to ribosomal RNA after rearrangement of nucleotides[18] shows that messenger can, in fact, be modified Some modification may be necessary for the coding triplets in messenger RNA which are known to contain relatively rather too much of uracil[19] Since the turnover of uridylic acid has been reported as being higher in nucleolar RNA than in nuclear RNA[20], this nucleolar turnover might suggest a modification of messenger to that end Similarly, the first synthesis of hæmoglobin in the cell was detected at the nucleolus[21], and this might indicate that the messengers are somehow first made functional in the nucleolus, although, since in this case also the chromosomes apparently later synthesize the protein, the earlier nucleolar synthesis could simply be due to the amplification rather than to the adaptation of messengers Modification of messenger may also be the mode of formation of transfer RNA[9] which is ultimately DNA primed[22] as nucleolar RNA itself is If transfer RNA is salvaged degraded messenger (rather than degraded ribosomal RNA as in ref 23), then the problem of accounting for the conferment of specificity to transfer RNA at the nucleolus becomes less The modification might include the appearance of the unusual bases of transfer RNA, even if it is complementarily replicated on messenger It is perhaps suggestive that a series of types of RNA which depart increasingly from the DNA type of base composition, that is, messenger–transfer–ribosomal, are located at correspondingly increasing distances from the genes in the series of structures chromosome–nucleolus–ribosome

What we now know of nucleolar evolution also suggests that the nucleolus is concerned with the function of messenger RNA We take it that the sciarid condition, although it can possibly be derived from the chironomid condition by a single gene mutation leading to the loss of the organizer, represents the primitive situation In this, elementary nucleoli are associated with single genes The evolved consolidated nucleolus is associated with the whole genome In the primitive nucleus, gene DNA primes the synthesis of messenger RNA, which may thereafter be amplified by self-priming in the multiplying elementary nucleolar particles (primer RNA is associated with particles also in the enzyme system[5]) The resulting elementary nucleoli are then gene products Any DNA which becomes trapped within these nucleoli during their formation later becomes waste or disappears In more highly evolved nuclei the elementary nucleoli (or prenucleolar bodies) from many genes become consolidated into a single nucleolus, presumably this saves reduplication of energy-consuming processes in the multiple elementary nucleoli, and assists the co-ordination of the output of messengers, etc The consolidation of the single nucleolus is the function of a mutated gene—the organizer—which, however takes no part in the synthesis of the nucleolar RNA although remnants of its DNA may be trapped inside the nucleolus It is, however, the messenger RNA, not the organizer DNA, which primes the intrinsic nucleolar RNA synthesis, itself becoming amplified and perhaps modified in the process It i this fact, which emerges from the effect of RNA inhibitors on the nucleolus, which makes it possible to regard the dispersed nucleoli of the sciarid and the consolidated nucleolus of the chironomid as two stages in the evolution of structures fulfilling the same cellular function

In the course of nucleolar evolution some changes in the topography of turnover have occurred The region of the nucleolus which actually synthesize the intrinsic RNA is the dense particulate region[14,24] This can be located either centrally or peripherally depending on whether, during the formation of th nucleolus, the organizer is centrally located as i chironomids or lies at one side of the nucleolus as i Drosophilids and many other metazoa When th organizer and the dense particulate region lie centrally, the RNA moves centrifugally When the dens particulate region is peripheral the RNA move centripetally, but in this case it is not related to th particular portion of the surface of the nucleolus a which the organizer is externally located, the sciar nucleolus without organizer shows the same pattern Whatever the pattern, the intrinsic RNA synthesize in the dense particulate region eventually fills th whole nucleolus As to the mechanism by whic the messenger RNA reaches the nucleolus, it i not yet quite clear whether this is by random collision after free diffusion through the nuclea sap, or whether there is any directed movemen of messenger towards the nucleolus The latte possibility is perhaps suggested by the kinetics o the orthophosphate pool[25] It also seems possibl that the nucleolus-associated chromatin of interphas nuclei may be a feeder of messenger to the nucleolus However, the fully formed nucleolus is usually quite large enough for it to seem plausible that the free diffusion of messenger would be a sufficient mechanism

As we see it, the interpretation which has been presented has several advantages It explains under a single scheme both nucleolar development and function for virtually any form which nucleoli may take, even down to presumable ultrastructural (elementary) nucleoli in lower cells It does so within a framework where all necessary genes participate Finally having regard to the function of messenger RNA, self-priming of messenger as the basis of nucleolar function seems to correspond well with the essentially auxiliary, although in the long run indispensable, general nature of nucleolar function[9]

It remains to say a word about nucleolar protein In both the primitive dispersed nucleoli, and in the more evolved consolidated nucleoli, the protein turnover occurs uniformly throughout the whole body of the nucleolus and shows no directional characteristics In adult cells it occurs mainly during the period of nucleolar growth and is only slight in the fully formed nucleolus This suggests that the protein synthesis is mainly an aspect of nucleolar growth, and not the production of something like RNA which is contributed to the rest of the cell In evolved nucleoli some protein could come to the nucleolus in the prenucleolar bodies, and it was actually shown that some of the protein comes from the nucleolus of the previous cell cycle[26] On the other hand, it might well be that the main synthetic function of the organizer could be the early production of an organizing nucleolar protein[24] This is perhaps suggested by the topographical relationships between the organizer and the dense particulate region mentioned above It should also be noted that in cleanly isolated nucleoli there is only one major protein[27] It has been suggested that the nucleolus synthesizes ribosomal protein[28] If so, this protein would presumably have to be made in the many dispersed elementary nucleoli in the less-evolved stage This possibility cannot be ruled out, but, since ribosomal protein has a similar overall composition in all species examined, the suggestion that many different genes all make essentially the same ribosomal protein does not appear to us very attractive Our view[24] is that the nucleolus (and possibly the nucleus as a whole) essentially contributes free informational RNA's and does not contribute protein, which is made outside the nucleus and possibly by the ribosomes themselves Evidence for this comes from the free state of messenger[12] and, possibly, from ribosome reorganization during regeneration[24]

We thank Prof C H Waddington for help in preparing the manuscript

This work is supported by the Damon Runyon Memorial Fund for Cancer Research (*DRG*-579)

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THE LOWER ARCHEGONIATE PLANTS

OVER the years the Linnean Society of London has had many important meetings and discussions to its credit The recent symposium on "The Growth and Development of the Lower Archegoniate Plants", held in the Society's rooms during April 5–6, had, however, the unique distinction, as the president, Prof T M Harris, stated in his opening remarks, of being the first truly international symposium ever arranged by the Society In addition to British representatives, the speakers included botanists from Canada, the United States, France, Germany and Finland, and, in all, sixteen papers were read About one hundred people attended each of the four sessions In passing, it may perhaps be noted that a group of this size proved admirable for the progressive development of full and friendly discussions

In opening the first session (over which Prof Harris presided) on the gametophyte, Mr P R Bell (University College, London), to whom much credit for arranging the symposium is due, referred to the value of ferns and mosses for studies of growth, development and differentiation He indicated that classical problems of long standing concerning the differences between the alternating phases of the life-cycle might perhaps be solved by the application of new techniques, and he affirmed that principles of cellular biology based on the lower archegoniate plants could have important applications to higher plants Prof H Mohr (Botanisches Institut, University of Freiburg, West Germany) then submitted observations on the influence of visible radiation on the germination of spores of archegoniate plants, in particular of *Dryopteris filix-mas*, *Funaria hygrometrica* and *Sphaerocarpus* sp, and on protonemal growth These and other investigations indicate clearly the importance of light and of photoreactive systems in growth and morphogenesis Spore germination in *F hygrometrica*, for example, is controlled by light, though the processes involved are complex The first stage of germination, involving the uptake of water, enlargement, rupture of the exospore and conspicuous greening of the plastids, can occur in darkness in some of the spores of a population The results so far obtained indicate that the control of this stage is mediated by the phytochrome system The second stage, which includes the outgrowth of the rhizoid and/or chloronema, is also controlled by light, photosynthesis being involved The spores of *Sphaerocarpus* are obligate light germinators, at least two photoreactive systems being involved

Since germination can be induced by both blue and far-red radiation, it is tentatively assumed that there is a promotive photoreactive system identical with the blue, far-red reaction system (the so-called 'high energy reaction of photomorphogenesis') The inhibiting reaction system, which is particularly effective with longer daily periods of irradiation, may be identical with the phytochrome system

In *Dryopteris filix-mas*, the relative and absolute protein contents of blue-grown prothalli increase much more than do the protein contents of the corresponding red-grown filaments (protonemata), even when the increase in dry weight is about the same under both light conditions After a transient increase the relative protein content remains constant in darkness and in red light, whereas in blue light the relative protein content increases steadily, at least during the experimental period If red-grown protonemata are placed under blue light and blue-grown prothalli under red light, rapid shifts in the relative protein contents are observed, indicating that, in a gametophyte, the protein content is actively brought to a certain level determined by light wave-length It thus appears that the increased rate of protein synthesis in blue light is intimately connected with the related critical developmental changes It is probable that photomorphogenetic light reactions determine into which metabolic pathways the primary products of photosynthesis are channelled In the plants under consideration, a photoreactive system which depends on blue light apparently directs this flow of metabolites primarily in the direction of protein synthesis

Prof A Vaarama (in collaboration with Nina Tarén, University of Turku, Finland) gave an account of the synergism of calcium and certain growth substances in the germination and protonemal growth of mosses, based on their experimental studies of certain lithophytic and non-lithophytic calcicolous species and a calciphobous species Among other observations, gibberellic acid inhibited protonemal growth in all the calcicolous species tested, but promoted growth in *Dicranella cerviculata*, a calciphobe

Prof M Bopp (Botanisches Institut, University of Freiburg, West Germany) discussed differentiation in moss protonemata and the factors controlling bud formation in them During the development of the protonema of *Fumaria hygrometrica*, two different substances diffuse into the substrate In the chloronema a heat-labile, growth-promoting substance is formed, in the caulonema, after about ten days, a heat-stable substance, soluble in amyl alcohol, is formed This substance functions as a growth inhibitor Both substances affect bud formation, which is at an optimum only when a certain balance between them exists This bud-inducing effect is fundamentally different from that due to kinetin, in that the latter only functions as an additional factor in promoting bud formation Kinetin is regarded as an agent which creates centres of activity toward which morphogenetic substances are drawn This view is supported by the facts that kinetin cannot be transported and therefore has no 'after-effect' and that it functions only when it penetrates a caulonema cell It converts every caulonema cell into a 'reaction cell' The opening session was completed by a contribution by three French workers, Messrs Kofler, Dutel and Nurit, on variations in the geotropic sensitivity of germinating *Funaria* spores in response to external factors such as light, and to applied growth substances, glucose, etc (This paper was read by Dr E Lodge)

The second session (under the chairmanship of Prof C W Wardlaw, University of Manchester) was mainly devoted to factors which affect the formation of antheridia Dr U Naf (Rockefeller Institute, New York) dealt with an antheridium-inducing effect of a specific substance which is apparently common to many polypodiaceous ferns, though it is inactive when applied to some Schizaeaceae, Osmundaceae and Cyatheaceae

The prothalli of *Onoclea sensibilis* respond to the active substances at an early stage of development but become incompetent to respond to it soon after they attain the adult heart-shape This loss of sensitivity occurs within less than two days Once they have become insensitive, prothalli fail to respond to the active substance even if they are supplied with very high concentrations, for example, 15,000 times higher than that which is sufficient to bring about a response in prothalli just two days younger Surgical studies by Dopp have shown that the meristem of the maturing prothallus begins to form a substance to which the loss of sensitivity can be attributed Dr Naf suggested that the antheridial factor acts by modifying a situation which blocks or inhibits antheridium formation In the course of a lively and entertaining discussion of this paper—to give one indication of the mood of the symposium—it emerged that the responses of antheridia in the common bracken, *Pteridium aquilinum*, were considerably different on the two sides of the Atlantic—varietal effects being no doubt involved

Dr D Wilkie (University College, London) then varied the general tenor of the session by giving an account of a genetical analysis of bracken (*Pteridium aquilinum*) prothalli In the course of this work, based on the pure culture of single spores, he frequently observed a form of prothallus lacking a meristem Actually, some 20 per cent of the spores yielded these small, ameristic prothalli, they bore antheridia and most of them usually reverted to normal forms The genetical properties of these variant forms were investigated by methods involving cross- and self-fertilization analysis A genetic analysis of the spores from the resulting sporophytes showed that control of the ameristic character could not be directly attributed to the action of nuclear genes Accordingly, a hypothesis of heritable cytoplasmic units was advanced to explain the results

The session was concluded by a paper from Prof L Bauer (Botanisches Institut, University of Tubingen, West Germany) on his experiments on the stabilizing of the male sexual tendency in mosses, *Splachnum* sp , that is, by the repression or exclusion of the female tendency by exposing plants to certain external conditions The persisting modifications which he obtained were attributed to cytoplasmic factors

In the third session (under the chairmanship of Prof L J Audus, University of London) the speakers were mainly concerned with the further differentiation of the protonema and prothallus and their products, especially as it related to the sporogonium in mosses and the ovum and young embryo in ferns Prof Bauer made a very interesting contribution on the physiology and organization of sporogonium differentiation in mosses He demonstrated that if different regions, apical, sub-apical and maturing, of the distal part of the young needle-like sporophyte were isolated and cultured, they typically gave rise to different morphological entities The more distal segments were capable of regenerative growth and eventually gave rise to sporogonia, but the more proximal maturing segments had apparently lost permanently some of their initial inherent potency and did not give rise to sporogonia when cultured under the same conditions as the other segments This observation in mosses appears to be in general accord with contemporary cytogenetical conclusions based on work in other groups

Mr P R Bell (University College, London) then gave an account of recent work on the cytochemical and ultrastructural peculiarities of the unfertilized egg in *Pteridium aquilinum* His lecture was illustrated by some excellent electron micrographs which not only shed new light on a classical botanical topic but also on fine-structural features of the nucleus and cytoplasm Critical observations on staining and cytochemistry were followed by an account of some novel observations on the nucleus and cytoplasm as disclosed by electron microscopy As the egg matures the nucleus appears to evaginate into the cytoplasm and there is evidence that, in the formation of the egg, the plastids and mitochondria derived from those of the gametophyte degenerate The mature egg, in fact, contains a new set of mitochondria, the evidence so far obtained from this material suggesting that they may be formed with the direct participation of the nucleus Although it has not yet been possible to correlate the peculiar cytochemistry of the egg with its unique ultrastructure, Mr Bell suggested that the evidence now accumulating admitted of the interpretation that the mature egg has a cytoplasm which is transformed under the influence of the nucleus, and this he considered may be responsible for the differences in complexity of the morphological configuration generated by the spore and the zygote

Dr A E De Maggio (Rutgers University, New Jersey) introduced the theme of the developing sporophyte by giving an account of morphogenetic factors which affect the development of the fern embryo In particular, he illustrated and discussed the effect of the physical restraint which may be exercised on the developing zygote by the archegonial wall and adjacent gametophyte tissues, and the effects of nutritional factors His surgical experiments showed that the early, complete removal of the natural, restrictive limitations imposed on the zygote and growing embryo seriously affects the potentiality of the young sporophyte for three-dimensional growth and normal sporophyte production Whether this single modification, which leads to what looks like a reversal of the regular phases in the alternation of generations, results in a loss of control over the planes of cell division, as previously suggested, or deprives the embryo of essential growth substances necessary for orderly sporophyte formation, remains to be seen In the species studied it is known that changes in carbohydrate nutrition do not account for permanent alterations in the morphological form Accordingly, the possibility cannot be overlooked that nutritional and hormonal interactions can give rise to changes in embryo development of such a magnitude as to suggest that these substances exert considerable control in determining morphological levels of development

These explorations of factors in the early embryogenesis in ferns were taken a stage further by Dr M Ward (Glenville State College, West Virginia), who discussed developmental patterns in adventitious, that is, non-sexual, sporophytes of *Phlebodium aureum* Such sporophytes are regularly formed when petiole and rhizome segments are grown in various culture media Gametophytic plants regularly develop from petiole segments but sporophytic plants are less common On rhizome sections in culture, sporophytic and gametophytic forms occur at random and often adjacent to each other. Apogamous sporophytes thus formed tend to give rise to secondary apogamous sporophytes on their own leaves In this fern, apogamous sporophytes are also regularly formed on the normal gametophyte under appropriate cultural conditions

The genetic constitution of these apogamous and aposporous forms has not yet been established Certain problems incident to the classical cycle of alternation of generations are indicated by the occurrence of these adventitious plants This session was concluded by an outline account of leaf regeneration in *Adiantum pedatum* given by Dr G Morel (Institute of Plant Physiology, Versailles) Since, in this fern species, juvenile leaves regenerate gametophytes, whereas adult leaves regenerate sporophytes, it may be inferred that during the process of ageing the factor controlling gametophyte formation, still present in juvenile leaves, tends to be lost If this be accepted, the problem is to ascertain how the same nucleus controls the development of these very different morphological entities—the small cordate laminæ with sex organs, and the sporophytic plant with roots and spore-bearing leaves, respectively In each, the information coded in the chromosomes should be the same but, nevertheless, the resulting organisms show marked differences in their morphology

The fourth and final session was held under the chairmanship of Dr C R Metcalfe (Royal Botanical Gardens, Kew) botanical secretary to the Linnean Society, who also had an important share in the arrangement of the symposium In an account of apical organization and allometric growth in ferns, Prof C W Wardlaw illustrated, by reference to a number of common British and some exotic ferns, that the main organizational features of the developing sporophyte of any particular fern and much of the morphological diversity in ferns in general can be referred (1) to the inception of a characteristic primary pattern of organs in the apical meristems of shoot and leaves, and (2) to various elaborations of these patterns by differential or allometric growth in the sub-apical and maturing regions

Factors of various kinds, genetical, nutritional, correlative and environmental, may, in different instances, affect the primary pattern and the nature of differential growth Accordingly, the view was advanced that the phenomena of organization in plants lend themselves to analytical and experimental investigation, but synthesis is evidently an essential part of the task

Dr T A Steeves (University of Saskatchewan) then contributed a detailed analysis of the growth and morphogenesis of the leaf of *Osmunda cinnamomea,* dealing first with the inception and development of the circinately-coiled primordium and of the maturing but still rolled leaf, and then with the further physiological activities which result in the uncoiling of the more or less adult leaf in spring Particular attention was given to the relative roles of cell division and cell elongation in the ground tissue of the leaf rachis throughout development The coiled condition of the rachis in the crozier was interpreted as the result of more cell division abaxially than adaxially during its formation, and uncoiling depends on the equalizing of cell numbers by cell division late in leaf development Cell elongation in *O cinnamomea,* except in the leaf base, is delayed until the fifth growing season, at which time it extends acropetally along the rachis Leaf elongation in several ferns depends on diffusible auxin, probably indolylacetic acid, from the pinnæ Leaf primordia, after being determined, can complete their development in isolation in sterile nutrient culture, indicating that the essential features of leaf formation are regulated within the organ itself The small size of cultured leaves is the result of diminished cell division in the rachis and appears to have a nutritional basis

Finally, in this session, Dr A Allsopp (University of Manchester) summarized and illustrated his many experimental investigations of morphogenesis in *Marsilea* spp, using aseptic culture technique Special attention was given to the effects of nutritional factors and growth-regulating substances in heteroblastic development and other organizational features, and to the physiological conditions which determine the characteristic features of land and water forms He concluded that the changes in leaf segmentation of *Marsilea,* or heteroblastic development are correlated with changes in the size of the apical meristematic regions of the shoot, which in turn are influenced by the nutritional status of the plant On the other hand, the differences between land and water forms are probably due to differences in the available concentration of dissolved carbohydrates in the growing parts

When I reflect on the symposium as a whole, my thoughts naturally turn to Hofmeister, Goebel, Campbell, Bower and Lang, and to the many other distinguished botanists of past times who contributed so much to our knowledge of the growth and morphology of archegoniate plants, and, not least, who were so profoundly interested in the causes underlying their singularly unlike alternating generations Had the shades of those eminent ones lingered awhile on this occasion, they might well have been stirred to surprise and wonder at the interest these plants and their problems still command, and, like myself, at the end of the meeting, they might perhaps have entertained the optimistic view that, although we evidently still have a very long way to go, we are at length coming within tangible distance of a more factual interpretation of some aspects of the growth morphology and life-cycle of these enigmatic and fascinating plants Accordingly, this is a suitable place to express the appreciation of all who participated in these sessions to the president and officers of the Linnean Society for arranging the symposium, and to the British Council and the International Union of Biological Sciences for very substantial financial assistance, without which, indeed, the symposium could not have been held

The papers contributed will be published collectively as a number of the *Journal of the Linnean Society* as soon as possible and this may be obtained from the Society C W Wardlaw

OBITUARIES

Dr L G G Warne

The University of Manchester has lost a valued member of staff in the sudden death of Leslie Gordon Glynn Warne on May 3, aged fifty-three years He had suffered a severe coronary thrombosis in 1955, which slowed his movements somewhat, he was well aware of a possible fatal recurrence, but he retained full mental vigour, wonderful personal equanimity, and continued his university activities as senior lecturer in horticulture and assistant director of the Experimental Grounds Actually, he was engaged on field work at the experimental grounds of the University, Jodrell Bank, Cheshire, when he collapsed

Warne was born at Faringdon, Berks, on June 25, 1908 On leaving St George's Secondary School, Bristol, he was employed for three years in investigational work in the laboratory of Messrs H J Packer and Co, Ltd, cocoa and chocolate manufacturers In 1928 he entered the University of Bristol, and in 1932 he graduated with first-class honours in botany In 1933 he was awarded M Sc and in 1936 Ph D He joined the staff of the University of Manchester in 1934 as assistant lecturer in botany, served the Department in many ways and was appointed senior lecturer in horticulture in 1948 This is an endowed post, the aim of which is to help gardeners and others to secure adequate scientific and professional training and to encourage horticulture in the region Warne had a considerable share in organizing the Experimental Grounds and evening classes in botany and horticulture in this connexion Indeed, his gifts as an organizer and as a practical gardener not only equipped him for this work and brought him success, but were also a great asset to the Department

In 1953 the University granted him three years leave of absence to occupy the chair of botany at Karachi, Pakistan There his varied ability again bore fruit, and his keenness and energy enabled him to acquaint himself with the work of other institutes in Pakistan and India, and to become familiar with tropical vegetation and agriculture.

During his earlier period in Manchester, Warne had complete responsibility for the degree course in plant physiology for about twelve sessions He also taught medical (1st M B) and dental students for several sessions, and served on various university committees His outside activities involved him in active membership of learned societies and of examining bodies He had a large number of published papers to his credit and was jointly responsible for the revision of Lowson's *Text-book of Botany* for use in Britain and also for India, Pakistan and Ceylon He was also joint author of a *Practical Botany* for home use, and one for use in tropical countries

In 1941 Warne married Miss M M Walker, whom he had met some years before at Long Ashton, and who was a lecturer at Swanley Horticultural College, Kent He is survived by his wife and three daughters Both in the Botany Department and more generally in the University he will be greatly missed as an unusually genial, even-tempered and helpful colleague

W O HOWARTH
C W WARDLAW

Dr T U Matthew

DR T U Matthew, director of manufacturing of Massey-Ferguson (United Kingdom), Ltd , and the first Lucas professor of engineering production in the University of Birmingham, died in Scotland, following a brief illness, on May 11, at the age of fifty-two

Thomas Urquhart Matthew was educated at George Heriot's School, Edinburgh, the Royal Technical College, Glasgow, and King's College, Cambridge He had production engineering and consultative experience in Great Britain and in South Africa, where he was technical adviser to the Government, before his appointment to the newly endowed Lucas chair in 1948 It was said, at the memorial service held on May 23, that the pioneer Department of Engineering Production which he developed at Birmingham would be his lasting memorial In 1955 Dr Matthew returned to industry as production director of Tube Investments, Ltd , and joined Massey-Ferguson a little more than a year ago

As Lucas professor, Dr Matthew was charged with determining and elucidating the principles of efficient production engineering, and the emergence of production engineering as a university discipline is, in large measure, a tribute to his ability He pioneered the application of scientific method to complex problems of production planning, organizing and control, and was one of the first to recognize the contribution that operational research could make in this field He was a modest, courteous and generous man, who devoted the whole of his time and energy, and considerable analytical gifts, to the service of industry and education for industry He served the Institution of Production Engineers in many capacities, and was a member of its Council, chairman of the Birmingham Section, and chairman of the Education Committee at the time of his death

In 1952, he led an I L O Technical Assistance Productivity Mission to the engineering and textile industries of India He was a founder-member of the International University Contact for Management Education, which brought him into close and continuing contact with his colleagues in Europe and America About the same time, he established, at Birmingham, the University Institute for Engineering Production to serve as a link between his postgraduate Department of Engineering Production and practising production engineers After his own return to industry, his keen interest and efforts on behalf of the Department remained undiminished

Dr Matthew leaves a widow and three sons

N A DUDLEY

Dr. H McCombie

DR HAMILTON MCCOMBIE, reader emeritus in chemistry in the University of Cambridge, died at Woking on May 31, aged eighty-two McCombie will be remembered for his distinguished service in the First World War, for his work in connexion with chemical defence, and by his many pupils in Birmingham and in Cambridge A Scot of the Scots, he was born in London, but in 1900 atoned by taking an ordinary M A of the University of Aberdeen A London B Sc with honours in chemistry followed, and he went on in the old tradition to Strasbourg to work under Thiele, getting his doctorate there in 1905

His first appointment was as lecturer in chemistry in the University of Birmingham, where he became interested in reactions in non-aqueous solvents, work which he continued later in Cambridge He was in Australia in 1914 to attend the meeting of the British Association There was the rush back home His enlistment in the Worcesters followed, but he was soon transferred to the Special Company, Royal Engineers, on its formation after the first German gas attack There his knowledge and steadfastness proved invaluable and were recognized by the awards of the M C and the D S O , the Croix de Guerre and two mentions in despatches

He was elected a Fellow of King's College, Cambridge, in 1919 and served his College and University until his retirement in 1945 In King's he became assistant tutor for natural sciences when C T Heycock retired, and he held this office until 1945, in the earlier years jointly with Sir Joseph Barcroft Kingsmen remember him with affection, and acknowledge the debt they owe to him He was wise in counsel, and long service on the College Council, on its Estates Committee and as an elector to fellowships was demanded from him and given ungrudgingly except, perhaps, when there was an important rugby match at Grange Road In the University he lectured on inorganic chemistry to the large class of second-year men about to take Part I of the Natural Science Tripos, and he ran a laboratory to which usually only scholars and exhibitioners were admitted Once in, a man could attend when he liked and do very much what he liked

Between the Wars, McCombie directed a very substantial volume of fruitful research, first on antidotes to mustard gas and, later, on the phosphorus gases During the Second World War he was a deputy chairman of the Joint Recruiting Board and acting head of the Chemical Laboratory after the death of Sir William Pope After his retirement he lived quietly at Woking to be near one of his daughters and his grandchildren

Prof P Hoffmann

PROF PAUL HOFFMANN, for many years professor of physiology in the University of Freiburg, died on March 9 in his seventy-eighth year He was a scientist of distinction and a man of great personal charm, modest, tolerant and wise His most important work dates from 1910, when he began the study of human reflexes by recording muscular activity with the string galvanometer His monograph, *Die Eigenreflexe*, published in 1922, remains a remarkable achievement, for it established for man many of the principles of reflex control of movement which have since been studied in greater detail in animals

In 1934 he succeeded von Kries as professor at Freiburg and became emeritus professor in 1954 His colleagues in many countries will sympathize with the University of Freiburg at the loss of an elder statesman, admired for his character as well as for his reputation as a scientist ADRIAN

NEWS and VIEWS

Editorship of *Science*

Dr. Graham P DuShane

DR GRAHAM P DUSHANE editor of *Science* (the official organ of the American Association for the Advancement of Science) since 1956, has resigned to become professor and chairman of the Department of Biology and dean of the Graduate Sciences at Vanderbilt University, Nashville, Tennessee Dr DuShane received his doctorate in zoology at Yale University in 1934 Following a research associateship at the University of Iowa and a National Research Council fellowship at Stanford University, he joined the faculty of the University of Chicago in 1936, where he taught for ten years before being appointed professor of biology in Stanford University in 1946 After six and a half years with the American Association for the Advancement of Science, he is returning to academic life in a newly created post that will give him responsibility for graduate education and research in the natural sciences, medicine and engineering at Vanderbilt University His appointment there will begin on September 1

Dr Philip H Abelson

As the new editor of *Science*, the Board of Directors of the American Association for the Advancement of Science has appointed Dr Philip H Abelson, member of the National Academy of Sciences and, since 1953, director of the Geophysical Laboratory of the Carnegie Institution, a position he will continue to hold while serving as editor of *Science* Prior to assuming the directorship of the Geophysical Laboratory, he was chairman of the Biophysics Section of the Carnegie Institution's Department of Terrestrial Magnetism During 1941–46 he was with the Naval Research Laboratory, engaged in the separation of uranium isotopes He received the doctorate in nuclear physics from the University of California in 1939

Convection Currents in the Earth

COMMENTING on the article by Prof J Tuzo Wilson, entitled "Cabot Fault, an Appalachian Equivalent of the San Andreas and Great Glen Faults and Some Implications for Continental Displacement" (p 135 of this issue), Prof J D Bernal, Birkbeck College, London, W C 1, writes "I find Prof Tuzo Wilson's account of convection currents in the Earth, in all but one respect, consistent and illuminating My objection is to the special and downward limited function he ascribes to the asthenosphere It seems to me to neglect the real evidence that exists for horizontal movements in the upper mantle

"If, as he implies in his Fig 2, the mid-ocean ridge cracks extend well into the mantle, why may not the return movements which occur under continental mountain chains do so as, indeed, is shown by the continuity of the epicentral surfaces of earthquakes above and below the asthenosphere? Furthermore, movement on these 40–50° shear surfaces may, as I have indicated elsewhere (*Nature*, 192, 123, 1961), be responsible for the building in of sial into the bases of the leading edge of continental sheets This might well take place by gravity differentiation of the intruded asthenosphere

"The puzzle of how the asthenosphere can appear on one hand as flowing and on the other as transmitting shear between mantle and crust may perhaps be solved by attributing to its substance a characteristic time of the order of some hundreds of years For stresses of shorter duration it would then, in common with the crust above and the mantle below, yield by shear faulting, for those of longer duration, by continuous plastic deformation"

Biochemistry at the Imperial College of Science and Technology

WITH the view of re-establishing biochemistry as a major discipline at the Imperial College of Science and Technology, Dr E B Chain has been appointed to the chair of biochemistry A gift of £350,000 was made by the Wolfson Foundation to provide the necessary laboratories, to be known as the Wolfson Laboratories It was intended that the new Wolfson Laboratories should be built behind the present Royal College of Science and that the rest of the space requirements of the Biochemistry Department should be met by adaptation of part of the existing building As a result of the University Grants Committee's subsequent approval in principle of the redevelopment of the site on which the Royal College of Science now stands, these plans were changed and a composite building for the whole of the Biochemistry Department has been planned, as the first stage of this re-development, near the west end of Imperial Institute Road The accommodation falls into the two distinct categories of laboratories and fermentation pilot plant The laboratories are designed and will be equipped for research in all important sectors of modern biochemistry, with special emphasis on the study of the reactions of intermediate metabolism and their regulation in higher animals and micro-organisms Laboratories will also be available for research in the disciplines bordering on this subject The fermentation pilot plant unit will consist of two separate elements a hall for vertical fermentation vessels and two chemical plant rooms, mainly for the extraction and purification of the desired substances and distillation of solvent A large underground solvent store is incorporated outside and adjacent to this building During the interim period until the building in South Kensington is ready, Prof Chain will continue his present activities as scientific director of the International Centre for Chemical Microbiology of the Istituto Superiore di Sanità, a position which he has held since 1949 when he left the University of Oxford Dr Ing R Falini, until recently in charge of the fermentation pilot plant at the Istituto Superiore di Sanità, will design the corresponding unit at the Imperial College

University of Saskatchewan Linear Electron Accelerator

THE Canadian National Research Council and the University of Saskatchewan Board of Governors have recently made available funds to the Department of Physics, University of Saskatchewan, for a linear electron accelerator and the erection of a building to house this machine and provide the ancillary research space Preliminary plans indicate that it will be a travelling wave machine composed of six separate accelerating sections each about 4 8 metres long operating in the $2\pi/3$, *TM* mode These will be powered by three *S*-band (2,856 Mc /s) klystron amplifiers of the type to be used on the 2-mile linear accelerator of Stanford University (Project *M*) Each klystron, delivering approximately 26 MW peak and 22 kW radio-frequency power average, has two output windows and will thus feed two of the accelerator sections An unloaded (zero beam current) energy of 250 MeV is anticipated, and, when loaded to deliver 20 kW of beam power, the energy should be in excess of 150 MeV Single shot operation is specified as well as repetition-rates up to 1,400 c /s

One feature of this machine will be the availability of exceedingly narrow pulses at the higher repetition-rates Widths of 10 mμsec are being specified in the design, although according to recent technological advances pulses as narrow as 1 mμsec are confidently expected The beam current in the narrow pulse is expected to exceed 2 amp Pulse widths may, however, be varied up to 1 μsec with correspondingly lower repetition-rates Since the *S*-band machine operates at 3×10^9 c /s and the electrons are bunched on the crest of each cycle with a phase spread of 20°, the electron beam is composed of individual electron bunches each 2×10^{-11} sec in duration with $\frac{1}{3}$ mμsec spacing between them A 1-mμsec pulse, as mentioned here, will thus be composed of 3–5 such bunches, each carrying 25 amp , that is, each bunch is $\frac{1}{2}$ cm diameter, $\frac{3}{4}$ cm long and contains 3×10^9 electrons The energy of the electron beam will be continuously variable from 5 MeV to the maximum value of 250 MeV Experiments in nuclear physics and radiation chemistry are being planned Intense beams of photons, electrons and neutrons will be available for these

Australian National Insect Collection

OVER the past thirty years the Division of Entomology of the (Australian) Commonwealth Scientific and Industrial Research Organization has been building up a collection of insects drawn from all States and Territories of the Commonwealth Intensive collecting by officers of the Division has been supplemented by numerous major donations and bequests, and to-day the collection is unquestionably the largest and most representative in existence of the insects of Australia and her dependent territories By proclamation in the *Commonwealth Gazette*, the collection has been named the Australian National Insect Collection in order to emphasize its status as a national heritage which it is the responsibility of the Commonwealth to preserve in perpetuity for scientific-study The title establishes an Australian equivalent of the Canadian and South African National Insect Collections and is to be welcomed for the assurance it implies regarding the future of the Collection The Collection will remain for the present in the custody of the Division of Entomology in Canberra, where it will play its part in the Division's research programme Co-operation with Australian and overseas museums will continue as in the past

The National Foundation for Scientific Research, Brussels

THE thirty-fourth annual report of the National Foundation for Scientific Research Brussels, for 1960–61 (Pp 384 Bruxelles Fonds National de la Recherche Scientifique, 1962), records the award of 104 research grants totalling 9 3 million francs to individual investigators, varying in amount from 25,000 to 200,000 francs Seventeen grants for special research work in the academic year 1961–62, totalling 11,308,088 francs, were also made and five extraordinary awards for 1960–61, amounting to 11,349,000 francs These include 2 25 million francs for the determination of coefficients of diffusion in alloys; 625,000 francs for an experimental study of the irreversible phenomena of relaxation of nuclear magnetism, 426,125 francs for a structural study of new amino-acids produced by different vegetables and fungi, including the isolation, determination of structure and synthesis of antibiotics, 750,000 francs for a study of high dispersion in the infra-red absorption spectra of vinyl and ethylene isotopes, 957,500 francs for the construction of an ultra-violet spectrograph of very high power, 994,845 francs for the purchase of apparatus for the study of electronic resonance in irradiation by X-rays in polymerization and in the radiolysis of organic liquids and alcohols in the solid state, 677,500 francs for the study of natural products, including indolic alkaloids, erythrophleums and *Morinda lucida*, 1,262,158 francs for the study of constructional materials in which deformation has an important influence in their behaviour in fatigue (including concrete, wood and plastics), and 588,900 francs for studies of the toxicology of pharmaceutical products, including the identification of metabolites and the standardization of pharmaceutical preparations based on enzymes A subsidy of 8 million francs was awarded to the Foundation for Scientific Medical Research for investigations on radioisotopes and cancer Other grants were made to assist attendance at scientific meetings in other countries Lists of awards are appended to the report, together with a list of publications by recipients of awards, 1958–61

Health in India

ON June 12, 1959, the Government of India set up a committee to review development in all matters relating to health and to formulate plans for the country in the third and subsequent Five-Year Plan periods The chairman was Dr A Lakshmanaswami Mudaliar, vice-chancellor of the University of Madras, who, with his distinguished colleagues, has now presented a report which should form a basis for improving health services in India for many years to come Even on such controversial issues as the limiting of population growth and the indigenous systems of medicine, the committee is clear and firm in its recommendations With all its implications for the future of India, the way in which the report has been prepared and presented merits praise, it could be read with profit by many health administrators outside India (Government of India Ministry of Health Report of the Health Survey and Planning Committee (August 1959–October 1961), Vol 1 Pp 482 + 56 Delhi Government of India—Ministry of Health, 1962)

Unesco and Termite Research

PROBLEMS of termite research were discussed at Unesco House in Paris during June 12–15 by a sub-committee recently set up on the recommendation of the International Advisory Committee for Humid Tropics Research Members of the sub-committee attending were Dr R L Araujo (Sao Paulo), Prof K Gosswald (Wurzburg), Prof P P Grassé (Paris), Mr W V Harris (London) and Dr M L Roonwal (Calcutta) Also present as observers were Dr E Ernst (Basle) for the International Union of Biological Sciences, and Dr L. G E Kalshoven (Blaricum) for the International Society of Soil Science The resolutions which had been adopted at the symposium on Termites in the Humid Tropics, arranged jointly by Unesco and the Zoological Survey of India at Delhi in October 1960, formed a basis for the discussions It was agreed that the international co-operation desirable in termite research, and in the dissemination of results of practical value in the control of termites as pests of buildings and crops, would best be achieved through Unesco Among the projects suggested for early consideration and action were (1) review of the influence of termite activity on soil fertility, (2) review of the methods used in combating termite damage to buildings and to tropical crops, (3) investigation of introduced termites, and of methods for restricting the spread of injurious species, (4) co-ordination of methods for the laboratory testing of termite resistance in materials, (5) standardization of measurements used in termite taxonomy, and of terminologies used in termite biology

Refuse Disposal

EVERY year local authorities in Great Britain dispose of nearly 100,000,000 cubic yards of refuse, weighing about 13,000,000 tons Up to 20 per cent by weight of this is worth salvaging, and, as living standards rise, the proportion increases Many local authorities have central refuse sorting plants where these materials are extracted and sold back to industry Stoke-on-Trent, for example, has an income from this source of 20*s* for every ton of refuse handled Local authorities are apt to be cautious in their approach to any positive change in their established practice of refuse disposal, but, if they had a statutory duty to protect the landscape, carrying equal weight with their public health obligations, it is likely that the advantages offered by new methods of refuse and sewage disposal would compel their speedy adoption The Sheffield and Peak District Branch of the Council for the Preservation of Rural England has published a useful pamphlet by J C Wylie on this subject which is specially valuable for the information it gives on composting in practice (price 2*s*) The main advantages of composting are that no large area of land is required, composting plants can be permanently placed in close proximity to the areas from which the refuse is collected, the composting process, when aerobically carried out, is hygienic and is not a nuisance It offers a solution to the difficult problem of sewage sludge disposal, no more manpower is called for than is required under present systems of disposal, the prospects for selling compost are excellent

Norsk Polarinstitutt, Årbok, for 1960

THE Norsk Polarinstitutt has recently published the first of a new series, its *Årbok* (Yearbook) for 1960 This addition to the existing *Skrifter* (papers) and *Meddelelser* (communications) which have been published by the Institute for some years will fulfil several functions It will contain the annual report of the Director, general information about the Norsk Polarinstitutt, information on scientific work carried out by foreign expeditions in Norwegian Arctic and Antarctic territories, and shorter scientific papers of general interest. In the 1960 *Årbok*, about 80 per cent of the text is in English, so its use will not be restricted to those with a knowledge of Norwegian Shorter papers cover items such as the discovery of footprints of the dinosaur in the lower cretaceous of Vestspitzbergen, a discussion of the fossil *Gigantaspis* which has only been reported once before, and a general paper surveying height limits for vascular plants in Svalbard (in Norwegian) A short report of a glaciological survey of Maudheim in 1960, eight years after the station of the Norwegian-British-Swedish Antarctic Expedition was abandoned, throws useful light on processes of bottom melting and surface accumulation on ice shelves A review in English on recently published Russian work on the bathymetry of the region between Spitsbergen and Greenland shows that the postulated Nansen Ridge connecting the two lands does not exist, and a trough of more than 3,000 m in depth separates the two regions Another useful survey is that on absolute age determinations on rocks from Dronning Maud Land, Antarctica, by recent Belgian, Japanese and Russian expeditions Several other articles, the annual report and a short history of the Institute complete the *Årbok*

Scientific Research in British Universities and Colleges, 1961–62

Scientific Research in British Universities has now been superseded by the Department of Scientific and Industrial Research's publication entitled *Scientific Research in British Universities and Colleges* (1961–62 Pp xvii+622 London H M S O, 1962 32*s* 6*d* net) The hand-book lists research being carried out at present in universities and colleges and advanced technical colleges and regional technical colleges in England and Wales and equivalent colleges in Scotland and Northern Ireland It describes the nature of the project in sufficient detail to indicate the sort of research done by the various scientific departments and individual teams of investigators, but does not refer to the social science and industrial administration except where work in this field normally occurs within the science faculty or technical department The format of the hand-book remains the same and the name- and subject-indexes are separate Copies of the hand-book are available from the Department of Scientific and Industrial Research, State House, High Holborn, London, W C 1

Publications of the Instituto Antartico Argentino

DURING recent years the Argentine Antarctic Institute has played an active part in developing scientific investigations of Antarctica In addition to being responsible for the operation of the Ellsworth-General Belgrano group of stations at the head of the Weddell Sea, the Institute has developed a good library, has a scientific staff working on Antarctic results for publication, and is the main centre of Antarctic research in the Argentine The symposium on "The Antarctic" held there in 1959 was attended by 57 representatives of other countries, and was most successful as a means of exchanging early information on the results of research of the International Geophysical Year The Institute publishes several series which include the *Boletin del Instituto Antartico*

Argentino which appears twice per annum and contains short articles of general interest on Antarctic and Institute activities *Contribuciones del Instituto Antartico Argentina* present scientific papers on original work and some review papers, such as No 49, "Progress Recientes en Glaciologia Antartica", by R N Panzarini Some 53 *Contribuciones* have appeared so far *Publicaciones del Instituto Antartico Argentino,* of which eight have now appeared, are monographs of greater length and wider scope than many of the *Contribuciones,* "Estudio Geologicao-Petrografico de Caleta Potter, 25 de Mayo, Islas Shetland del Sur" by N H Fourcade being the most recent one The Institute also publishes a translation in Spanish of the *SCAR Bulletin,* which records the activities of the Scientific Committee on Antarctic Research of the International Council of Scientific Unions The Instituto Antartico Argentino has thus become the most important source of Spanish language publications dealing with Antarctic scientific research, and it is playing a valuable part in the twelve-nation co-operative research programme in Antarctica

Library Reference Services in the United States

Scientific Information Notes for April–May 1962, issued by the National Science Foundation, reports the selection of twelve universities and libraries in the United States to make more widely available the unclassified results of Federally sponsored research and development The Department of Defence, National Aeronautics and Space Administration and the Atomic Energy Commission, which it is estimated produce 90 per cent of the Government s unclassified technical reports, have agreed to provide copies of their reports for deposit in these centres, which will organize and maintain a cumulative library collection of all such material, providing personal reference service for the general public inter-library loans, and, where appropriate, assistance in obtaining copies for retention The National Federation of Science Abstracting and Indexing Services is developing, with the support of the National Science Foundation, a programme defining the present and future role of United States scientific abstracting, indexing and reviewing services The Biological Sciences Communication Project of the American Institute of Biological Sciences has inaugurated a programme for training graduate-level students to become "Science Information Specialists" The same Institute is conducting a literature survey to determine the proportion of biological research supported by the Federal Government

Assessing Reference Library Efficiency

In an article in the *Journal of Documentation* for June, P F Cole takes a new look at 'reference scattering' He claims that the slope of the curve obtained by plotting the cumulative fraction of total references against the logarithm of the cumulative fraction of total titles gives a 'reference-scattering coefficient' which may be characteristic of the subject field The reference-count method is regarded as giving untrustworthy results, but this reference-scattering coefficient can be used to derive a general relation between number of references and number of titles and provides a yardstick for assessing the efficiency of correlation between the holdings of a petroleum industry library, for example, and the needs of its users It can also be used as a criterion for detecting abnormalities in the results of literature usage surveys In the same issue, Dr A N Oppenheim contributes an interesting survey of the reading habits of students at the London School of Economics where Dr Oppenheim is a lecturer in the Department of Social and Industrial Psychology.

Magnesium Oxide and Hydrochloric Acid from Concentrated Sea Bittern

The mother liquor that remains after sodium chloride has been crystallized out of sea-water is termed bittern The production of magnesium oxide and hydrochloric acid by the thermal decomposition of crude magnesium chloride, recovered from sea bittern, has been attempted under different conditions In a procedure recently outlined by R K. Sapre and M P Bhatt, of the Central Salt Research Institute, Bhavnagar, debrominated bittern is concentrated first by solar evaporation and then, by forced evaporation, into a liquor which, on cooling, sets into a cake containing 46–50 per cent magnesium chloride (*J Sci Indust Res* , 21D, No 2, February 1962) The cake is then decomposed in a furnace by direct firing with an oil burner at 500°–700° C and the gaseous products cooled and condensed to give hydrochloric acid of 25 per cent concentration The residue containing 82 per cent magnesium oxide, on washing and dehydration at 500°–600° C , yields magnesium oxide of 96 per cent purity The recovery of hydrochloric acid is 76–78 per cent

Epidemiology of Skin Diseases in Africa

Dr James Marshall, head of the Department of Dermatology, University of Stellenbosch, writes that a pilot survey on the epidemiology of skin diseases in Africa has yielded such interesting material that he is preparing, with the assistance of the South African Council for Scientific and Industrial Research, to make a large-scale investigation of the distribution and incidence of skin diseases on the continent of Africa The ultimate aim is to establish a central office for information and a reference library of literature and photographs Dr Marshall wishes to approach not only those in scientific and academic institutions, but also anyone who practises or has practised in Africa who could supply information, even if it were only on a limited field of the question Questionnaires can be obtained from Dr James Marshall, Simon's View, Sorrento Road, St James, C P , South Africa

Meteorology and Poliomyelitis

Bio-meteorology is a branch of atmospheric science which has not received a great deal of attention in Britain In particular, the relationship between health and the weather is a subject of wide interest, a better understanding of which could be of considerable practical benefit to many Some of the relatively few investigations which have been made on such relationships in the United Kingdom are summarized in a note on the "Importance of Meteorological Factors to the Incidence of Poliomyelitis" by E N Lawrence (*Brit J Prev Soc Med* , 16, 46, 1962) The main parameters which have been investigated are dry-bulb temperature, vapour pressure and relative humidity Of these, it appears that the incidence of polio is highly correlated with temperature There are also indications of a positive correlation with humidity, though Spicer, a co-worker in these investigations, found a negative correlation with relative humidity and suggested that atmospheric drying power may be the important factor with which to correlate the incidence of the disease

Other workers, such as Hemmes, Winkler and Kool (*Nature*, 188, 430; 1960), conclude that relative humidity indoors is an important environmental factor contributing to the seasonal fluctuations of the morbidity of the disease, and show that the death-rate of the virus is diminished at higher relative humidities. Since the normal range of indoor temperature is restricted, indoor relative humidity may well be governed by the outdoor vapour pressure. Thus, high outdoor vapour pressure could be associated with high indoor relative humidity and both these parameters could be positively correlated with poliomyelitis incidence.

Anopheles hyrcanus and Malaria in East China

Anopheles hyrcanus sinensis, the chief component element of the anopheline fauna in South-east Asia, is not a single taxonomic unit but a species of the *Anopheles hyrcanus* group. This group includes a number of closely allied species which show only slight differences in adult morphology but diverge widely in behaviour, physiology and in their relation to malaria transmission. Both malaria and filariasis are prevalent in various areas in the lower Yangtze Valley, where the vector is said to be '*Anopheles hyrcanus sinensis*'. In the hilly tracts of Anhwei, Chekiang and Kiangsu provinces, malaria and filariasis are both prevalent. Investigations show that the major anophelines are two sibling species of the *Anopheles hyrcanus* group, one laying wide-decked eggs and the other narrow-decked eggs. The former is much more numerous than the latter and is more widely distributed. In their investigations for distinguishing characters of the two sibling species in the adult stage, Ho Ch'i *et al.*, of the Laboratory of Malariology, Institute of Parasitic Disease, Chinese Academy of Medical Sciences, Shanghai, found that the presence or absence of *T*-shaped cloudy markings on the membranous pleuron of the female abdomen is a reliable method of identification (*Chinese Medical Journal*, 81, No. 2; February 1962). Studies on the bionomics of the two anophelines—their food habits, resting habits, breeding habits, nocturnal activities, seasonal distributions, and hibernating characteristics—showed that differences in food habits, breeding habitats and hibernations are of epidemiologic importance. In the hilly tracts of the lower Yangtze Valley, where both malaria and filariasis occur and both anophelines are present, *A. lesteri* is the more important vector; *A. sinensis*, though also a vector, is of minor importance. In large open plains, where malaria and filariasis are endemic in a low degree, and only *A. sinensis* is found, it is responsible for the continuous occurrence of malaria, its seasonal variations, and at times outbreaks of malaria epidemics.

Flint Burins from California

WHEN making a flint implement from a blade or flake the object is held horizontally, and vertical blows are given to produce a trimmed edge. In the case of a burin, however, the blade is itself held vertically and a blow given, resulting in a flake scar which runs down the edge of the blade. When two such flake scars on the opposite sides of the blade intersect the result is a *bec de flute* or ordinary burin, a sort of flint screwdriver in appearance; when the burin facet is backed against a trimmed edge the tool is called an angle burin. Burins were for a long time considered to belong exclusively to the Upper Palæolithic and Mesolithic cultures of western Europe. From time to time an odd example occurred elsewhere from Australia to America, but these were usually considered to be chance manufactures. A whole collection of these tools, associated with small blades and cores, which in Europe would be classed as Mesolithic but which are said to come from Santa Cruz Island off California, have now been described. They were collected by Léon de Cessac as long ago as 1878 and have since been in the Musée de l'Homme at Paris. Cessac made many excavations in the Santa Barbara region on the mainland, as well as on several of the offshore islands. For the first time the collection has been studied by R. F. Heizer, and the results published with the collaboration of Harper Kelley in the *Proceedings of the American Philosophical Society* for 1962. The collection, as judged from the illustrations, is certainly extraordinary as coming from California. Accepting that there has been no admixture since 1878 when they were given to the Musée de l'Homme, it is not too easy to believe that an assemblage of tools so typical of Western European cultures could have occurred all together in sites off the west coast of America.

The Red Ochre Culture of the Upper Great Lakes of North America

THE March issue of *Fieldiana: Anthropology* contains an article on "The Red Ocher Culture of the Upper Great Lakes and Adjacent Areas" by R. E. Ritzenthaler, the curator of the Milwaukee Museum and G. I. Quimby, the curator of the Museum at Chicago (Vol. 36, No. 11. Pp. 243–275. Chicago: Chicago Natural History Museum, 1962. 1 dollar). The name "Red Ocher Culture" was first used in 1937 and refers to finds made in Wisconsin, Iowa, Illinois, Indiana, Ohio, Michigan and Ontario. It thus has a wide distribution. Common features at all sites are the use of a layer of red ochre to cover the graves, the burying of the corpses in a flexed position in pits and in sand, the occurrence of large ceremonial flint blades (sometimes up to 19 in. in length) and other stone tools, and of some copper awls, celts, knives and points.

Tenth Pugwash Conference

MORE than two hundred scientists from all over the world will meet in London during September 3–7 to consider how science can help to solve some of the world's problems. The Conference will be the tenth organized by the Pugwash organization of scientists, and the first to be held in London (Pugwash is the name of the village in Nova Scotia where the first conference was held in 1957). Among the participants will be sixty scientists from the United States. The British delegation will comprise thirty, and a similar number will come from the U.S.S.R. At least thirty other countries will be represented. The Royal Society will act as host for the London Conference, which will be held in the Russell Hotel and will be in private. An account of the proceedings will, however, be made public by participants at the Conference at the end of each day.

Among the subjects to be discussed at the Conference are the position of the scientist in the community, the problem of world security, international collaboration in science and the part which science can play in the developing nations. There will also be discussions of the ways in which science can assist in education. The Conference will also receive a report on measures for arms control prepared by a conference to be held at Cambridge in the week of August 27.

Operational Research for Management

The University of Hull is offering as from October a one-year postgraduate diploma course in operational research for management Operational research is one of the several management tools designed to provide industry with the means of reaching informed decisions It is an important basis of modern scientific management In no sense does it attempt to replace management Although related to them, it is to be distinguished from such other management aids as work study, ergonomics and organization and methods The techniques of operational research are mainly mathematical and statistical in character, many of them were used primarily for military purposes, but to-day they are used increasingly in modern industry Applicants for the course should have some prior knowledge of mathematics, but it will otherwise be open to graduates in any subject and, in special circumstances, to non-graduates also In addition to graduates in economics and statistics, a training in operational research can add considerably to the qualifications of physicists, chemists and engineers intending to go into industry, and much of the course will be concerned with the application of scientific techniques to practical management and industrial problems Further information can be obtained from the Registrar

The Paul Instrument Fund Awards

The Paul Instrument Fund Committee has made grants as follows £2,736 to Dr S Evans, senior assistant in polar research, University of Cambridge, for the development of an instrument for measuring the depth of continental ice sheets by a radar technique, £10,500 over a period of two years to Dr W R S Garton, reader in physics, University of London, and Dr R C M Learner, research associate, Imperial College of Science and Technology, for the construction of a high-resolution broad-range vacuum spectrograph, £3,600 to Prof J W S Pringle, Linacre professor of zoology, University of Oxford, for the construction of apparatus for making various physical measurements on muscle by sinusoidal dynamic analysis The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much-improved types of physical instruments and apparatus for investigations in pure or applied physical science"

The Leopoldina German Academy of Sciences Elections

The Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated *Mathematics*, Prof L Collatz (Hamburg), Prof L Rédei (Szeged), Prof R Sauer (Munchen) *Physiology*, Prof G von Békésy (Cambridge, Mass) *Physiological Chemistry*, Prof F B Straub (Budapest) *Pharmacology*, Prof J H Burn (Oxford), Prof U S von Euler (Stockholm), Prof J H Gaddum (Cambridge), Prof O Krayer (Boston, Mass), Prof L Lendle (Gottingen), Prof K Repke (Berlin-Buch), Prof B K M Uvnas (Stockholm), Prof W Wilbrandt (Berne) *Hygiene*, Prof P Grabar (Paris), Prof E G Nauck (Hamburg) *Internal Medicine*, Prof M Gulzow (Rostock), Prof N Henning (Erlangen)

Announcements

Dr C Bloomfield has been appointed by the Lawes Agricultural Trust Committee to succeed the late Dr A Muir as head of the Pedology Department of Rothamsted Experimental Station, Harpenden, Herts

At the annual general meeting, held on June 20, Dr J O Irwin was elected president of the Royal Statistical Society for the ensuing year Prof P Armitage, Mr B P Emmett and Miss S V Cunliffe were elected honorary secretaries, and Mr C T Saunders was elected honorary treasurer Prof P Armitage was awarded the Society's Guy Medal in Bronze

The Council of the Plastics Institute has accepted an offer from Temple Press, Ltd , to mark the Centenary of Plastics year This award, to be known as the 'Plastics' Centenary Award, will comprise a cash award of £75 to be given every two years, to the candidate who achieves the best overall results in the Plastics Institute Examination A detailed specification for the award can be obtained from the Plastics Institute, 6 Mandeville Place, London, W 1

The North Atlantic Treaty Organization is offering financial support for short courses, of two weeks or more duration, at which scientific topics are discussed at an advanced level The grant may be used for administrative expenses, publication of proceedings, or to subsidize the expenses of foreign visitors Further information can be obtained from the Scientific Affairs Division, North Atlantic Treaty Organization, Place du Maréchal de Lattre de Tassigny, Paris 16^{e}

The fifth international congress on "Electron Microscopy", organized by the Electron Microscope Society of America, will be held in Philadelphia during August 29–September 5 Further information can be obtained from the Fifth International Congress for Electron Microscopy, 7701 Burholme Avenue, Philadelphia 11, Pa

The ninth international symposium on "Combustion", arranged by the Department of Thermal Engineering at Cornell University under the auspices of the Combustion Institute will be held at Cornell University during August 27–September 1 Further information can be obtained from the Combustion Symposium Office, Upson Hall, Sibley School of Mechanical Engineering, Cornell University, Ithaca, New York

The third international meeting of the Collegium Internationale Neuro-Psychopharmacologicum will be held in Munich during September 2–5 The programme will include the following symposia methods of comparison of behaviour changes with drugs in animals and man, effectiveness of drugs in relationship to psychological and social forms of treatment, ten years of psychopharmacology, critical assessment of the present and future Further information can be obtained from Prof W de Boor, Kerpenerstrasse 4, Cologne, or Prof F A Freyham, Saint Elizabeth's Hospital, Washington, D C , or Dr P D Bradley, the Medical School Birmingham

Erratum In the biographical note concerning Mr A Stratton that appeared on p 918 of the June 9 issue of *Nature*, the penultimate sentence should read "In 1951 he obtained an M Sc (London) degree in mathematics and in 1957 a B Sc (Exeter), *ad eundem*"

RECRUITMENT OF BRITISH PERSONNEL FOR SERVICE OVERSEAS

IN reply to a question in the House of Commons on May 22, the Secretary for Technical Co-operation, Mr Denis Vosper, said that grants of £11,000 in 1959–60, £2,000 in 1960–61 and £17,500 in 1961–62 had been made to Voluntary Service Overseas, the grant for 1962–63 was under consideration He fully agreed as to the value of the work and hoped that he might have something to add after his talks with the voluntary bodies concerned on May 23 It was part of his task to meet the requests of overseas countries for technical people, and this could not be met on a voluntary basis

On May 29, Mr Vosper said that as a result of discussions with representatives of many voluntary societies and other interested people, a scheme had been worked out to co-ordinate and stimulate the recruitment of volunteers for overseas service These steps are supplementary to the general recruitment activities of his Department described in a White Paper also issued on May 29 He thought comparison of Voluntary Service Overseas with the American Peace Corps was accordingly misleading The present programme of Voluntary Service Overseas would send 160 individuals overseas, but others were sent by other organizations He hoped for a considerable advance on this number and welcomed the suggestion that voluntary service should be put on a wider basis One proposal of the statement circulated by Mr Vosper was for a co-ordinating committee on which would be represented all the bodies concerned and which would co-ordinate existing and stimulate further activities

The statement refers to the great and growing interest in the service overseas by volunteers and to the meetings held by the Department with voluntary societies and other bodies concerned At these meetings it was agreed that recruitment dispatch overseas of volunteers would best be discharged by the voluntary bodies, but that their work could be made more effective by co-ordination A committee, known as the Voluntary Societies Committee for Service Overseas, had been established to co-ordinate generally the work of voluntary societies in the whole field of voluntary service overseas by school-leavers, young graduates and other qualified or trained personnel and to work out a scheme for graduates It would formulate general policy for recruiting graduate volunteers, maintain contact with universities, colleges, industry and commerce to encourage applications for voluntary service, and disseminate information about the progress of the scheme The voluntary societies would remain responsible for all executive action connected with recruitment dispatch and administration of volunteers, including graduates, as well as the selection of graduates Dr J F Lockwood would be chairman of the Committee, which would include representatives of the National Council of Social Service, the British Council of Churches, the Overseas Development Institute, Voluntary Service Overseas, the Department of Technical Co-operation, the British Council, the National Union of Students, and six members from universities, colleges of advanced technology, schools, industry and commerce

The National Council of Social Service has agreed to provide a secretariat and the Committee would start at once to plan a campaign for recruitment of graduates as volunteers for the summer of 1963, while for 1962 it was planned to send fifty graduates to Africa on a pilot scheme for volunteer teachers The British Government would pay for return passages and terminal grants and meet some other incidental expenses In addition, the United Nations Association, the National Union of Students and International Voluntary Service were planning to send abroad this year a few graduate volunteers for a year or more, and Voluntary Service Overseas continue to build up their existing substantial programmes for sending school-leavers overseas If suitable schemes were presented by these bodies, the Department of Technical Co-operation would be ready to provide financial support up to an amount similar to that for the pilot scheme for volunteer teachers

The White Paper *Recruitment for Service Overseas Future Policy** sets forth the present nature of recruitment through the British Government for service in both independent and dependent countries overseas and for international organizations It examines the probable development of demand for such staff in the next four years and the problems likely to arise, and describes the steps being taken by the Department of Technical Co-operation to meet the demand Apart from taking account of the recommendation of the Select Committee on Estimates for the establishment of a Commonwealth Advisory and Technical Service, the White Paper is of interest as recognizing the existence of the Overseas Research Council Besides providing a concise statement of the policy the Department proposes to pursue, it includes a detailed summary of action being taken in specific fields and some statistics of appointments

The White Paper draws the conclusion that the proposal of the Select Committee on Estimates does not take sufficient account of the nature of the demand or the character of the supply The Department therefore proposes to apply the basic principle that most recruitment in future for service overseas within the sphere of the Department should be for limited periods which form part of a career based primarily in Britain or in the service of a British institution The way in which this principle is applied will vary widely from one profession to another, but the major thread is that people should find it easy, and preferably advantageous, to resume their professional employment in Britain (or with a British Institution) after a period of service overseas

The formal terms of appointment will be either contract, loan or secondment A new and more flexible form of contract, adapted to present overseas conditions, is now being prepared The National Council for the Supply of Teachers Overseas has asked all United Kingdom educational institutions to allow teachers going to suitable appointments overseas to preserve the continuation of their pensions and to have the right of return to their previous employment (though not necessarily their previous post). In the university field the flow of teachers on long-term conditions of service overseas requires supplementing by arrangements for supplying staff for short-term appointments tailored to meet the specific needs of overseas universities. A small committee has been set up by the Department to make possible and stimulate such arrangements through the British universities. Arrangements for the loan of staff already exist in the Civil Service and nationalized industries. It will be the aim of the Department of Technical Co-operation to develop and maintain existing arrangements of this kind and to introduce them in appropriate fields where they do not exist, in general, to encourage large employers in the public and private sector to accept the need to release qualified staff as required for service overseas.

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* Department of Technical Co operation *Recruitment for Service Overseas—Future Policy* Pp 29 (Cmnd 1740) (London H M S O , 1962) 2*s* net

For arrangements of this kind to be effective, it is important that the employers concerned should take an active interest in the encouragement of such service overseas, and that the value of such a service should be recognized by employers in Britain and taken into account in considering employees for promotion. It is a major task of the Department of Technical Co-operation in conjunction with other Government departments and the relevant professional bodies to achieve this.

The White Paper recognizes that the lending of staff in this way may be troublesome and inconvenient, but none the less, from the point of view of Britain, it is important that all in a position to do so should co-operate in making such service possible. Further, the Government must give the lead and all Government departments will make a special new effort to release staff for overseas service when they can, and staff will be encouraged to volunteer for this purpose. It is also proposed to examine the position profession by profession, and to discuss the matter with the Federation of British Industries and with certain large-scale employers. In the field of scientific research it has been possible to appoint people to home-based establishments on terms which require their release for overseas assignments from time to time, so that when the scientist concerned is not undertaking research overseas, he can be suitably employed on research in Britain. It is recognized, however that the number of people employed in this way is unlikely ever to be high.

CHEMICAL APPLICATIONS OF ELECTRON SPIN RESONANCE

A SHORT course dealing with "Chemical Applications of Electron Spin Resonance" was held at the Department of Chemical Technology, Bradford Institute of Technology, on February 15–16, 1962. It followed an analogous symposium last year on 'Applications of Nuclear Magnetic Resonance Spectroscopy" and was attended by about fifty chemists and physicists from the chemical and electronics industries and the universities and technical colleges.

Opening the meeting, Dr. E. G. Edwards, principal of Bradford Institute of Technology, noted the interaction between science and technology, appropriate to a College of Advanced Technology, which was implicit in the application to chemistry of a technique based on microwave equipment and large magnets of variable field.

In his introductory survey of electron spin resonance, Prof. D. J. E. Ingram (University of Keele) showed that the enhanced magnetic energy separation in a given field arising from the larger magnetic moments possessed by electrons than nuclei led both to a higher absorption frequency (microwave rather than radio) and also to a higher net absorption and sensitivity. Indeed, as few as $10^{11}\Delta H/\tau^{1/2}$ unpaired spins, where ΔH is the line width in gauss and τ is the time-constant in seconds, could be detected from the integrated line intensity. Discussing the hyperfine interaction between the unpaired electron and nearby nuclear spins, on which much of the chemical utility of electron spin resonance depends, Prof. Ingram outlined the two contributions to the Hamiltonian: the dipolar effect, angular dependent unless averaged out, and the isotropic Fermi contact term.

Dr. B. A. Coles (University of Oxford) contrasted the experimental techniques of shorter wave-length spectroscopy with those appropriate to operation in X (around 9,400 Mc./s.) and Q (around 35,000 Mc./s.) bands, and illustrated the mode patterns in wave guides and cavities. Replacement of the simple transmission cavity by a reflexion cavity and a microwave 'crossroads' bridge or preferably one of the 'roundabout' or ring type increases the useful proportion of microwave power measured. Further, the high sensitivity associated with a low band-width/frequency ratio is achieved by (1) adopting phase-sensitive detection (with small modulation amplitudes, the first differential of the absorption is recorded), and (2) modulating the field at 100 kc./s. rather than 50 c./s. However, if the resulting modulation satellites at about 35 mG limit resolution, it is preferable to superheterodyne sources of, say, 10,000 and 10,030 Mc./s. and to use low-frequency modulation.

A manufacturers' session followed, in which Mr. I. G. Morgan (Hilger and Watts, Ltd., and Microwave Instruments, Ltd.) described the 100 kc./s. Microspin spectrometer for Q and X band operation, Mr. J. M. Babb (Varian A.G.) emphasized the research and service activities in electron spin resonance of his organization, and Mr. J. L. Williams (Associated Electrical Industries, Ltd.) and Mr. M. J. Amos (Mullard, Ltd.) referred to magnet systems suitable for electron spin resonance.

The next three speakers discussed the application of electron spin resonance to free radicals. Dr. P. B. Ayscough (University of Leeds) showed how the nuclear hyperfine structure of narrow lines from free radicals in dilute solution could reveal the detailed unpaired electron distribution. According to the complexity of the spectrum and the condition of the specimen, a concentration of 10^{-4}–10^{-6} M was needed for identification rather than mere detection, for varying concentrations, the minimum was about ten times higher. Since these are larger than the stationary concentrations met in alkyl systems, aromatic and other comparatively stable radicals had been most studied. Dr. Ayscough then dealt with the problems of distinguishing between equivalent and non-equivalent protons and of synthesizing spectra for various values of hyperfine splitting constants In some radicals, for example, triphenylmethyl, which can be used as a reference, the total of spin densities computed was greater than unity because densities at some carbon atoms have spin opposite to that of the unpaired electron (although the signs are not determined by the electron spin resonance data)

On irradiation at low temperatures, solid polymers frequently yield measurable free radical concentrations which decay slowly at room temperature Mr M G Ormerod (Royal Military College of Science, Shrivenham) described the use of electron spin resonance as a check of purity, for detection of intermediates, and for the estimation of degree of cross-linking, future work might include the effects of water and of radiation protection additives Measurements with drawn fibres sometimes enabled the nature of the radical(s) to be identified, but resolution was lower than with irradiated single crystals, which formed the subject of the talk by Dr A Horsfield (Varian A G , Zurich) In dilute free-radical systems trapped among the host molecules of the crystal matrix, not only can the irradiation damage, such as stripping of a hydrogen atom, be investigated, as with polymers, but also the anisotropic hyperfine splitting associated with specific chemical groups may be obtained Among the simple negative inorganic radicals studied recently by D H. Whiffen and collaborators, Dr Horsfield mentioned the $SO_3^{\cdot -}$ ion obtained from salts containing the $—SO_3^-$ group, the single line spectrum is split into a quadruplet by the sulphur-33 nucleus ($I = 3/2$) in its 0 7 per cent natural abundance In $\dot{C}H(SO_3)_2^{2-}$, hyperfine splitting due to protons on nearby $CH_2(SO_3)_2^{2-}$ host ions has also been detected in crystals of the potassium salt

Dr A Carrington (University of Cambridge) turned from free radicals to the other main field of application of electron spin resonance to chemistry, transition metal ions The effect of the exact ligand symmetry on g-values and the fine structure resulting from several unpaired electrons were considered for dilute crystals of complexes Hyperfine interaction with ligand nuclei possessing magnetic moments as with the nitrogens in hæmoglobin, could provide crystallographic information Finally, Dr Carrington pointed out that, whereas the meeting had been devoted so far to the study of ground states, electron spin resonance could provide a means of studying excited states Recently, Hutchison had detected resonance in the phosphorescent state obtained from ultra-violet irradiation of a dilute solid solution of naphthalene in durene D W JONES

SOUTH-EASTERN UNION OF SCIENTIFIC SOCIETIES ANNUAL CONGRESS

AT the invitation of the Horsham Urban District Council and the Horsham Natural History Society, the South-Eastern Union of Scientific Societies held its sixty-seventh annual congress at Horsham during May 25–27 under the presidency of Dr Maurice Burton The headquarters of the congress was at Collyer's School, by kind permission of the headmaster

The delegates were welcomed by the chairman of the Urban District Council, Councillor Cullen, and J Southwood, the president of the Natural History Society They emphasized the need of collecting information regarding the footpaths in and around Horsham, continual development had caused the loss of many paths and often there was considerable difference between the amenities of an ancient pathway and the alternative diversion

The young naturalists' evening consisted of a 'brains trust', questions being debated by a panel under the chairmanship of Maxwell Knight The panel particularly stressed the desirability of biological control where damage was caused by animals, pointing out that spraying with insecticides over long periods cannot be justified There was an increase in the mortality-rate of birds last year, one cause being that insufficient notice was taken of the manufacturers' instructions, another was the careless throwing aside of used containers with contaminated water

The presidential address was given by Dr Maurice Burton, whose subject was "The Fallibility of Human Observation" Wishing to know the difference between biology and natural history, he had consulted *The Oxford Dictionary*, which defined natural history as "the unintelligent study of biology" Although startled at first, he had to agree that this was correct, although the choice of wording was unfortunate Seeing is a purely physical process, to be likened to the operation in a camera, while observing is this plus other mental processes It has often been said that it is "far more virtuous to observe than to see", but this is not always correct Dr Burton spoke of his experience when surprised by the appearance of a white bird in his holly tree On using his binoculars he discovered he had been looking at the underside of the holly leaves, members of his family at the same time experienced the same illusion He followed with the example of a class shown a drawing of an isosceles triangle with a rectangle attached to the centre of the base Halves of the class were shown the diagram independently, one having it described as a trowel, the other as a Christmas tree Later, when asked to draw what they had seen, they showed all graduations from a trowel with a long handle to a Christmas tree On another occasion he asked a friend, well informed on the subject-matter, to help by proof reading The galley and paginated proofs were passed, but, on receiving his author's copy, he was dismayed to find a caption under an illustration spelt incorrectly With the book open at this page on his desk, he was visited by the proof reader who, glancing from a distance of 3 ft , exclaimed "Did I pass that ?" His observation during proof reading made him see what he had expected, not what was

The important thing in studying animal behaviour is to look, look and look, to allow the impression to soak in, not permitting a previous impression to be formed Dr Burton considered that verbal pictures ran the risk of being wrong, drawings were better He showed and commented on a number of slides dealing with the 'Loch Ness monster', his examination had convinced him that the creature drawn and photographed was an otter, the other pictures were of a motor-boat. He concluded by saying that his analysis of this problem showed that maybe the dictionary was right and a naturalist could be an unintelligent biologist

The presidential address to the Zoological Section was given by R M Long, on "Butterflies and Moths of the Horsham Area" The address was illustrated by colour slides from the president's photographs, dealing mainly with the camouflage of many of the species in the area He spoke of the success achieved with his mercury-vapour-lamp trap as many as a thousand specimens were taken on a good night He had recorded 31 species of butterfly and 347 of moth in his garden near the Carfax at the town centre He asked that, when particular trees and hedgerows were the habitat of the rarer species, the Council for Nature and the County Naturalist Trust be notified to enable an order to be obtained for their preservation

The Archæological Section was addressed by the president, G P Burstow, on "Recent Excavations in Sussex" He spoke of the excavations with which he had been associated, especially at Monkdown Court, near Findon, where three years work had revealed 900 post holes of an iron-age village occupied between 500 and 300 B C There was evidence of drying racks, storage pits and a quantity of pottery with hæmatite surface The site was later occupied by the Romano-British, a shrine connected with the worship of the ox had been uncovered, a possible association with a local Celtic god Vast quantities of Roman pottery had been recovered, and many bronze objects, notably a plaque of a charging boar Mr Burstow described other excavations at Ranscomb Hill near Mount Caburn and Balcombe quarry It was in middle Sussex where research work was most needed, a mistaken idea that it was unoccupied in ancient times being due to insufficient archæologists having been interested in the area

The president of the Botanical Section, D McClintock, chose as the title of his address "Alien Plants in South-east England" He spoke of the recent introductions and of the probable manner in which they arrived Most were introduced by man, many from the Mediterranean area, then distributed by animals, being carried from place to place in their wool or fur The bomb craters of the War were favourite sites, providing a large number of species *Buddleia* was an outstanding example, being introduced from China as a rather rare plant in 1887, it was widely distributed in the countryside by 1926

Dr R Thurrell, as president of the Geological Section, gave an address on "Aspects of the Geology of the Horsham District" His talk was introductory to the excursion he was conducting during the congress After mentioning that the area was now being examined in detail by the Geological Survey, he outlined the history of its investigation from early times The Romans used Horsham Stone together with hardened clay as the foundation of the Stane Street at Slinfold Iron was obtained from the ironstone in the Hastings Beds up to 1550, later limonite, from the base of the Wadhurst Clay and from the Weald Clay underlying the Horsham Beds, was the basis of the iron industry

Mantell could be regarded as the pioneer of the systematic study of Wealden geology, his discovery of the remains of giant reptiles attracting interest as well as adding considerably to the knowledge of the conditions prevailing at the time of the deposition of the beds The main uses of the Horsham Stone have been for roofing tiles, flag-stones and walls for farm buildings Dr Thurrell concluded by describing the method of capture of minor streams by the River Arun when, on surface examination, there would appear to be more likely directions for their flow.

Miss Joyce Pope addressed the delegates on the upbringing of young animals by human beings Most people liked watching the development of young animals whether they be white mice or gorillas At the onset Miss Pope said she wished to emphasize that both young birds and mammals are around alone before they had finished with parental care and it was a mistake to take them and attempt hand-rearing, for the parents were often close by and would be tending them The exception was with those which had been abandoned or injured, often wished on you by friends Even then the difficulty of the task should be realized and, where recovery seemed hopeless, it would be kinder to dispatch them painlessly Voles were very good pets and when they were in the suckling stage she had found it better to feed with a small paint brush rather than with a fountain pen filler Friendship with a hand-reared animal was apt to end with the animal 'becoming itself', becoming nervous and bad tempered, so one had to prepare it to fend for itself before liberating it in its native habitat Miss Pope then showed the film "Elsa, the Lioness" to illustrate some of the points of her address

Weather conditions, although troublesome, permitted the full excursion programme to be carried out The botanists were conducted by O Buckle on the chalk downs surrounding Chanctonbury Ring, the zoologists visited the secluded parts of St Leonards Forest, under R. M Long's guidance, to study the effects of reafforestation on wild life and to examine the working of the charcoal industry The geological party, numbering seventy, visited the more important outcrops and exposures of the Wealden Series in the Horsham district under the direction of Dr R Thurrell

The excellent arrangements for the congress were undertaken by the Horsham Natural History Society, for whom Mrs R Hurst acted as honorary secretary

F J EPPS

TARONG PYGMIES IN NORTH BURMA

DURING March–April 1962 a team from the Burma Medical Research Society carried out an expedition to the Arumdum area (97° 40′–97° 45′ E, 28° 5′–28° 10′ N) in the Adung Long Valley in the northernmost part of Burma The primary object of the expedition was to study the recently discovered Tarongs—a pygmy tribe living in that area

The team was led by Prof M Mya-Tu of the Department of Physiology, University of Rangoon, other members being Dr. Ko Ko, Dr Aung Than Batu and Dr Kywe Thein of the Directorate of Health, Burma, and Dr C J R Francis and U Than Tun Aung Hlaing of Prof Mya-Tu's Department

The expedition left Rangoon for Putao (Fort Hertz), some 800 miles north, in a Burma Air Force plane From Putao it travelled 60 miles by car until the road ended From there on, it proceeded on foot over mountain, river gorge and valley for nine days until Arumdum was reached

The whole population of Tarongs (69) in that region was examined The team spent seven days in the area during which time it collected anthropometric, hæmatological and other physiological data, and also conducted dietary, goitre and intestinal parasite surveys It also obtained information regarding the customs and beliefs of the Tarongs

Detailed analyses of the results obtained are still in progress, but some preliminary conclusions may be drawn Anthropologically, the Tarongs are of Mongolian stock, and are believed to be ethnologically closest to the Rawangs, a tribe of the Kachins The majority of them are between 4–5 ft in height, very few being taller than 5 ft

The Tarong blood groups show a high predominance of group B with group O coming next No person belonging to group A was found Only two were of group AB Group M was twice as common as MN N was rare Of the Rh group, CDe was most common CDE was also fairly common

Electrophoresis of hæmoglobin solutions, alkaline denaturation test for hæmoglobin F, sickling and osmotic resistance tests were also performed No hæmoglobinopathies were found

Vital registration records are non-existent, but judging from the family-histories, there is a high infant mortality, but once the difficult childhood years are survived, the Tarongs seem to enjoy reasonably good health. There is little evidence of gross malnutrition, and they seem to be relatively free from serious infections like smallpox and plague Intestinal diseases such as dysentery and worm infections also appear to be uncommon As is to be expected in most hill tribes, a high incidence of goitre was evident and, side by side with it cretinism

The team also brought back samples of blood, stool and urine from the Tarongs for further examination, and also collected insects, arrow poisons and medicinal plants from the region for examination in Rangoon

The report of the expedition will be published in the *Proceedings of the Burma Medical Research Society*

M MYA-TU
U KO KO
AUNG THAN BATU
U KYWE THEIN
C J R FRANCIS
THAN TUN AUNG HLAING

AN ELEMENTARY INTERPRETATION OF SCIENCE

IN January 1961, Purnell and Sons, Ltd , printed the first copy of *Knowledge*, a weekly colour journal edited by John Chancellor and an advisory board of Lady Violet Bonham Carter, Prof C N L Brooke, Mr Norman Fisher, Mr Walter Hamilton, Mr John Sparrow, Dr Dudley Stamp and Sir George Thomson The success of this magazine in the field of general education has apparently encouraged the publication of a very similar but more specialized magazine entitled *Understanding Science**, under the senior editorship of Leslie Basford, with a consulting editorial board comprising Sir James Chadwick, Mr Norman Fisher, Sir Harry Melville and Prof J Z Young

According to the chief editor it is "a new weekly magazine intended both for the general public and to help in developing the teaching of Science in schools" The general plan is that each issue will contain a selection of articles, each of which is a member of a series of articles outlining a particular branch of science The first issue, for example, contains articles on biology ("Living Cells"), chemistry ("Atomic Structure of Elements"), electricity ("An Electric Circuit"), heat ("The Fireless Locomotive"), hydrostatics ("Floating and Sinking"), magnetism ("The 'Loves' and 'Hates' of Magnets") and technology ("Using a Micrometer"), as well as an article on Sir Isaac Newton The cover design and article printed on the front and back constitute a special feature on topics of general scientific interest the first of these is entitled "Water and Life on Earth" Every twelfth issue will contain an index and title-page to facilitate collecting and binding of the copies· this habit will be further encouraged by the offer of special volume binders both for the main articles and for the special features

The text is very clearly printed, and on the whole the large diagrams and illustrations are good The sections on the micrometer (issue No 1, pp 8–9), the chemical balance (No 2, pp 24–25) and the microscope (No 3, pp 42–43) which would, as Mr Basford suggests, provide science teachers with material for visual aids, are quite impressive On the other hand, some of the biological drawings such as the worm, flowering plant and *Spirogyra* (No 1, pp 10–11) and the plankton (No 4, pp 56–57) are rather poor Some of the material and illustrations are taken directly from the *Junior Science Encyclopaedia* (Sampson Low, Marston and Co , Ltd) The topics are always treated as clearly and as simply as possible Occasionally, therefore, they suffer the inevitable consequences of oversimplification and contain somewhat misleading statements such as "Atoms are the smallest possible pieces of the basic pure materials that make the universe" (No 1, p 5) and "At another certain temperature water turns into a gas—steam On the centigrade scale of temperature the point at which this happens is defined as 100°" (No 1, back cover)

The editor claims that the magazine will present science in a way which is easy to understand He also claims that it will cover the equivalent of the Ordinary Level syllabus of General Certificate of Education in chemistry, physics, biology, etc Unfortunately, he does not indicate how many years this will take Nevertheless, the parents of young aspiring scientists may, perhaps, still consider 2*s* every Monday a worth-while investment.

MICHAEL C COX

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* *Understanding Science*, No 1 (A New Colour Magazine which becomes an Encyclopedia) Pp 16 Subscription rates 52 weekly copies, £6 10*s* 0*d* , 26 weekly copies, £3 5*s* 0*d* (Paulton, Nr Bristol Purnell and Sons Ltd , 1962)

U.S. COMMITTEE ON EXTENSION TO THE STANDARD ATMOSPHERE*

A REVISED *U.S. Standard Atmosphere to 700 km* is about to be adopted by twenty-nine U.S. scientific and engineering organizations, the U.S. Committee on Extension to the Standard Atmosphere (COESA). This Committee has also recommended the lower 32 km of this representation of the atmosphere to the International Civil Aviation Organization (ICAO) for international standardization. The lowest 20 km of this new COESA atmosphere coincides with the current 20 km ICAO Standard Atmosphere except for minor differences due to revised standardization of some physical constants. The new COESA atmosphere is in general agreement with the Committee on Space Research International Reference Atmosphere (CIRA) 1961, but follows the rules established for the previous U.S. and current ICAO Standard Atmospheres, and will provide more detail and more parameters than CIRA. It includes refinements, in matching the data, which were not possible in the earlier COSPAR report. Fortunately, continued scientific communication between COSPAR and COESA reduced discrepancies between the two atmospheres to a minimum.

The complete report, including tables, of the revised atmosphere is being prepared for U.S. Government publication to replace the *U.S. Extension to the ICAO Standard Atmosphere—Tables and Data to 300 Standard Geopotential Km* (G.P.O., Washington, D.C., 1958).

The National Aeronautics and Space Administration (NASA), the Geophysics Research Directorate, Air Force Cambridge Research Laboratories, and the U.S. Weather Bureau jointly sponsored the movement which led to this new representation of the atmosphere. Participating organizations included Aeronautical Systems Division, AFSC, Air Force Deputy Commander Aerospace Systems, AFSC, Air Weather Service, Applied Physics Laboratory (Johns Hopkins University), Army Ballistic Missiles Agency, Army Signal Research and Development Laboratory, Ballistics Research Laboratory (Aberdeen Proving Grounds), Battelle Memorial Institute, The Boeing Co., Federal Aviation Agency, General Dynamics, Geophysics Corporation of America, Geophysics Research Directorate, AFCRL, Goddard Space Flight Center, NASA, Harvard College and Smithsonian Astrophysical Observatory, High Altitude Engineering Laboratory (University of Michigan), Jet Propulsion Laboratory (California Institute of Technology), Langley Research Center, NASA, Lockheed Missile and Space Co., Marshall Space Flight Center, NASA, NASA Headquarters, National Bureau of Standards, Naval Proving Grounds, Naval Research Laboratory, Navy Weather Research Facility, The Rand Corporation, Space Technology Laboratories, Inc., Weather Bureau, Department of Commerce, White Sands Missile Range.

COESA is an unofficial group of scientific and engineering organizations, each holding national responsibilities related to the requirement for accurate tables of the atmosphere to high altitudes. They joined forces toward developing such an atmosphere in November 1953. Several new organizations have since been added to the ranks. A working group, appointed at the first meeting, met frequently between 1953 and the end of 1956, at which time the entire committee accepted their recommendations, published by the G.P.O. in 1958.

Scientific progress could scarcely wait for the publications of this 1958 document, when orbital periods of the first *Sputnik* indicated that densities at the upper altitudes were of more than an order of magnitude. A new Working Group was convened in January 1960 to review all available satellite and rocket data as related to the COESA table and to consider the need for revision. It recommended, on an interim basis, the ARDC 1959 Model Atmosphere, GRD, AFCRC-TR-59-267, August 1959. Up to about 50 km this atmosphere was the same as the recently published U.S. extension to the ICAO Standard Atmosphere. Above that altitude some International Geophysical Year rocket and early satellite data were used. Since all these data were obtained during the period of maximum solar activity, the resulting model was more representative of these conditions than

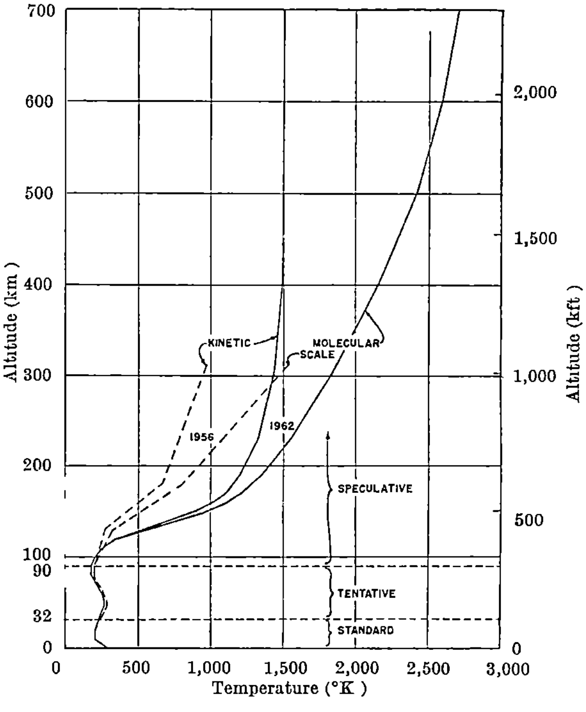

Fig. 1. Defining temperature-altitude of new and old COESA atmosphere

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* Address: U.S. Committee on Extension to the Standard Atmosphere, c/o Meteorological Development Laboratory, GRD, AFCRL, 1065 Main Street, Waltham 54, Mass.

Table 1 SKELETON OF THE *U S Standard Atmosphere, 1962*

Defining temperature and molecular weights of the proposed U S standard atmosphere and computed pressures and densities

Z (km)	h (km)	T_M (°K)	L (°K /km)	M	T (°K)	P (mb)	ρ (g/m ^{3})
0 000	0 000	288 15	−6 5	28 966	288 15	10 1325+2*	1 2250+3*
11 019	11 000	216 65	0 0	28 966	216 65	2 2632+2	3 6392+2
20 063	20 000	216 65	+1 0	28 966	216 65	5 4747+1	8 8033+1
32 162	32 000	228 65	+2 8	28 966	228 65	8 6798 0	1 3225+1
47 350	47 000	270 65	0 0	28 966	270 65	1 1090 0	1 4275 0
52 429	52 000	270 65	−2 0	28 966	270 65	5 8997−1	7 5939−1
61 591	61 000	252 65	−4 0	28 966	252 65	1 8209−1	2 5108−1
79 994	79 000	180 65	0 0	28 966	180 65	1 0376−2	2 0009−2
90 000	88 743	180 65	+3 0	28 966	180 65	1 6437−3	3 1698−3
100 000	98 451	210 65	+5 0	28 88	210 02	3 0070−4	4 9731−4
110 000	108 129	260 65	+10 0	28 56	257 00	7 3527−5	9 8277−5
120 000	117 777	360 65	+20 0	28 07	349 49	2 5209−5	2 4352−5
150 000	146 542	960 65	+15 0	26 92	892 79	5 0599−6	1 8350−6
160 000	156 071	1,110 65	+10 0	26 66	1,022 20	3 6929−6	1 1584−6
170 000	165 572	1,210 65	+7 0	26 40	1,103 40	2 7915−6	8 0330−7
190 000	184 485	1,350 65	+5 0	25 85	1,205 40	1 6845−6	4 3450−7
230 000	221 968	1,550 65	+4 0	24 70	1,322 30	6 9572−7	1 5631−7
300 000	286 478	1,830 65	+3 3	22 66	1,432 10	1 8828−7	3 5831−8
400 000	376 315	2,160 65	+2 6	19 94	1,487 40	4 0278−8	6 4945−9
500 000	463 530	2,420 65	+1 7	17 94	1,499 20	1 0949−8	1 5758−9
600 000	548 235	2,590 65	+1 1	16 84	1,506 10	3 4475−9	4 6362−10
700 000	630 536	2,700 65		16 17	1,507 60	1 1908−9	1 5361−10

* Power of 10 by which preceding number must be multiplied

Z = geometric altitude h = geopotential altitude

T = kinetic temperature M = mean molecular weight.

L = gradient of molecular scale temperature = $\mathrm{d}T_M/\mathrm{d}h$ (below 79 geop km).
= $\mathrm{d}T_M/\mathrm{d}Z$ (above 79 geop km)

T_M = molecular scale temperature = $(T/M)\,M_0$

M_0 = sea-level value of M.

average atmospheric properties For the next year, the Working Group studied new data and theories from more refined satellite flights Greater confidence was required than could be obtained using U S S R satellite orbital periods, since the U S S R aerodynamic configurations were not accurately known A few years of observations at satellite altitudes were also required to understand the effects of solar activity and position The American satellites provided the necessary extreme altitude data Additional rocket flights provided needed detail for levels above those of balloon flights The COESA Working Group prepared a recommendation from this new scientific inventory which was accepted by the entire committee on March 15, 1962

The *Revised U S Standard Atmosphere* will depict typical mid-latitude year-round conditions averaged for daylight hours and for the range of solar activity which occurs between sunspot minimum and sunspot maximum Supplementary presentations will also be provided to indicate variability of density above 200 km with solar position

The new atmosphere is defined by linear-segmented temperature–height relationship (Fig 1) The last COESA standard is included for comparative reasons The terminology of 'standard' in the figure was applied to the layer of the atmosphere in which it is believed there will be international agreement established by the ICAO The next layer covers an altitude interval much explored by rocketry, yet subject to some modification in order to be consistent with the revisions of the atmosphere above 90 km , which are certain to be required as more data become available The speculative altitude interval 90–700 km is, of course, a zone about which there is still much to learn

Although density is the primary atmospheric property measured at, or deduced for, very high altitudes, it was necessary to define the atmosphere in terms of temperature, in order that the extension above ICAO levels be continuous with the ICAO atmosphere Temperature segments at higher altitudes, developed from the barometric equations, are consistent with observed density data The basic framework for the extensive tables of the *U.S Standard Atmosphere, 1962*, which will be published through the G P O , appears in skeleton form in Table 1 The completed tables will follow closely the format developed in the Committee's *1958 Standard Atmosphere*

COESA is also developing a set of supplemental atmospheres which will represent mean summer and winter conditions by 15 deg latitude intervals from the tropics to the poles to an altitude of 90 km These atmospheres will appear some time later as an appendix to the revised *U S Standard Atmosphere*

The final report will be prepared for publication by NASA, under the monitorship of a three-man editorial board, representing each of the co-sponsoring organizations The published volume will not be part of any existing series but a separate publication of the U S Government Co-sponsors and all organizations of the Parent Committee will be prominently indicated, as will the editors and contributing scientists and engineers Much of the work to date has been accomplished by four Task Groups, into which the Working Group was divided to provide recommendations for various altitude intervals

The preparation of the *Tables* and final report will be under the supervision of Mr William J O'Sullivan, Langley Research Center, National Aeronautics and Space Administration, assisted by Mr Richard Hord Mr O'Sullivan and Dr K S W Champion, Geophysics Research Directorate, Air Force Cambridge Research Laboratories, assisted by Dr Hartley Pond, and Mr Sidney Teweles, U S Weather Bureau, will be principal editors

CABOT FAULT, AN APPALACHIAN EQUIVALENT OF THE SAN ANDREAS AND GREAT GLEN FAULTS AND SOME IMPLICATIONS FOR CONTINENTAL DISPLACEMENT

By Prof. TUZO WILSON, O.B.E.

Institute of Earth Science, University of Toronto

The Cabot Fault, its Trace and Age

FROM northern Newfoundland across Nova Scotia and the Bay of Fundy to Massachusetts and Rhode Island there extends a narrow belt of large faults. Although all parts are well documented, little attention has been paid to the apparent continuity of this dislocation which in its entirety will be called the Cabot fault after the strait it crosses.

In Newfoundland, the Cabot fault (Fig. 1) forms the east side of the Northern Peninsula[1] at the southern end of which it bifurcates into several subparallel branches in the valley containing Deer and Grand Lakes[2–9]. Thence it crosses to the south-west corner of the island[2,10–12].

H. L. Cameron has suggested that the fault crosses Cabot Strait[13,14], a view supported by the strike of anomalies on aeromagnetic maps[15], and that it continues across Nova Scotia. In northern Cape Breton Island[16] and across the isthmus from Cape George to Cape Chignecto[17–20] faults are clearly mapped, but in southern Cape Breton the published maps[21–25] disagree and Cameron has also suggested privately that the main fault may lie off the west coast of Cape Breton. If so, its extension may cross the isthmus farther west under cover of a deep basin of Pennsylvanian and Permian rocks[26]. From Cape Chignecto anomalies shown on aeromagnetic maps[27] and an observation by S. Powers[28] suggest that the fault connects across the Bay of Fundy with one postulated by P. B. King[29] to lie off the New Brunswick coast. D. W. Johnson[30] has suggested that faults which appear to form "one of the great fracture lines of North America" continue across the Gulf of Maine to Massachusetts. Recent seismic measurements barely intersect the fault, and are said to provide no basis for any conclusions about it[31]. This zone strikes the coast where five basins of late Palæozoic rocks and many large faults cross Massachusetts and Rhode Island. Extensive glacial cover, complex structure, lack of continuous marker horizons and few fossils have made this a difficult region to map so that a variety of views has been expressed, but recent ones involve large faults striking south-west[32–36]. Without attempting to resolve these ambiguities, this much is sufficient to suggest that a late Palæozoic fault zone does reach this vicinity.

Members of the committee preparing a new tectonic map of Canada have stated that in uppermost Devonian time a phase of deformation began in the Canadian Appalachians which was "restricted to a narrow belt which extends from the Bay of Fundy through Cape Breton Island north-east to Cape Anguille and beyond to White Bay, Newfoundland. Folding of the Carboniferous rocks varied in time and intensity from place to place within this belt. It was chiefly controlled by movements of rigid blocks of pre-Carboniferous rocks. Late Pennsylvanian (Pictou) sediments and block-faulted Triassic red beds and basic volcanics cover strata of this late Palæozoic folded belt"[37]. This description also seems valid for the southern part of the belt, although there is disagreement about the precise age of the basins in New England.

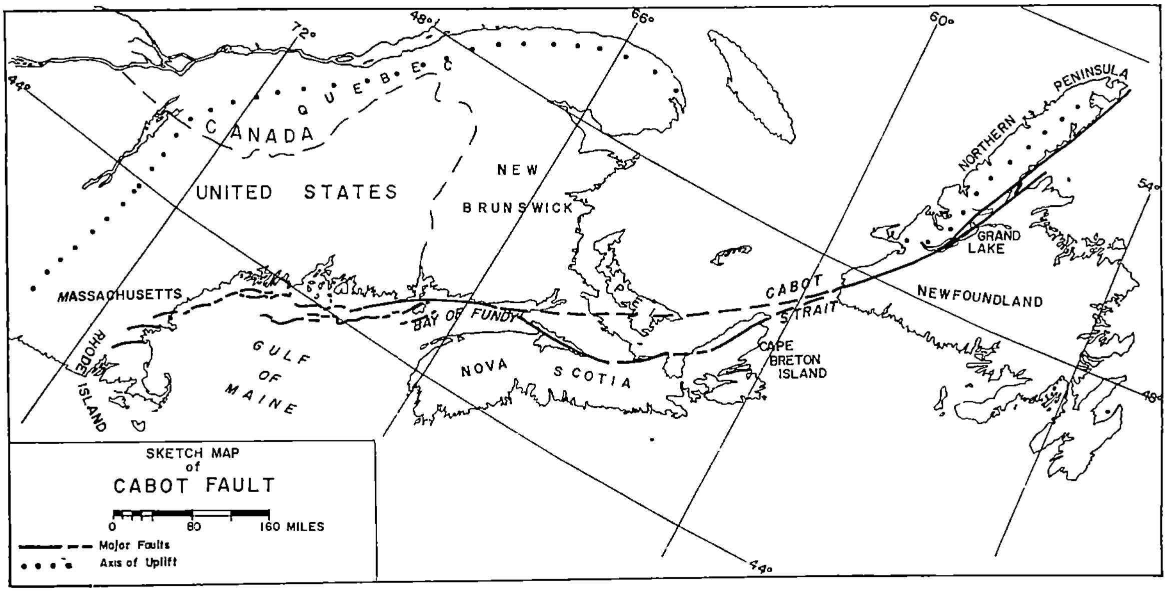

Fig. 1. Sketch map of the Cabot fault

Comparison between the Cabot and San Andreas Faults

Detailed study shows that this deformation conforms to the characteristics used by Hill and Dibblee[38,39] to suggest that the San Andreas rift is a great transcurrent fault. These include (1) long sections of straight trace, (2) steep but variable dips, (3) a disturbed zone varying from a few feet to a few miles in width, (4) apparent reversals in throw along the fault, (5) local drag folds and thrusts across it, (6) profound differences in older rocks on opposite sides of the fault.

It has therefore been suggested that the Cabot fault is a great transcurrent fault similar to the San Andreas[40], but active in late Palæozoic time. It will be observed that both faults formed late in their respective orogenies and that both occupy similar geographical and tectonic positions relative to the continent and the mountain belts.

It is tempting to go further and to compare the Bay of Fundy and the Gulf of Maine with the Gulf of California, but this is premature, for the crust beneath the Gulf of California is oceanic[41], whereas that beneath the Gulf of Maine is transitional between continental and oceanic[31] and the displacement on the Cabot fault is unknown. It is more justifiable to point out that the fault cuts like a bow-string across the curve of the Appalachians in New England and southern Quebec.

An anorthosite body in south-western Newfoundland of which the offset part does not re-appear within the region which has been mapped might suggest a minimum lateral displacement of more than 80 miles, whether dextral or sinistral, but officers of the Geological Survey now feel that this fault, along much of its trace, forms a complete separation between pre-Cambrian and younger basement complexes. If the branch faults[4,9] which turn off to the east from Grand Lake to Notre Dame Bay are due to 'horsetailing' their orientation suggests a sinistral displacement. If the large, younger transcurrent faults of post-Triassic age recently reported in New Jersey[42] should prove to be related to the Cabot fault, then their north–south strike and dextral motion would suggest a conjugate relation.

Comparison between the Cabot and Great Glen Faults

Most reconstructions of the continents based on the assumption of continental drift bring Newfoundland into juxtaposition with the British Isles[43,44].

On any such map it will immediately be seen that the Cabot and Great Glen faults[45] are opposite to one another and have the same orientation. Since both are the same age (uppermost Devonian and Lower Carboniferous), are transcurrent, are large, are perhaps sinistral and cut rocks well known to be similar[46,47], it is reasonable to speculate that, if drift has occurred, they are two ends of the same fault. It follows that the Lewisian might have been part of the Labrador province[48,49] and the granodiorite stocks in north-eastern Newfoundland such as those in the Bay of Exploits[50] and Fogo Island[51] might be other volcanic centres of the Ben Nevis type[52,53], for they are of the same age and composition and can be seen to have swarms of narrow, pink-weathering, acid dikes striking north-east from the centres.

Acceptability of Continental Drift

When the theory of continental drift was first proposed it was supported by the parallelism of some adjacent coasts on opposite sides of oceans and by the faunal, structural and stratigraphic coincidences which would result if these coasts had once been in contact[43]. Opponents pointed out that to have fitted more than a few coasts together would have required unacceptable distortions of the continents. They felt that, in fauna, coincidences could be explained by migration, in structure, by mountain chains crossing oceans, and in stratigraphy, by the same reasons plus climatic similarities[44,47]. Most physicists[54] considered that the crust of the Earth was too strong and the interior too viscous for continents to have been moved by the proposed dynamic forces.

In spite of these objections, evidence supporting drift has continued to accumulate. Some aspects are of new kinds like that from palæomagnetism[55] and that of transcurrent motions of more than 1,400 km. on the Pacific Ocean floor[56], but some concern closer geological relations[57]. If to the generalized suggestions of fit formerly advanced could be added several precise structural relationships, then coincidence would become too improbable an explanation and drift would have to be accepted. The continuation of the Moine thrust, if it could be traced in western Newfoundland, might provide another connexion like that just discussed.

The discovery[58–60] of a low velocity layer at a depth of 100–200 km. in the upper mantle has provided a possible lubricating channel on which the crust may move. Arguments have been advanced that in some places the crust has developed weak zones along which it can break far more easily than had been supposed[61]. Three further points may make drift more acceptable.

First, it would seem that the proponents of drift did their cause great harm by their too rigid concepts of the proto-continents of Pangaea or Laurasia. If, instead of believing that the present continents had all been confined in neat packets before Carboniferous time, they had assumed that continents and fragments of continents had always had a random distribution and motion, now joining together in one place and now rifting and separating elsewhere, they could have explained the good fits of some coasts without the necessity of introducing any hypothetical and unnecessary distortions[62]. If they had considered that continents may grow along their leading edges[63] they could have explained why coasts which may once have fitted together are now so altered by growth that the fit is no longer obvious.

It may be that the crust breaks and moves as a result of dynamical forces, but my second point arises if alternatively the crust is considered to move because of convection currents in the mantle[64]

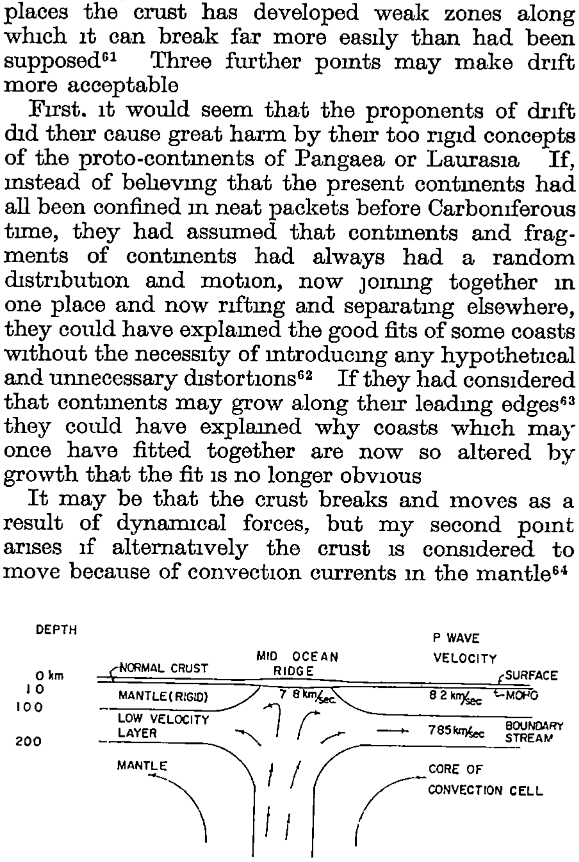

Fig. 2. Section across a mid-ocean ridge suggesting one possible underlying structure

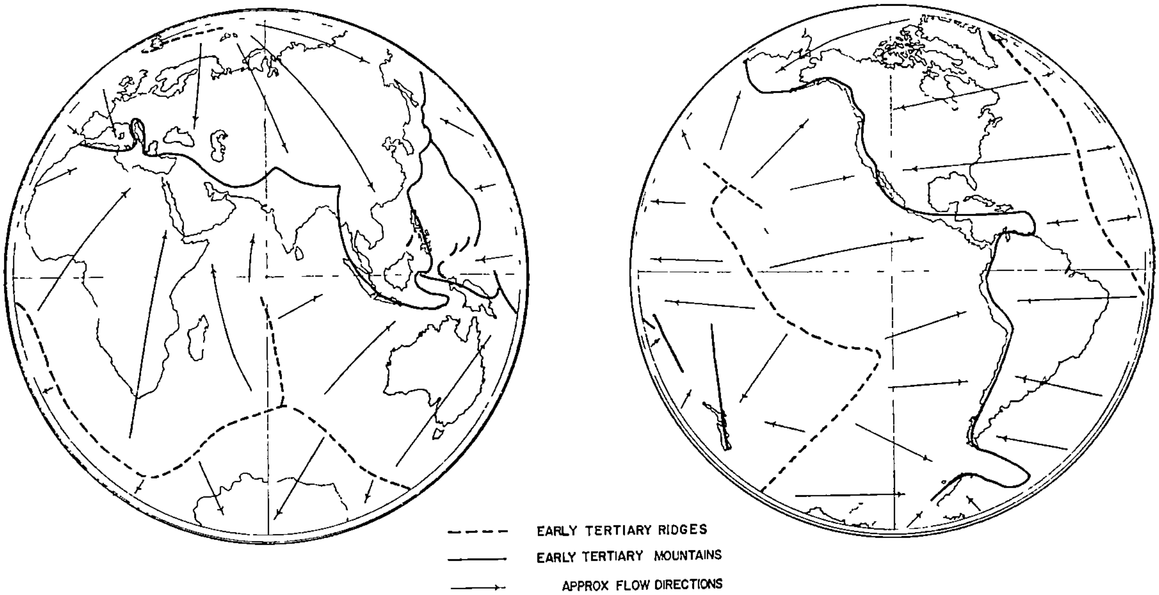
---- EARLY TERTIARY RIDGES
—— EARLY TERTIARY MOUNTAINS
—→ APPROX FLOW DIRECTIONS

Fig 3 World map of continental and mid-ocean mountain systems in early Tertiary time showing directions in which sub-crustal currents may have moved

If there is convective overturn it is presumably driven by relatively narrow streams of heated matter rising along the boundaries between cells If these cells behave as in other convecting systems the cores can be presumed to turn over rather as though they were solid rollers, the adjustment to the boundaries being confined to narrow peripheral streams Hence, following the usual assumption that the currents rise under the mid-ocean ridges, we might suppose that, as the rising convection currents approach the surface, adjustments between them and the cooler and more brittle uppermost layers of the mantle and crust would take place in a comparatively thin sub-surface layer Such adjustments are to be expected because the uppermost layers are cool and presumably withstand stress differences over longer periods of time than does the interior The segments of the uppermost layer may move at different velocities from the currents beneath them If so, it is reasonable to ask whether the zone of adjustment is not connected with the low velocity layer Fig 2 illustrates why, under the mid-ocean ridges[65], the velocities immediately beneath the Mohorovičić discontinuity might be about equal to those in the low velocity layer elsewhere[59,60]

Thirdly, in spite of the difficulty just mentioned, if convection currents are supposed to rise under mid-ocean ridges and sink under continental mountains, the location of the systems is known and the approximate flow-pattern can be plotted on a globe[66] One possible pattern for early Tertiary time has been plotted in Fig 3 and it will be noticed that the whole surface of the Earth is involved in a pattern separated by the two continuous lines of mountains and ridges respectively The whole mantle can be thought of as behaving as a single, continuous torus twisted about the Earth and rotating in a uniform direction, although different segments may have moved at different rates producing differential motions in the crust above the segments[56]

The possible pattern from early Tertiary time was chosen because at that time the pattern was straightforward Since then a change has been supposed to have occurred in the Pacific[67,68] It is confusing because it has superimposed the supposedly rising current of the East Pacific Rise on the sinking current postulated for the Cordillera A complexity has also arisen in East Africa

While considering convection currents, R Hyde demonstrated by a model that the currents between two rotating cylinders tended to follow one stable mode or pattern until the thermal conditions were changed sufficiently to cause the currents to change abruptly to some other mode

If there are convection currents in the mantle and they behave in a similar manner, we might suppose that changes in the thermal state of the Earth have from time to time produced changes in mode, each of which has corresponded to one major, world-wide orogenic cycle and that the intervening periods of stable modes have corresponded with times of fixed patterns of mountain building[69]. The irregularities mentioned suggest that such a change may now be starting

It is also easy to see that currents rising and diverging will rarely produce longitudinal shears along the mid-ocean ridges whereas converging currents approaching the continental mountains from different directions might produce shear faults along the mountain belts of which the Cabot fault may be one of many examples

I thank Prof A F Pillow for advice on the behaviour of convection currents, and Drs D M Baird, D G Kelley, E R W Neale and G C Riley for information about the geology of eastern Canada

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[For comments by Prof J D Bernal see p 122 of this issue.]

DEFORMATION OF URANIUM DURING ALPHA/BETA CYCLES UNDER SMALL STRESSES AND A QUANTITATIVE INTERPRETATION OF THE MECHANICAL WEAKNESS OF METALS UNDERGOING PHASE TRANSFORMATIONS

By Dr R. H. JOHNSON and Dr G. W GREENWOOD

Central Electricity Generating Board, Materials Division, Berkeley Nuclear Laboratories, Gloucestershire

THOUGH the mechanical weakness of metals when undergoing phase transformations is well known, no analyses have been developed which explain the extent of deformation occurring when small stresses are applied We have examined this phenomenon experimentally for the case of α/β cycling of uranium, and propose a quantitative theory to explain our results and the observations of other workers on other metals, notably iron

Specimens of pure (Springfields Dingot) uranium 0 025 cm thick, 1 cm wide and 2 cm in gauge-length were subjected to tensile stresses in the range 24–430 gm /mm ^{2} while undergoing α/β cycles at a rate of 3 cycles/hr about a temperature-range of 665° ± 50° C The specimens were situated in the centre of a vertical furnace and the thermal flux was approximately perpendicular to the applied stress The deformation was measured continuously and after the first one or two cycles the elongation per complete cycle ($\alpha \rightarrow \beta \rightarrow \alpha$) was constant for a given load The strain per cycle increased with applied stress as illustrated in Fig 1 Such a linear relationship has already been noted in the case of $\alpha \rightarrow \gamma \rightarrow \alpha$ cycles for iron[1]

It is suggested here that our results for uranium and those obtained for iron can be explained quantitatively by a development of recent theoretical models[2,3] of the deformation which occurs when a material is highly stressed internally and is subjected to a small external stress In the case of uranium, the first region which transforms into the β-phase increases in volume by an amount $\frac{\Delta V}{V}$ Since this phase is considerably harder than the α-phase, the latter deforms plastically Assuming that plastic deformation can be considered exclusive to the α-phase, its mode of deformation can be described by the matrix·

$$\begin{bmatrix} \left(\frac{\Delta V}{V}\right)_x & 0 & 0 \\ 0 & \left(\frac{\Delta V}{V}\right)_y & 0 \\ 0 & 0 & \left(\frac{\Delta V}{V}\right)_z \end{bmatrix}$$

where $\left(\frac{\Delta V}{V}\right)_x$, etc , are the components of plastic deformation in the x, y and z directions respectively and ΔV is the volume difference between the α- and β-phases If a small stress σ is applied in the z direction, and an increment of plastic strain ε occurs for each $\alpha \rightarrow \beta$ transformation, then the deformation matrix is expressed as

$$\begin{bmatrix} -\varepsilon/2 & 0 & 0 \\ 0 & -\varepsilon/2 & 0 \\ 0 & 0 & \varepsilon \end{bmatrix}$$

Making the assumptions that each grain undergoes the same average strain as the aggregate and that the deformation obeys the Lévy–von Mises criterion, then

$$\left[-\varepsilon/2 - \left(\frac{\Delta V}{V}\right)_x\right]^2 + \left[-\varepsilon/2 - \left(\frac{\Delta V}{V}\right)_y\right]^2 + \left[\varepsilon - \left(\frac{\Delta V}{V}\right)_z\right]^2 = \frac{2}{3}\lambda^2 Y^2$$

where Y is the yield stress of the α-phase at the transformation temperature and λ is a constant Now

$$\sigma = 3/2\lambda\left[\varepsilon - \left(\frac{\Delta V}{V}\right)_z\right]$$

so that λ can be eliminated from the two equations, and for the condition that $\varepsilon < \frac{\Delta V}{V}$, we obtain a strain for each α/β cycle given by

$$\varepsilon \sim \sqrt{\frac{2}{3}}\,\frac{\sigma}{Y}\left[\left(\frac{\Delta V}{V}\right)_x^2 + \left(\frac{\Delta V}{V}\right)_y^2 + \left(\frac{\Delta V}{V}\right)_z^2\right]^{\frac{1}{2}} + \left(\frac{\Delta V}{V}\right)_z$$

Now

$$\left(\frac{\Delta V}{V}\right)_x^2 + \left(\frac{\Delta V}{V}\right)_y^2 + \left(\frac{\Delta V}{V}\right)_z^2$$

is invariant and can be shown to have the value $\frac{2}{3}\left(\frac{\Delta V}{V}\right)^2$ This implies that the effect of the applied stress is to give an increment of deformation for each transformation which is independent of the orientation of the phase front and the direction in which it moves The term $\left(\frac{\Delta V}{V}\right)_z$ represents the component of the strain which occurs in the direction of measurement due to the change in volume alone and depends strongly on the shape of the transformation front It has been shown[4] that this term need not be zero for a complete transformation cycle $\alpha \rightarrow \beta \rightarrow \alpha$, and in the extreme case[5] of a plane transformation front of constant orientation it can approach a value $\sim \frac{\Delta V}{V}$. Representing the component of strain due to the change in volume alone by $\left(\frac{\Delta V}{V}\right)_T$ for a complete $\alpha \rightarrow \beta \rightarrow \alpha$ cycle, the total deformation ε_n after n complete cycles is given by

$$\frac{\varepsilon_n}{n} \sim \frac{4}{3}\,\frac{\sigma}{Y}\,\frac{\Delta V}{V} + \left(\frac{\Delta V}{V}\right)_T \qquad (1)$$

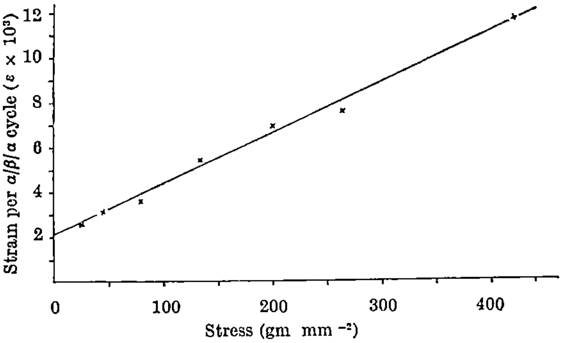

Fig 1

From equation (1) and the experimental results in Fig 1 the value of Y, the yield stress of the α-phase at the transformation temperature, is deduced to be 600 gm /mm ², which is similar to the experimentally measured yield stress on our material There is thus close correlation between theory and experiment

We have also compared equation (1) with experimental results on iron[5] Here work on material with 0 008 wt per cent nitrogen and with 0 2 wt per cent carbon has been reported with values of the yield stresses of these materials at the transformation temperature of 1,000 and 2,000 gm /mm ² respectively These values are in reasonable agreement with values of 600 and 1,200 gm /mm ² respectively, deduced from equation (1)

The intercept in Fig 1 which corresponds to the extent of deformation without applied stress must now be compared with equation (1), where it is identified with the term $\left(\frac{\Delta V}{V}\right)_T$ We find $\left(\frac{\Delta V}{V}\right)_T \sim 0\ 2\left(\frac{\Delta V}{V}\right)$ This is roughly comparable with values generally less than 0 3 $\left(\frac{\Delta V}{V}\right)$ on cycling without applied load[4] Such values, however, are extremely sensitive to the shape of the transformation front and $\left|\left(\frac{\Delta V}{V}\right)_T\right|$ is likely to be a small fraction of $\frac{\Delta V}{V}$ unless the conditions are such that the transformation front remains plane and of constant orientation

We conclude that the strain ε_n occurring after n complete cycles under small applied stress σ can be described by an equation of the form

$$\frac{\varepsilon_n}{n} = A\,\frac{\sigma}{Y}\left(\frac{\Delta V}{V}\right) + B\left(\frac{\Delta V}{V}\right)$$

where Y is the yield stress of the phase which deforms plastically A is independent of and B is very sensitive to the orientation of the phase front Both theory and experiment lead to the values $A \sim 4/3$ and $|B| < 1$

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ELECTRON MICROSCOPY AT HIGH BEAM ACCELERATING VOLTAGES

By DR. R. E. BURGE and G. H. SMITH

Department of Biophysics and Medical Research Council Biophysics Research Unit,
King's College, University of London, Strand, W.C.2

THE practical aspects of electron microscopy at beam accelerating voltages in the range 300–1,000 kilovolts (kV.) have recently been given a good deal of attention. The French machine was the first to yield results[1-3] in this voltage-range, and other similar projects are being developed in a number of countries and at the Cavendish Laboratory, Cambridge. The theory of the contrast expected in the images of biological specimens and in the images of amorphous specimens in general has not, however, received so much attention. A few calculated values, at 500 and 1,000 kV., of the electron-scattering cross-sections for a number of elements as functions of angle of scattering have been published by us[4] and it is proposed to extend these data here. Since at high voltage it is the hope that thick specimens may be examined under conditions of adequate image contrast, clearly the two factors, penetration and contrast, must be considered together.

The contrast, C, in the image of a specimen of mass-thickness $\omega(\omega = \rho t$ where the specimen is of density ρ and thickness $t)$ is given by the equations

$$C = \ln I_0/I = S_t\omega = (S_e + S_i)\omega \qquad (1)$$

Here the incident intensity I_0 of the electron beam is reduced to I by the scattering of electrons by the specimen at angles greater than the semi-angular aperture α of the objective lens. The total mass-scattering cross-section of the amorphous specimen for scattering outside α is S_t, S_t represents the sum of the elastic and inelastic scattering components S_e and S_i. When $\alpha = 0$, S_t becomes S_T, the total mass-scattering cross-section for scattering at all angles, also S_e and S_i become respectively S_E and S_I under this condition.

It has been shown both theoretically and by experiment[4] that, at voltages in the 50–100 kV. range, S is virtually independent of the composition of the specimen. For a given specimen examined at constant α, S decreases as the accelerating voltage increases, whereas, at a given accelerating voltage, S decreases as α increases. Thus, to maintain the highest possible level of contrast in the image at high accelerating voltages α must be made as small as possible. The smallest value of α is limited ultimately by diffraction effects and the astigmatism in the image caused by the rapid contamination of small objective-lens apertures in the electron beam.

On theoretical grounds, S (equation 1) is expected to remain constant under single scattering conditions, that is, provided specimens are of a mass-thickness such that, on the average, each electron is not scattered more than once. Multiple scattering sets in for mass-thicknesses greater than a certain value—the critical mass-thickness, $\omega_{\lim}$. Now $\omega_{\lim}$ may be defined in two ways[5] according to whether elastic scattering only or total scattering is considered. It is proposed to use both definitions of $\omega_{\lim}$, that is, $\omega_{\lim} = 1/S_e$, $1/S_t$; when $\alpha = 0$ then $\omega_{\lim}$ is expressed[5] as $1/S_E$ and $1/S_T$. Thus, $\omega_{\lim}$ gives a measure of (a) the contrast in the image which may be ascribed to either elastic or total electron scattering for a given specimen examined at a given beam accelerating voltage and a given value of α, (b) the penetration of electrons in amorphous specimens under single scattering conditions.

The theory used in the calculations is that published by us[4], which essentially represents a redevelopment of the Lenz[6] single-scattering theory on the basis of recently published theoretical descriptions of the atom. Calculations with full relativistic corrections were made for the elements carbon and gold. These two elements were chosen in order to approximate the electron scattering by organic specimens (carbon, oxygen and nitrogen are very similar) and to give an example of an element of high atomic number (gold). Three apertures were chosen, $\alpha = 0$ (a purely theoretical case corresponding to conditions of maximum contrast), $\alpha = 10^{-3}$ radian, and $\alpha = 5 \times 10^{-3}$ radian. The variations of $\omega_{\lim}$, on the definitions already given, and for beam accelerating voltages up to 1,000 kV., are shown in Figs. 1–3.

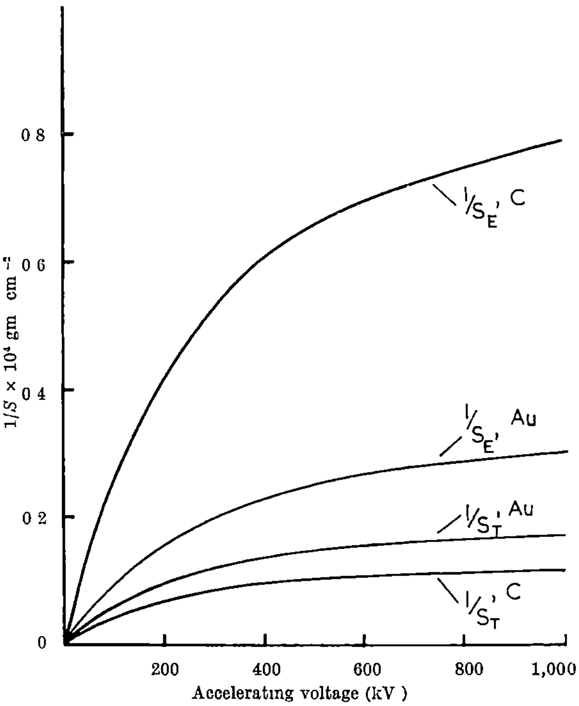

Fig. 1. The variation of $\omega_{\lim}$ (= $1/S$) with accelerating voltage for carbon and gold, $\alpha = 0$

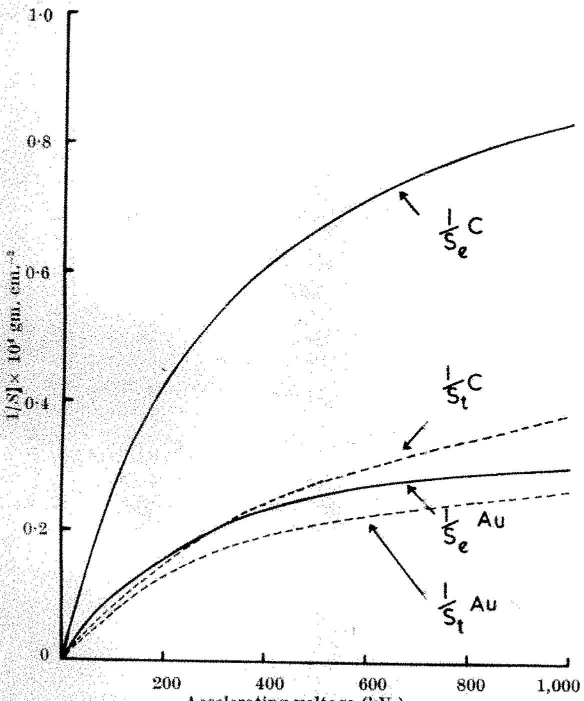

Fig. 2. The variation of ω_{lim} ($= 1/S$) with accelerating voltage for carbon and gold; $\alpha = 10^{-3}$ radian

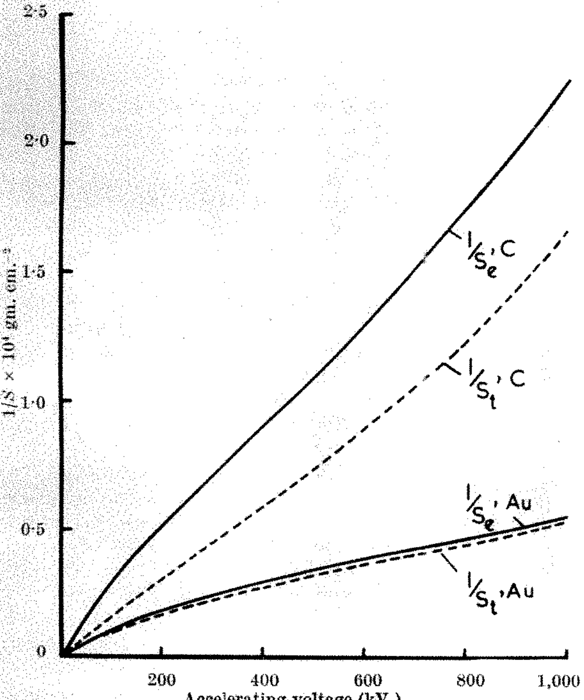

Fig. 3. The variation of ω_{lim} ($= 1/S$) with accelerating voltage for carbon and gold; $\alpha = 5 \times 10^{-3}$ radian

To interpret these curves in terms of a critical specimen thickness t_{lim} ($\omega_{\text{lim}} = \rho t_{\text{lim}}$) the densities of amorphous carbon and of gold should be used; these values are about 2 and 20 gm. cm.$^{-3}$ respectively.

It is clear from Figs. 1–3 that an assessment of the dependence on accelerating voltage of the electron penetration under single scattering conditions depends critically on the angular aperture of the objective lens. Fig. 1 ($\alpha = 0$) shows that the limiting thicknesses tend to constant values above an accelerating voltage of about 500 kV. Fig. 2 ($\alpha = 10^{-3}$ radian) shows that all the values of ω_{lim} increase fairly slowly with increasing accelerating voltage above 500 kV., while in Fig. 3 ($\alpha = 5 \times 10^{-3}$ radian) the values of ω_{lim} increase fairly rapidly with increase of accelerating voltage over the entire range.

Thus, at very small objective apertures ($\alpha <$ say 10^{-3} radian), corresponding to relatively high image contrast, the increase in ω_{lim} with accelerating voltage above, say, 500 kV. is scarcely worth while. However, for $\alpha > 10^{-3}$ radian, where image contrast is lower, ω_{lim} increases with increasing voltage over the entire range considered; the larger the value of α the faster ω_{lim} increases with increase of voltage.

In Table 1 are given some examples of the calculated values of t_{lim} at 100 kV. and 1,000 kV.

Table 1. VALUE OF t_{lim}

Definition	Voltage (kV.)	t_{lim} Å. (carbon)	t_{lim} Å. (gold)
$1/S_T$	1,000	596	86
$1/S_T$	100	225	31
$1/S_t$ ($\alpha = 10^{-3}$ radian)	1,000	1,920	132
$1/S_t$ ($\alpha = 10^{-3}$ radian)	100	420	38
$1/S_t$ ($\alpha = 5 \times 10^{-3}$ radian)	1,000	8,325	263
$1/S_t$ ($\alpha = 5 \times 10^{-3}$ radian)	100	823	48

Although these theoretical results may be in error, it is a reasonable assumption that the qualitative agreement between theory and experiment found at accelerating voltages below 100 kV. (ref. 4) will extend to higher voltages. It appears therefore that, for amorphous specimens in general and for biological specimens in particular, examined in the electron microscope with objective apertures which are not too small ($\alpha \nless$ say 10^{-3} radian), ω_{lim} increases in a favourable manner with increase of accelerating voltage up to 1,000 kV. Since the increased penetration is at the expense of image contrast, electron-staining techniques to increase contrast should be especially valuable at very high voltages.

It should be noted that the rate of change of S_t with voltage for finite apertures (Figs. 2 and 3) depends on the element concerned and increases with increase in size of the objective aperture; substance differentiation may be possible by this means. The rough constancy of S_t with atomic number found at voltages in the 50–100 kV. range evidently does not extend to higher voltages.

Although thicker specimens than those useful at accelerating voltages up to 100 kV. may be used with benefit at very high voltages, presumably the resolution in the recorded image will be limited to 1/10th–1/20th of the specimen thickness[7,8].

We thank Prof. J. T. Randall for facilities and advice, Dr. V. E. Cosslett for discussion, and the University of London Computer Unit for the use of the digital computer *Mercury*. One of us (G. H. S.) is indebted to the Medical Research Council for a research scholarship.

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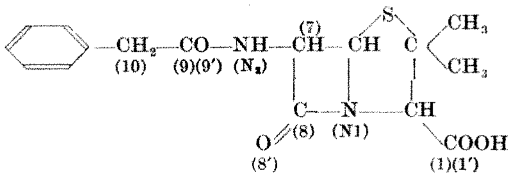
A STRUCTURAL SIMILARITY BETWEEN N-ACETYLMURAMIC ACID AND PENICILLIN AS A BASIS FOR ANTIBIOTIC ACTION

By J. F. COLLINS and M. H. RICHMOND

National Institute for Medical Research, Mill Hill, London, N.W.7

THERE is considerable evidence supporting the idea that the antibiotic activity of penicillin is connected with the presence of *N*-acetylmuramic acid in the mucopeptide of bacterial and rickettsial cell walls, and that the antibiotic acts in sensitive strains by interfering with the function of an enzyme which normally handles some derivative of *N*-acetylmuramic acid as an intermediate during mucopeptide synthesis[1-5]. Inhibition of this type could occur if penicillin were bound to the part of the active centre that normally handles the *N*-acetylmuramic acid residue of the intermediate. To do this, the binding groups in penicillin must be able to form bonds to the active centre similar to some of those which bind the *N*-acetylmuramic acid residue.

To see whether *N*-acetylmuramic acid and penicillin showed any similarity in this respect, wire models of the two molecules were constructed to a scale of 2·5 cm./Å. Penicillin (I) was made in its correct absolute configuration[6], and *N*-acetylmuramic acid (3-*O*-D-α-carboxyethyl-*N*-acetyl-D-glucosamine[7]) (II), being derived from D-glucose, was constructed from crystallographic data for D-glucose, *N*-methylacetamide and D-tartaric acid[8]. *N*-Acetylmuramic acid is not a rigid molecule, and the configuration considered here is not the most stable, but one which could occur on the active site of an enzyme. Penicillin, in contrast, has a rigid configuration across both rings in the nucleus. There is restricted rotation about the bond C_7–N_2; the amide linkage (C_9–N_2) is rigid, but rotation is possible between C_9 and C_{10}.



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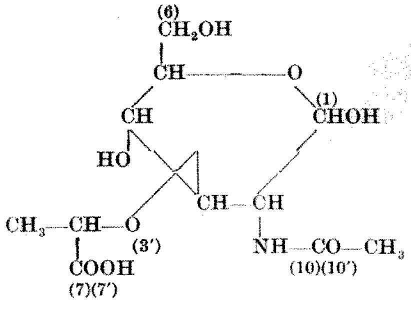

II

The binding of an *N*-acetylmuramic acid residue to an active centre will depend primarily on ionic and hydrogen bonding. Examination of the two models showed that $O_{1'}$, N_1 and $O_{9'}$ of penicillin could occupy identical positions to $O_{7'}$, $O_{3'}$ and $O_{10'}$ in *N*-acetylmuramic acid. The antibiotic could therefore bind with part of an active centre normally binding an *N*-acetylmuramic acid residue, for the following reasons:

(1) All but one of the hydrogen bonding groups in penicillin are exposed on one face of the molecule (Fig. 1*b*), which closely resembles part of the face of the *N*-acetylmuramic acid molecule shown in Fig. 1*a*.

(2) The carboxyl groups in both molecules are in similar positions in the configurations shown in Fig. 1. They are both strongly acidic, with similar *p*K values (penicillin: *p*K 2·7 (ref. 9); *N*-acetylmuramic acid: *p*K 2·6 (ref. 7)), and therefore have the same ionic and hydrogen bonding possibilities at physiological *p*H values.

(3) The nitrogen atom N_1 in penicillin and the oxygen atom $O_{3'}$ in *N*-acetylmuramic acid occupy identical positions in the two models, and the nitrogen atom can form a hydrogen bond in a similar direction to one of the two that can be formed by the oxygen atom.

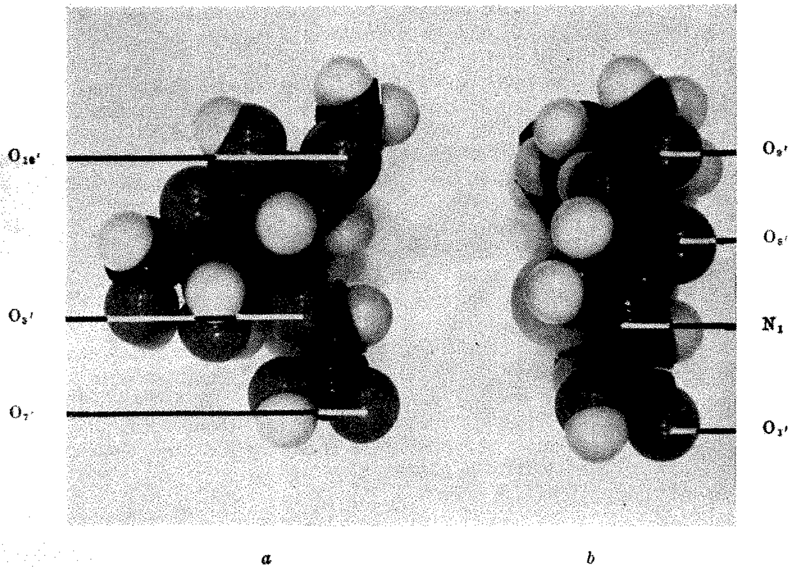

Fig. 1. Solid models of *N*-acetyl muramic acid (*a*) and benzyl penicillin (*b*) to show the similar distribution of reactive groups. *N*-acetyl muramic acid: $O_{3'}$, ether oxygen of the lactyl side-chain; $O_{7'}$, carboxyl group; $O_{10'}$, carbonyl oxygen of *N*-acetyl group. Penicillin: N_1, triply substituted nitrogen of β-lactam ring; $O_{1'}$, carboxyl group; $O_{9'}$, carbonyl of benzoyls ide-chain; $O_{8'}$, β-lactam carbonyl group

(4) The carbonyl groups $C_9O_9{}'$ in penicillin and $C_{10}O_{10}{}'$ in *N*-acetylmuramic acid arise in slightly different directions, but the two oxygen atoms occupy identical positions in the two molecules, and can each form a hydrogen bond in a common direction

(5) There are no large groups in penicillin which protrude beyond the face of the molecule shown in Fig 1*b* and which could interfere with binding of the antibiotic at the active centre The side-chain is curled back in the configuration illustrated (as found in the crystal[6]) and rotation about C_9–C_{10} cannot bring the phenyl ring near the reacting face of the molecule

Further evidence supports the hypothesis that these groups are involved in the binding of penicillin at a specific site Alterations in the structure of penicillin that do not affect the positions of these groups (for example, *N*-acyl derivatives of 6-aminopenicillanic acid) do not necessarily result in the loss of antibiotic activity[9,10] In contrast, loss of one of the reactive groups (for example, the carbonyl group $C_9O_9{}'$ as in 6-aminopenicillanic acid[11,12] and 6-picramidopenicillanic acid[13]) results in a several thousand-fold fall in antibiotic activity Changes which alter the relative positions of the binding groups (for example, in penicilloic acid[9], desthiopenicillin[9], the cyclic tripeptide derived from *N*-phenacetyl-L-cysteinyl-D-valine[14], compounds in which the β-lactam ring is replaced by a five- or six-membered ring[15] and penicillin hydroxamate[9]) result in total loss of activity

These considerations, if correct, give a rational basis for the action of penicillin at a molecular level The kinetics of penicillin action suggest that the compound has a very high affinity for the active centre[12] This could be achieved if the carbonyl group $C_8O_8{}'$ of penicillin (which has no counterpart in *N*-acetylmuramic acid) also binds to the active centre If penicillin binds through the groups that have been suggested, the $C_8O_8{}'$ carbonyl group will be favourably placed to interact with the site Since the carbonyl group is also the most reactive group in penicillin, it is possible that the molecule may first be bound to the active centre by ionic and hydrogen bonds described earlier, and then by reaction of $C_8O_8{}'$ with the active centre to form a covalent linkage that breaks down relatively slowly

It is not possible from a consideration of the structure of penicillin to identify the derivative of *N*-acetylmuramic acid involved in mucopeptide synthesis nor to decide the precise reaction inhibited by penicillin The *N*-acetylmuramic acid residue could be substituted at C_1 and C_6 without impairing the similarity of the molecule with penicillin Thus the sensitive reaction could involve a uridine nucleotide derivative of *N*-acetylmuramic acid undergoing a uridine transglycosidation at C_6, a reaction thought to be involved in mucopeptide synthesis[16–18]

The cephalosporins act in a similar way to penicillin[19] and contain the same binding groups in almost identical conformations[20] The same explanation could also account for their antibiotic activity

We thank many of our colleagues for help in the course of this work, Drs J Walker, Olga Kennard and J W Cornforth in particular Mr F Doré assisted in the construction of the wire models, and Miss P M Woodward made some of the calculations involved

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DISTRIBUTION OF 5-BROMOURACIL AMONG THE PYRIMIDINE CLUSTERS OF THE DEOXYRIBONUCLEIC ACID OF *E. COLI*

By Dr RIVKA RUDNER, Prof HERMAN S. SHAPIRO and Prof ERWIN CHARGAFF

Cell Chemistry Laboratory, Department of Biochemistry, College of Physicians and Surgeons,
Columbia University, New York

SINCE the discovery of the biological specificity exhibited by certain microbial deoxyribonucleic acids[1], there has been much discussion of the function of these polymers as genetic determinants or as the carriers of biological information (cf refs 2 and 3) The possible importance, in this respect, of the specific sequence of the nucleotide constituents, and of its alteration as the cause of mutations, has been pointed out very early[4,5] Changes in the nucleotide pattern of a nucleic acid should, consequently, have far-reaching biological effects

The 6-ketopyrimidine 5-bromouracil is known to be accepted by certain bacterial cells as an analogue of thymine, being incorporated into the newly forming deoxyribonucleic acid in considerable quantities[6–8]. There are many biological consequences attending the incorporation of the halopyrimidine, an incorporation limited essentially to cells incapable of the synthesis of thymine[9] a gradual loss of viability[9] and, of particular interest, a very much increased rate of formation of mutants[10,11], sometimes with preferred sites of very high mutability[12]. At the same time,

Table 1. COMPOSITION OF DEOXYRIBONUCLEIC ACID OF TWO THYMINE AUXOTROPHS OF *E. coli* GROWN IN THE ABSENCE AND PRESENCE OF 5-BROMOURACIL

Mutant strain	Preparation No.	Growth supplements	Moles constituent per 100 moles total					Molar ratios			
			A	G	C	T	B	$A+T+B/G+C$	$A+C/G+T+B$	A/T or $A/T+B$	$B/T+B \times 100$
t^-arg$^-$	1	T	25.0	24.9	25.6	24.4	—	0.98	1.03	1.02	—
	2	$T+B$	24.4	25.6	25.7	10.6	13.7	0.95	1.00	1.00	56
I	3	T	24.6	25.5	25.6	24.3	—	0.96	1.01	1.01	—
	4	$T+B$	24.9	25.1	25.4	13.5	11.1	0.98	1.01	1.01	45

A, Adenine; *G*, guanine; *C*, cytosine; *T*, thymine; *B*, 5-bromouracil.

it is known that bacteriophage T_2 particles containing a high proportion of 5-bromouracil retain their infectivity[13] and that the transforming activity of nucleic acid preparations from *B. subtilis* is not annihilated by the incorporation of the analogue[14].

The interest of this laboratory in the problems relating to the arrangement of nucleotides in deoxyribonucleic acids[15] and the observation that the two normal 6-aminopyrimidine constituents, cytosine and 5-methylcytosine, apparently do not substitute for each other haphazardly within the nucleic acid chain[16] prompted two of us to investigate the effects of the intrusion of an abnormal analogue. The question to be answered was whether the living cell possesses a selection mechanism capable of distinguishing between the normal and the fraudulent 6-ketopyrimidine. A preliminary examination of one preparation of deoxyribonucleic acid of a thymine auxotroph of *E. coli*, strain I, led to the conclusion that the incorporation of 5-bromouracil gave rise to a severe distortion of the normal nucleotide pattern of the polymer[17]. The most remarkable observation was the high proportion of the halopyrimidine (nearly one-third of the analogue) that was found in the form of solitary units[18], that is, interjacent between purine nucleotides. This seemed to indicate a certain bias in the 'replacement' of thymine by 5-bromouracil. Further investigations have, however, furnished no evidence of gross non-randomness in the ability of the halopyrimidine to substitute for thymine within the polynucleotide chain.

The previous observations[17] are thus shown to be inconsistent with the weight of evidence, though we still lack an explanation of this anomaly. It should be emphasized that the original finding cannot have been due to the inadequacy of the hydrolysis procedure, which has in the meantime been shown to give nearly quantitative results[19]—and this is confirmed in the studies to be discussed presently—nor to an unusual lability of the glycosidic linkage of 5-bromodeoxyuridine. The rate of decomposition of this nucleoside has been found to follow first-order kinetics with a rate constant of 0.853 mole per cent per min. in 0.2 *M* sulphuric acid at 100°, a value not unduly different from those found for the other pyrimidine deoxyribonucleosides[20].

The present series of experiments started with an investigation of the mode of replication of a bacterial deoxyribonucleic acid in which the uptake of 5-bromouracil by another thymine-requiring mutant of *E. coli*, namely, 15 t^-arg$^-$, was followed. This auxotroph represents the 15 t^- strain[21] in which an additional requirement for arginine was produced with 2-aminopurine[22]. This strain offers the opportunity of achieving the synchronization[23] of the culture by depleting the cells of thymine during a starvation period of 30 min. and has the advantage of a readily controlled incorporation of 5-bromouracil. With 6 μgm./ml. of the halogenated analogue as the only supplement, the deoxyribonucleic acid produced in the first replication cycle contains nearly equal amounts of thymine and of the analogue (ref. 24 and unpublished observations). These studies revealed no gross alterations of the nucleotide sequence concomitant on the insertion of the halopyrimidine into the newly formed nucleic acid chain, as would have been predicted on the basis of the original findings[17]. A renewed investigation of the problem of 'replacement' seemed in order, with attention being paid this time not only to solitary pyrimidine units and a few of the coupled sequences but also to as complete a balance as could be established. The improvement in the separation methods achieved in the meantime[19] made this possible.

With the auxotroph t^-arg$^-$ grown in the presence of 10 μgm. of 5-bromouracil and 1 μgm. of thymine per ml. of synthetic medium[25] the contents in thymine and 5-bromouracil of the deoxyribonucleic acid were approximately equal after two cycles of replication (see Preparation 2 in Table 1). The same conditions, but with the omission of the halogenated pyrimidine were observed for the isolation of Preparation 1. The second mutant of *E. coli* examined here was strain I, which had been used previously[17]. It was grown, exactly as before (18 hr. at 37°) with the addition of 2 μgm./ml. of thymine, either alone (Preparation 3 in Table 1) or together with 10 μgm./ml. of 5-bromouracil (Preparation 4).

Deoxyribonucleic acid was isolated from the organisms by a procedure using a buffered phenol–water system at *p*H 9 (ref. 26). The bulk of contaminating ribonucleic acid was removed by precipitation with 10 per cent sodium chloride and the remainder by digestion with cryst. pancreatic ribonuclease. We reserve a complete account of all our experiments for a detailed publication to be submitted in due course, limiting ourselves here to a selection from the findings. The base composition of the preparations under comparison is shown in Table 1. In the preparation of nucleic acid from strain I investigated here the substitution of 5-bromouracil was more extensive (45 per cent of the sum of the 6-ketopyrimidines) than in the specimen studied previously (35 per cent)[17].

The hydrolysis of the preparations was performed as described in a previous publication[20] (2 per cent solution of nucleic acid in 0.1 *M* sulphuric acid, 100°, 35 min.). In all experiments but one, the pyrimidine nucleoside 3′,5′-diphosphates, indicative of sequences of the type purine–pyrimidine–purine, and the pyrimidine oligonucleotides carrying terminal phosphate groups at positions 3′ and 5′, respectively, indicative of pyrimidine clusters of varying length enclosed by purines, were separated by fractional chromatography on DEAE-cellulose[19]—a procedure permitting a much more complete accounting of all pyrimidine sequences released by acid hydrolysis than had been possible in the previous studies. Fig. 1 shows examples of the profiles of two separation experiments. It will be noticed that from the nucleic acid preparation produced in the absence of the halopyrimidine at least eight distinct fractions,

Table 2 DISTRIBUTION OF PYRIMIDINE NUCLEOTIDE CLUSTERS IN DEOXYRIBONUCLEIC ACID OF TWO THYMINE AUXOTROPHS OF *E coli* GROWN IN THE ABSENCE AND PRESENCE OF 5-BROMOURACIL

Strain		*t*⁻arg⁻		I	
Growth supplements		*T*	*T*+*B*	*T*	*T*+*B*
DNA Prep No		1	2	3	4
Fraction No	Number of pyrimidine nucleotides in fragment (*n*)	Proportion of DNA phosphorus, per cent			
I	1	13 7	13 4	13 9	13 9
II	2	12 1	12 3	12 2	11 7
III	3	7 7	7 6	7 4	7 6
IV	4	5 4	5 5	5 1	5 6
V	5	3 4	3 6	3 7	4 0
VI	6	2 2	8 1†	2 3	6 5†
VII	7	1 3		1 4	
VIII	8	1 1		1 2	
IX		3 3*		3 5*	
Total		50 2	50 5	50 7	49 3

* Based on an average chain length of $n = 11$
† Based on an average chain length of $n = 8$

Table 3 FREQUENCY OF SOLITARY AND COUPLED PYRIMIDINE NUCLEOTIDE UNITS IN DEOXYRIBONUCLEIC ACID OF THYMINE AUXOTROPH OF *E coli* *t*⁻arg⁻ IN THE ABSENCE AND PRESENCE OF 5-BROMOURACIL

Growth supplements		*T*	*T*+*B*
DNA Prep No		1	2
		Moles of pyrimidine per 100 gm atoms of DNA phosphorus	
Solitary units	Tp_2	3 81 (0 05)	1 60 (0 05)
	Bp_2	—	2 02 (0 08)
	Cp_2	4 53 (0 17)	4 84 (0)
	Total *T* or *B*+*T*	3 81	3 62
Coupled units	T_2p_3	2 42 (0 01)	0 66 (0 10)
	TBp_3	—	1 32 (0 24)
	B_2p_3	—	0 97 (0 05)
	TCp_3	5 42 (0 06)	2 50 (0 14)
	BCp_3	—	3 18 (0 10)
	C_2p_3	3 44 (0 02)	3 27 (0 11)
	Total *T* or *B*+*T*	5 13	5 79
B/*T* + *B* × 100 in solitary units		—	56
B/*T* + *B* × 100 in coupled units		—	56
B/*T* + *B* × 100 in total DNA		—	56

The nucleic acid preparations are numbered as in Table 1 The same abbreviations as in Table 1 are used, with *p* indicating the number of phosphate groups in the fragments having the general formula Py_np_{n+1} (Py = pyrimidine) No distinction is made between sequential isomers of the coupled units The figures are the averages of the results of two separate hydrolysis experiments, each chromatographic component being determined twice, the deviations from the averages are given in parentheses

going from solitary units to octanucleotide sequences, could be isolated, whereas with the preparation containing 5-bromouracil the longest runs separated clearly were those of five pyrimidine nucleotides in a row The significance of this difference, though observed often, cannot yet be appraised

The distribution of organic phosphorus among the fractions, summarized in Table 2, was essentially similar in preparations containing the bromopyrimidine and in those devoid of it In all chromatographic fractions, except Fraction I, the agreement between organic phosphorus (corrected for the fact that all nucleotide sequences carried an additional terminal 3′-phosphate) and pyrimidines recovered (see Tables 3 and 4) was satisfactory Only in Fraction I was the first figure higher than the second This points to the presence in this fraction of some non-nucleotidic organic phosphate arising, presumably, from the degradation of purine nucleotide units, this matter is being studied now

The hydrolysates of all preparations were, after separation into distinct size fractions (Fig. 1), studied further, as described in a previous publication[19] The results are shown in Table 3 with regard to the solitary and coupled pyrimidine units found in the preparations from strain 15 *t*-arg⁻, in Table 4, the same information, but extended to the pyrimidine triplets, is provided for strain I In the latter case, a second analytical procedure, more rapid and economical (1 mgm of nucleic acid being required instead of 20 mgm), but less adapted to specimens comprising three different pyrimidines, was applied to one hydrolysate In this method the hydrolysate is subjected directly to two-dimensional chromatography on filter paper in *iso*propanol-ammonia and in buffered *iso*butyrate[16] The Δε values used for quantitative spectroscopy have been given before[16], for 5-bromodeoxyuridine 3′,5′-diphosphate a $\Delta\varepsilon_{280-320}$ of 8,500 was used

Table 4 FREQUENCY OF SOLITARY, COUPLED AND TRIPLET PYRIMIDINE NUCLEOTIDE UNITS IN DEOXYRIBONUCLEIC ACID OF THYMINE AUXOTROPH OF *E coli* I GROWN IN THE ABSENCE AND PRESENCE OF 5-BROMOURACIL

Growth supplements		*T*	*T*+*B*	
DNA Prep No		3	4	
Analytical procedure		I	I	II
		Moles of pyrimidine per 100 gm atoms DNA phosphorus		
Solitary units	Tp_2	3 84 (0 09)	2 33 (0 12)	2 07 (0 16)
	Bp_2	—	1 82 (0 09)	1 86 (0 03)
	Cp_2	4 50 (0 12)	4 61 (0 13)	4 49 (0 04)
	Total *T* or *B*+*T*	3 84	4 15	3 93
Coupled units	T_2p_3	2 45 (0 03)	0 71 (0 03)	0 99 (0 06)
	TBp_3	—	1 61 (0 07)	
	B_2p_3	—	0 51 (0 07)	
	TCp_3	5 40 (0 02)	2 78 (0 02)	2 69 (0 10)
	BCp_3	—	2 55 (0 09)	
	C_2p_3	3 73 (0 29)	3 36 (0 16)	2 53 (0 08)
	Total *T* or *B*+*T*	5 15	5 49	
Triplets	T_3p_4	1 30 (0 03)	0 21 (0 05)	
	T_2Bp_4	—	0 51 (0 04)	
	TB_2p_4	—	0 39 (0 09)	
	B_3p_4	—	[0 17]	
	T_2Cp_4	2 24 (0 01)	0 69 (0 10)	
	B_2Cp_4	—	0 51 (0 03)	
	$TBCp_4$	—	1 14 (0 07)	
	TC_2p_4	2 73 (0 18)	1 62 (0 05)	
	BC_2p_4	—	1 29 (0 15)	
	C_3p_4	1 04 (0 15)	1 02 (0 14)	
	Total *T* or *B*+*T*	3 70	3 64	
B/*T*+*B*×100 in solitary units		—	44	47
B/*T*+*B*×100 in coupled units		—	47	
B/*T*+*B*×100 in triplets		—	43	
B/*T*+*B*×100 in total DNA		—	45	

The nucleic acid preparations are numbered as in Table 1, for abbreviations and manner of presentation see Tables 1 and 3 In analytical procedure I the separation of pyrimidine nucleotide units was carried out by means of a DEAE-cellulose column, as shown in Fig 1, in procedure II two-dimensional chromatography on filter paper was performed directly The value for B_3p_4 is an estimate based on the results of another experiment and has been excluded from the balances For other details, see Table 3

Tables 3 and 4 show that in all nucleotide sequences under comparison, and in both mutant strains examined, the relative contribution of 5-bromouracil to the sum of 6-ketopyrimidines equalled the relative

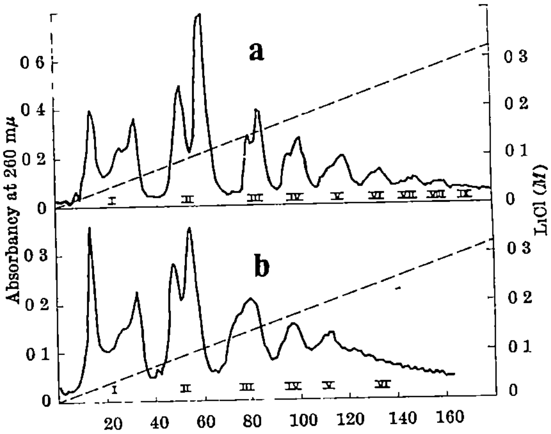

Fig 1 Chromatography of hydrolysates of deoxyribonucleic acid of *E coli*, mutant strain I *a*, grown in the absence, *b*, in the presence of 5-bromouracil Elution from DEAE-cellulose with an increasing linear gradient of lithium chloride in lithium acetate buffer (*p*H 5 0) and other techniques as in ref 19 Total volume of eluent 1,350 ml, 7-ml fractions

abundance of the analogue in the total nucleic acid Thus, in Preparation 2 the halopyrimidine accounted for 56 per cent of the sum of the 6-ketopyrimidines of the nucleic acid (Table 1) and the same figure was found for its relative frequency in the solitary and dinucleotide units (Table 3) The agreement was nearly as good in the preparation from strain I extending, in this case, even to the trinucleotides (Table 4) Moreover, the frequencies of thymine in the several units from Preparations 1 and 3 were quite similar to the frequencies of the sum of thymine and 5-bromouracil in the corresponding fractions from Preparations 2 and 4 While the final version of this article was being written, a note by Burton[27], dealing with another mutant strain of *E coli*, came to our attention, so far as comparison can be made, his findings are similar to those presented here

An observation pertaining to the structure of the normal nucleic acids produced without the interference of an analogue, deserves mention One gains the impression, also from previous observations[19,28], that there exists a certain bias in the disposition of thymine and cytosine, many nucleic acids appear to contain polythymidylic acid tracts that are much longer than those of polycytidylic acid This apparent trend is particularly noticeable in the case of the deoxyribonucleic acid of *E coli* which contains nearly equimolar quantities of thymine and cytosine One would have expected similar proportions of the stretches of varying length comprising only thymine or cytosine No units of the latter longer than C_3 have, however, been observed in hydrolysates of Preparation 1 (Table 1), whereas T_4 and T_5 units have invariably been noticed together with the various mixed fragments such as T_3C, T_2C_2, TC_3, T_4C, T_3C_2 and T_2C_3 The relatively greater abundance of cytosine among the small units, and the converse tendency of thymine, will be apparent even from the limited data in Tables 3 and 4

It must be understood, in conclusion, that the observation of an ostensibly random replacement of thymine by 5-bromouracil does not exclude the possibility that the analogue may exert certain non-random preferences for neighbours For example, we have no information about the relative frequencies of *pTpBp* and of *pBpTp* among the coupled units or about the frequency of adenine–thymine–adenine as compared with that of adenine–bromouracil–adenine among the solitary pyrimidine units Whatever may be the final explanation of the loss of viability and the occurrence of preferred locations of mutability, arising from the incorporation of 5-bromouracil, of which mention has been made before, it is clear that the substitution of the brominated analogue for thymine cannot be an inconsequential event

This work was supported by research grants from the National Institutes of Health, U S Public Health Service

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INACTIVATION OF EGG-WHITE LYSOZYME BY ULTRASONIC WAVES AND PROTECTIVE EFFECT OF AMINO-ACIDS

By DR F M DIETRICH*

University of Pittsburgh School of Medicine, Pittsburgh

DENATURING effects of ultrasonic waves on proteins have been demonstrated in many instances The subject has been reviewed by Grabar[1] and Griffin[2] In more recent studies, Kanig and Kunkel[3] reviewed the complex problem of ultrasonic effect on different proteins and discussed probable mechanisms involved in the denaturation processes Several enzymes are known to be inactivated by ultrasonic treatments, for example, blood catalase[4], salivary amylase[5], DNA-depolymerase[6], ribonuclease[7], pepsin, trypsin[8], milk reductase and peroxidase[9], succinic dehydrogenase[10], hyaluronidase[11], alkaline phosphatase[12], and diastase[13]

As shown in the following report, egg-white lysozyme is inactivated by ultrasonics and can be protected by several substances, including single amino-acids

Egg-white lysozyme, three times crystallized (Sigma Chemical Corporation) was used and activities were measured by a turbidimetric technique as described in another study[14] The standard concentration was 1 mgm /ml, dissolved in 0 6 *M* phosphate buffer (*p*H 6 2) The activity was shown to be linear

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* Present address Scripps Clinic, Division of Experimental Pathology, 476 Prospect Street, La Jolla, California

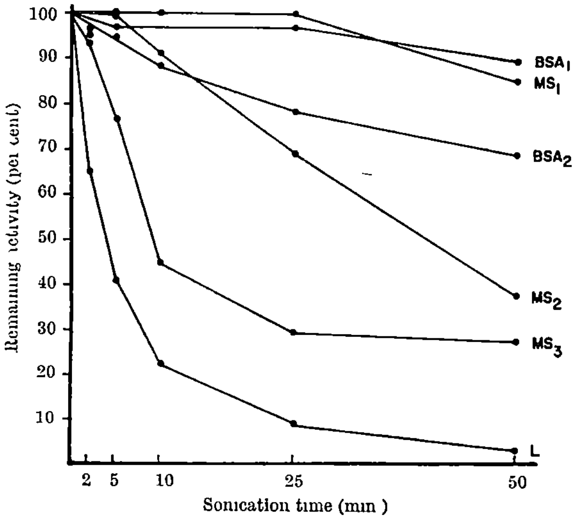

Fig 1 Sonication of lysozyme Protective effect of bovine serum albumin (BSA_1, 0 1 per cent BSA_2, 0 01 per cent) and 'Bentonite'-treated mouse serum (MS_1, 1 per cent , MS_2, 0 1 per cent , MS_3, 0 01 per cent) , *L*, lysozyme alone

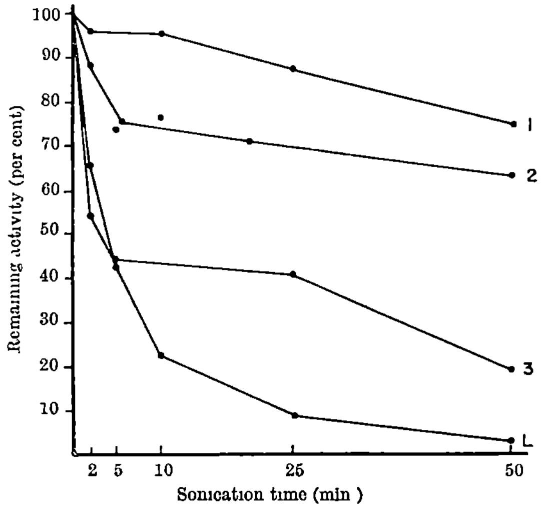

Fig 2 Sonication of lysozyme Protective effect of glycogen (1, 0 3 per cent, 2, 0 1 per cent, 3, 0 01 per cent, *L*, lysozyme alone)

during a 20-min incubation time and, in this assay, the activity was directly proportional to even higher enzyme concentrations (2 mgm /ml) Thus, enzyme activities determined under these experimental conditions can be regarded as initial rates Only in some experiments which were designed to determine the inactivation constant was the standard enzyme concentration changed When it was higher than 1 mgm /ml , the incubation time was reduced in order to get the true value for the initial rate In all inactivation and protection experiments, samples of 4-ml buffer, containing lysozyme (1 mgm /ml) and different amounts of L-amino-acids (only histidine was in the DL-form) or other substances were subjected to ultrasonic treatment for 2, 5, 10, 25 and 50 min Enzyme determinations were carried out in duplicate and the remaining activities reported as per cent of the original activity The values of the blank were subtracted from experimental values The *p*H of the amino-acid-buffer solutions was found to range from 5 8 to 6 3 However, in the experiments with DL-histidine (0 1 *M*) and arginine (0 1 *M*), the *p*H was 6 72 and 9 0 respectively Sonication was carried out by a 200-W , 10-kc /s oscillator (Raytheon, model *DF* 101) set at 1 25 amp The system was cooled with ice

Bovine serum albumin and mouse serum, previously treated with 'Bentonite' in order to absorb the lysozyme-like substance in the serum[14], showed a definite protective effect on lysozyme subjected to ultrasonic treatment (Fig 1) The protection depended on the concentration and was almost total with 0 1 per cent bovine serum albumin or 1 per cent mouse serum after 25 min sonication and fell slightly after 50 min In comparison, the activity of lysozyme solutions without bovine serum albumin or mouse serum was reduced after 25 and 50 min to approximately 8 and 3 per cent respectively

Glycogen also had a protective effect on lysozyme under the same experimental conditions (Fig 2) On the other hand, sodium chloride, magnesium sulphate, magnesium chloride and D-glucose tested in different concentrations had no protective effect Figs 3 and 4 summarize the data obtained with single amino-acids Several concentrations were

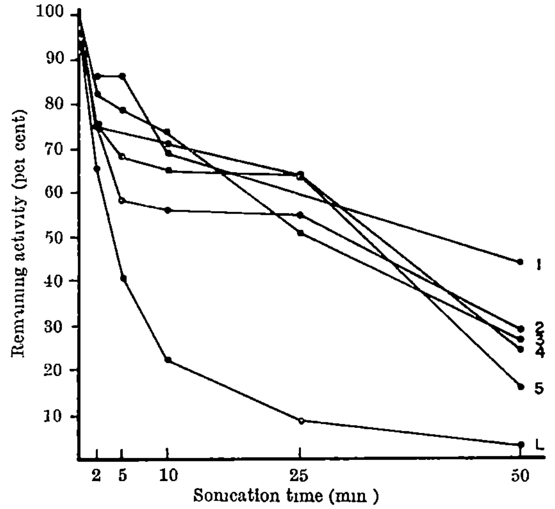

Fig 3 Sonication of lysozyme Protective effect of amino-acids and glutathione (1, glutathione, 0 01 *M* , 2, alanine, 1 *M* , 3, leucine, 0 01 *M* , 4, histidine, 0 1 *M* , 5, valine, 0 1 *M* , *L*, lysozyme alone)

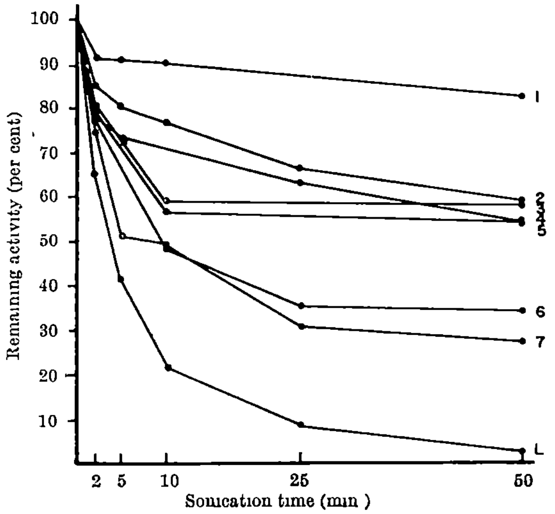

Fig 4. Sonication of lysozyme Protective effect of amino-acids (1, methionine, 0 08 *M* , 2, serine, 0 1 *M* , 3, arginine, 0 1 *M* , 4, asparagine, 0 1 *M* , 5, proline, 0 1 *M* , 6, glycine, 1 *M* , 7, lysine, 0 1 *M* , *L*, lysozyme alone)

tested Only the highest concentrations used in the experiments are presented Solubility, change in pH and inhibition of the enzyme activity were limiting factors in using higher concentrations Two different protection patterns were observed alanine (1 M), leucine (0 01 M), histidine (0 1 M) and valine (0 1 M) gave a high protection up to 25 min , but were less active thereafter (Fig 3) With lower concentrations, the activity at 50 min was often reduced to the level of the control, whereas at 25 min a protection effect was observed On the other hand (Fig 4), several amino-acids kept their protective effect for longer sonication periods and the remaining activity between 25 and 50 min was not significantly changed There was no correlation between molecular weight and protective effect Sulphur-containing substances, as methionine and glutathione, gave the best results

The ultrasonic inactivation of lysozyme did not show first order kinetics This behaviour was clearly demonstrated by several inactivation experiments using different enzyme concentrations In no instance did plotting of $\log \frac{A_0}{A}$ (where A_0 is the original activity and A the activity at time t) versus sonication time give a straight line By plotting $\log \frac{\mathrm{d}c}{\mathrm{d}t}$ versus $\log c_1$, the slope indicating the order (n) of the reaction ($\log \frac{\mathrm{d}c}{\mathrm{d}t} = \log k + n \log c$) was between 1 5 and 1 8 This finding was quite unexpected and indicates that ultrasonic inactivation of lysozyme follows more complicated kinetics Shugar[15] reported the ultra-violet-inactivation of lysozyme to conform to a first-order reaction

The enzyme assay, where ultra-violet-killed whole organisms (*M lysodeikticus*) are used as 'substrate' and the change in optical densities is measured spectrophotometrically, is not accurate enough to evaluate exact reaction constants

The mechanism of protection by single amino-acids, proteins and glycogen is now known Protecting agents may act by forming compounds with the enzyme active site, similar to an enzyme-substrate complex, or else they may act by protecting the entire molecule rather than peculiar areas Taking into consideration the molar ratio of enzyme to protective agents, it is more likely that another mechanism is involved Ultrasonic inactivation of enzymes and denaturation of proteins are often believed to be due to surface inactivation Protection may then occur by competition for available surface at the gas liquid interphases

This work was supported by a U S Public Health Service grant (2*E*–80)

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ACID MUCOPOLYSACCHARIDES OF THE HUMAN UTERUS

By G LOEWI and R CONSDEN

Rheumatism Research Unit (Medical Research Council), Canadian Red Cross Memorial Hospital,
Taplow, Maidenhead, Berks

THIS article reports an analysis of the acid mucopolysaccharides of human uteri of several ages and conditions This was done in an effort to correlate any differences in such analyses with different conditions of the uterus The uterus was considered to be particularly suitable for such an investigation, since it undergoes marked alterations with age and pregnancy In addition, uterine collagen has been found to differ in its shrink temperature and fluorescence[1] from other adult human collagen, a difference which might be reflected in the associated mucopolysaccharide pattern Previous investigations of uterine acid mucopolysaccharides[2,3] have suggested the presence of hyaluronic acid and chondroitin sulphate, but these constituents were not isolated or individually characterized

Uteri were obtained at operation or at autopsy within a few hours of death The available material consisted of two uteri obtained respectively 2 and 4 days after parturition, and of a fibromyoma from a 38-year-old woman, normal pre-menopausal uteri were pooled from three women aged 36, 45 and 47 years, and post-menopausal uteri from six women aged 60–80 Uterus, including cervix, was freed from adhering tissue, secretions and loose tissue were, so far as possible, removed from the endometrium The tissues were then ground in a meat-mincer dehydrated in several changes of acetone and air-dried Fat was afterwards removed by washing with petroleum ether (b p 40°–60°) The minced, defatted material was digested with activated papain according to Scott's method[4] This was followed by the removal of nitrogenous contaminants and eventually by fractional precipitation of the calcium salts of polysaccharides by ethanol These methods and those used for analysis and identification of the mucopolysaccharides were as described by Meyer *et al* [5,6] Ethanol fractions were analysed for hexosamine (quantitative and qualitative[7]), uronic acid (orcinol and carbazole), nitrogen (micro-Kjeldahl) sulphate and acetyl (Laboratory Alfred Bernhardt Mulheim), specific rotation and susceptibility to testicular and bacterial hyaluronidase The 28 per cent ethanol fraction was subjected to further fractionation by the copper sulphate method of Cifonelli *et al* [8] Small amounts of hyaluronic acid were isolated from 36 per cent ethanol fractions by the cetyl pyridinium chloride method of Scott[4] 36 and 50

Table 1 ACID MUCOPOLYSACCHARIDE AND HYDROXYPROLINE CONTENT OF UTERINE TISSUE

Source of tissue	Dry defatted weight (gm)	Total acid polysaccharide (mgm) (combined wt of ethanol fractions)	Chondroitin sulphate *B*		Heparitin sulphate		Other polysaccharide		OH-proline (N as per cent total N)
			mgm	Total polysaccharide (per cent)	mgm	Total polysaccharide (per cent)	mgm	Total polysaccharide (per cent)	
Pre-menopausal uteri	76	220	98	44 5	30	13 6	85	38 6	3 6
Post-menopausal uteri	90	160	86	53 7	16	10 0	57	35 6	4 0
Post-partum uterus (2 days)	117	207	87	42 0	26	12 5	80	38 6	1 9
Post-partum uterus (4 days)	132	297*	—	—	—	—	—	—	1 8
Fibromyoma	85	219	98	44 7	28	12 8	93	42 5	3 4

* Actual weight Material not further fractionated

per cent ethanol fractions were analysed for keratosulphate by examination of hydrolysates for galactose by paper chromatography and by the anthrone reaction[9] Ethanol fractions (20–30 μgm each) were examined for acid polysaccharides by electrophoresis on cellulose acetate membranes (30 min at 15 V /cm in $M/40$ phosphate buffer, *p*H 7, stained with toluidine blue) Hydroxyproline was estimated in dried, defatted tissue after hydrolysis in 6 *N* hydrochloric acid for 16 hr by the method of Neuman and Logan[10]

Table 1 shows the types and quantities of acid mucopolysaccharide fractions obtained from the various groups of uteri The 18 per cent ethanol fraction and the precipitate obtained by treatment of the 28 per cent ethanol fraction by copper sulphate together constitute chondroitin sulphate *B*, which amount to about a half the acid polysaccharide isolated Its identity was confirmed by a low carbazole orcinol ratio (range 0 3–0 5) by liberation of galactosamine on hydrolysis, by negative rotation and by complete resistance to hyaluronidase The polysaccharide obtained from the supernatant part of the 28 per cent ethanol fraction after copper sulphate treatment accounted for about 10 per cent of the total material isolated from each batch of tissue The following properties suggested that it was heparitin sulphate positive rotation, liberation of glucosamine with only a trace of galactosamine on hydrolysis, sulphate, 4–8 per cent, acetyl, determined for one sample only, 4 2 per cent Carbazole orcinol ratio was relatively high (range 1 5 2 1), and the material was resistant to hyaluronidase Both the 36 and the 50 per cent ethanol fractions (sulphate, 6-12 per cent), consisted largely of chondroitin sulphate *A* and/or *C*, as evidenced by carbazole orcinol ratios of approximately 1 0, galactosamine as the predominating amino sugar, and digestibility by testicular hyaluronidase Slight degree of digestion by bacterial hyaluronidase and the presence of some glucosamine in the cases of the pre- and post-menopausal uteri suggested the presence of some hyaluronic acid This was confirmed by the isolation of a few milligrams of hyaluronic acid from each of these two groups by Scott's method[4] No evidence for the presence of keratosulphate was found in any of the 36 or 50 per cent ethanol fractions

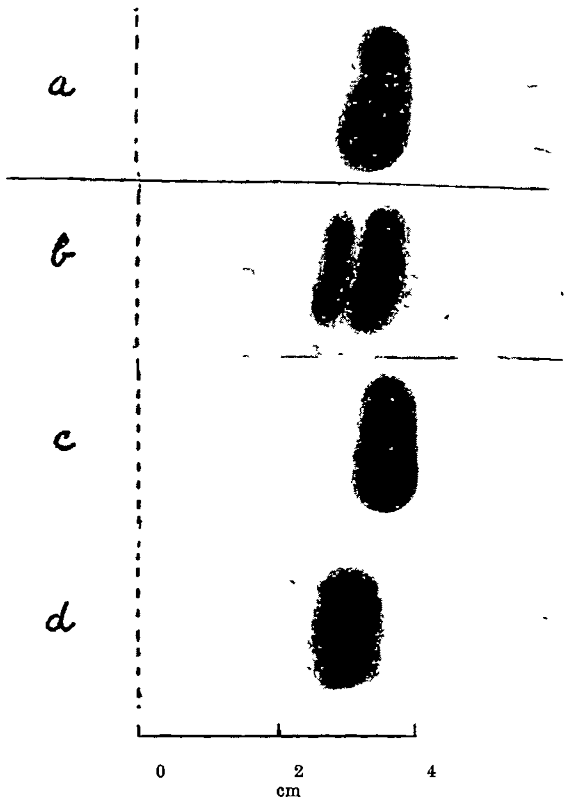

Fig 1. Electrophoretic pattern on cellulose acetate membranes *a*, 18 per cent ethanol fraction, *b*, 28 per cent ethanol fraction, *c*, precipitate from copper sulphate treatment of 28 per cent ethanol fraction, *d*, the copper sulphate supernatant Broken line represents position of origin

Fig 1 shows electrophoretic patterns given by (*a*) the 18 per cent ethanol fraction which was typical for chondroitin sulphate from a variety of sources, (*b*) the 28 per cent ethanol fraction, showing the presence of chondroitin sulphate together with the slower heparitin sulphate The copper sulphate treatment of this ethanol fraction resulted in almost complete separation of chondroitin sulphate which was precipitated (*c*) from heparitin sulphate, (*d*) which remained in the supernatant Electrophoresis of the 36 and the 50 per cent ethanol fractions showed that substantially only chondroitin sulphate was present, the former containing a trace of heparitin sulphate in addition

Meyer *et al* [5] found roughly equal proportions of chondroitin sulphate *B* and of *C*, with hyaluronic acid as a minor component, in adult connective tissue, such as pig heart valve and pig or calf tendon This pattern appears to be somewhat similar to that described here in uterine tissue, with, however, the interesting addition of heparitin sulphate Other collagen-rich tissues of human origin, such as skin[11], while containing amounts of total acid polysaccharides similar to those found in uterus, were relatively rich in hyaluronic acid, but no heparitin sulphate was found This polysaccharide was found in bovine lung and liver[12], in aorta[13] and in large amounts in the tissues from cases of Hurler's syndrome[14] This distribution does not suggest an association of heparitin sulphate with any particular tissue component

With the exception of a slightly higher proportion of chondroitin sulphate *B* in post-menopausal uteri, there are no marked differences in the distribution of individual acid polysaccharides in the four types of uterine tissue examined There were some differences in the yields of total acid polysaccharides extracted, but no clear correlation with age or type of uterus was apparent. Collagen content, as reflected by hydroxyproline–nitrogen values, was lower in post-partum than in other uteri. This difference was not, apparently, accompanied by differences in acid polysaccharide content.

We thank N. V. Organon of Oss, Holland, for a gift of bacterial hyaluronidase.

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CONTROL OF ENDOGENOUS ADENOSINE TRIPHOSPHATASE ACTIVITY BY ENERGY-LINKED PYRIDINE NUCLEOTIDE REDUCTION IN MITOCHONDRIA

By Prof. BRITTON CHANCE and Dr. TAKERU ITO[*]

Johnson Research Foundation, University of Pennsylvania, Philadelphia

IN previous work[1] the existence of a pathway for the reduction of diphosphopyridine nucleotide (DPN) by succinate has been demonstrated and this pathway causes more DPN$^+$ reduction than any other substrate. In pigeon heart mitochondria, succinate-linked reduction of DPN$^+$ shows a specific requirement for adenosine triphosphate (ATP)[2,3] and the breakdown of the ATP expended in this reduction can be recorded as a change in *p*H by the use of a sensitive glass electrode technique[4]. Our results show that the oxidation-reduction state of DPN$^+$ exerts a powerful control over the endogenous ATPase of aerobic pigeon heart mitochondria; succinate-linked DPN$^+$ reduction inhibits endogenous ATPase of aerobic pigeon heart mitochondria by a very large factor. The measurement of ATP breakdown is sufficiently sensitive to detect amounts equivalent to carrier concentrations (~ 1 mμmole H^+/mgm. protein). The amount of ATP breakdown caused by succinate-linked DPN$^+$ reduction is measured and the stoichiometry of the reaction is evaluated.

The control of ATPase activity by the oxidation-reduction state of the respiratory carriers is one of the few links between this activity of the mitochondria and their property of oxidative phosphorylation. Investigations of ATPase activities in the presence of uncouplers such as dinitrophenol[5,6] also show inhibition by the oxidation-reduction state of the carrier[7-9]; 'complete reduction' appears to cause less than 70 per cent inhibition in liver mitochondria[8]. ATPase observed with no added activators is nearly 100 per cent inhibited by 'complete reduction'[9]. On the basis of these and related experiments, unique interpretations have been made regarding the role of respiratory carriers in oxidative phosphorylation[9], but other interpretations are being actively considered[8,10-12]. The basic question is whether the ATPase activity requires formation of a high energy intermediate with the oxidized or reduced forms of the respiratory carriers[10].

Here we observe the ATPase activity of mitochondria which contain both oxidized and reduced carriers; it is found that activity of endogenous ATPase is highly inhibited when energy-linked DPN has been reduced by succinate[1-3], but cytochromes remain partly oxidized. These results are consistent with the hypothesis that the reduced form of a respiratory carrier (DPNH) binds one of the intermediates of the energy transfer reactions of mitochondria[11,12]. The idea that the 'energy rich' form of the carrier involves the oxidized state[9] does not receive experimental support from these data.

These investigations shed some light on reaction mechanisms of reversed electron transfer, oxidative phosphorylation, and ATPase activity. The results illustrate a competition between 'hydrolysis' of an essential intermediate in ATPase activity (a 'dissipative' reaction) and the utilization of such an intermediate in reduction of DPN$^+$ (a 'conservative' reaction)[13-15].

The pigeon heart mitochondria were prepared as described previously[16] and were suspended in a magnesium-free medium containing 0.225 *M* mannitol, 0.075 *M* sucrose, and 0.003 *M* *tris*, at *p*H 7.4 (termed 'MST solution'). The temperature was 25°, protein was determined by the biuret method and ATP was determined optically. Determinations of hydrogen ion concentration were based on standard phthalate solutions.

The hydrolysis of ATP involves a formation of hydrogen ions which at *p*H 7.4 amount to 0.89 H^+/ATP (refs. 4 and 17). This change in *p*H was continuously recorded by a sensitive meter (Radiometer type 22) supplemented with an amplifier and chart recorder. Under the buffering conditions used, a change of hydrogen ion concentration of approximately 1 μ*M* (1.0 mμmoles) could be detected. Mitochondria were suspended in a continuously rotating cup in which the electrodes were immersed[4].

Inhibition of ATPase. The formation of hydrogen ions due to hydrolysis of ATP added to a suspension of pigeon heart mitochondria in the absence of added substrate is indicated in Fig. 1 (trace *A*) (the downward deflexion of the trace indicates formation of hydrogen ions); the addition of 85 μ*M* ATP is expended in an approximately first-order reaction in about 2½ min. The *p*H is reset to the initial value by two additions of sodium hydroxide, and a second addition of 85 μ*M* ATP reproduces the effects of the first addition.

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[*] Present address: Ohio State University.

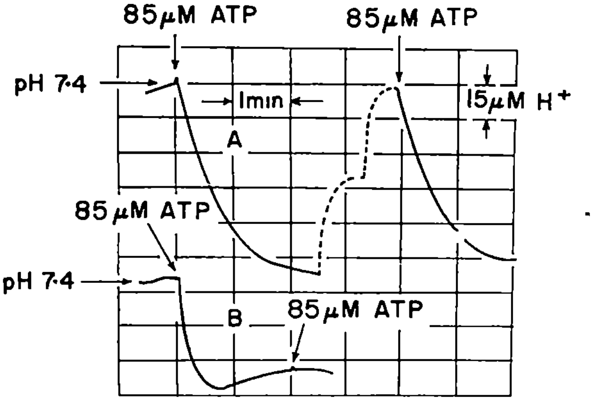

Fig 1 Hydrogen ion production from ATPase activity Trace *A*, endogenous ATPase activity of the pigeon heart mitochondrial suspension as measured continuously by a glass electrode The initial *p*H is 7 4, the arrows indicate the addition of 62 μM ATP, the downward deflexion of the trace indicates an increase of hydrogen ion concentration at a sensitivity of 15 μM H$^+$/large division 2 3 mgm /ml mitochondrial protein are suspended in MST medium at *p*H 7 4, 25° The dotted portions of the trace indicate the additions of sufficient sodium hydroxide to return the *p*H to the original level Trace *B*, hydrogen ion production from ATP breakdown in mitochondria supplemented with 0 6 m*M* succinate, initial *p*H 7 4 Other conditions identical to trace *A*

If the mitochondrial suspension is supplemented with 0 72 m*M* succinate prior to the addition of ATP, the formation of hydrogen ions is approximately twice as rapid (Fig 1, trace *B*) and the hydrogen ion production is much smaller Furthermore, a second addition of ATP under these conditions causes a scarcely measurable formation of hydrogen ions (the ratio of rates for the first and second additions of ATP exceeds 25)

The succinate concentration required to inhibit the breakdown of ATP according to the conditions of Fig 1*B* is about 400 μM These concentrations are of the same range as required for activation of succinate-linked DPN$^+$ (ref 18)

Effect of DPN-linked substrates Experiments with a variety of DPN-linked substrates show that the effect is highly specific for succinate, in the presence of malonate no inhibition is caused with malate, malate and glutamate, and α-ketoglutarate These substrates cause partial and not complete reduction of DPN$^+$ (ref 18)

Stoichiometric aspects A series of experiments have been carried out under the conditions of Fig 1*B* and in which the protein concentration was varied at constant concentrations of succinate and ATP A linear relation obtained so long as the added ATP was not expended The slope was 35 mμmole H$^+$/mgm protein for pigeon heart mitochondria 25°

Effects of inhibitors In agreement with spectrophotometric observations of inhibitors of the succinate-linked DPN reduction, similar effects were found for the ability of the succinate-linked pathway to inhibit endogenous ATPase (Fig 1*B*) The ATPase was reactivated by dinitrophenol, antimycin *A*, and hydroxyquinoline oxide (unpublished observations of Reiko Oshino) The effect of dinitrophenol is expected not only from its well-known ability to activate ATPase[5,6], but also from its inhibition of succinate-linked DPN reduction[1] The effects of antimycin *A* and hydroxyquinoline oxide are interpreted on the latter basis because they specifically interfere with DPN$^+$ reduction (see discussion later)

Reaction-rates The formation of hydrogen ions from ATP proceeds about twice as rapidly when DPN$^+$ is being reduced (Fig 1, trace *B*) (130 mμmoles/min /mgm protein) as when it is not (Fig 1, trace *A*) (40 mμmoles/min /mgm protein) The latter rate is in the range of values obtained with dinitrophenol at these concentrations of ATP

Correlation with DPN$^+$ reduction In order to demonstrate that DPN$^+$ reduction actually occurs under the conditions where the hydrogen ion formation is being observed, we illustrate in Fig 2 consecutive measurements of hydrogen ion formation (upper trace as recorded by the glass electrode) and DPN$^+$ reduction (lower trace recorded with the double beam spectrophotometer) 31 mμmoles H$^+$/mgm protein are produced as are 10 mμmoles DPNH/mgm protein

Steady-state hypothesis One explanation of the observed results is that sufficient ADP and phosphate are formed from the breakdown of the added ATP to allow rephosphorylation to occur On this basis the cessation of ATP breakdown represents a steady state rather than the end-point of the titration A number of considerations suggest that this is not the case

(1) The amounts of phosphate formed are well below the K_M value for oxidative phosphorylation[16,19] If a steady state exists, it should respond to further additions of ATP with a measurable hydrogen ion production at approximately the same initial rate as the first addition (Fig 1, trace *B*)

(2) The addition of phosphate to mitochondria which have been treated as indicated by Fig 1*B* allows an assay of ADP remaining in amounts which roughly account for the breakdown of ATP (refs 16 and 20)

(3) Addition of ATP in the absence of magnesium ions can lead to an inhibition of respiration and leads to conditions favourable to the accumulation of low concentrations of ADP and inorganic phosphate[21]

(+)DPN-linked substrates causing the reduction of only part of the DPN, but nevertheless activating respiration, do not inhibit the endogenous ATPase

These and other considerations lead us to set aside the 'steady-state' hypothesis in favour of the one outlined here

Competitive inhibition of endogenous ATPase by energy-linked DPN$^+$ reduction It is proposed to explain two characteristics of Fig 1 (1) That the formation of hydrogen ions, attributed to breakdown of ATP, is accelerated by the concomitant reduction of DPN$^+$ (2) That further breakdown of ATP after the completion of DPN reduction is severely inhibited

These results lead us to consider a competitive reaction between the hydrolysis of an intermediate in ATPase action and the utilization of such intermediates in DPN$^+$ reduction

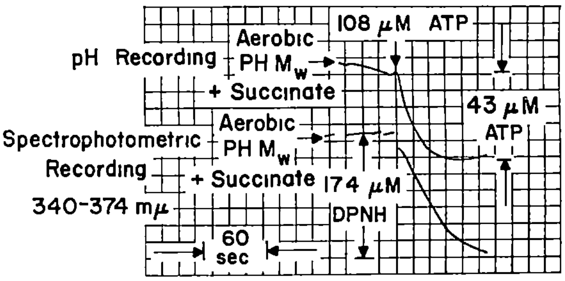

Fig 2 Consecutive glass electrode recordings of increases of hydrogen ion concentration following addition of ATP to succinate-treated mitochondria (1 4 mgm /ml pigeon heart mitochondria) (top trace) and spectrophotometric recordings of DPN reduction (1 7 mgm /ml pigeon heart mitochondria) (bottom trace) The calibration of the glass electrode trace was made by the addition of known concentrations of sodium hydroxide The calibrations of the spectrophotometric trace were based on the extinction coefficient of DPNH of 2 3 mM^{-1} × 0 5 cm^{-1} at wave-lengths of 340–374 mμ (212–4)

Equations representing the competitive inhibition of endogenous ATPase activity follow

$$\text{ATP} + X \rightleftharpoons X \sim \text{P} + \text{ADP} \quad (1)$$

$$X \sim \text{P} + \text{I} \rightleftharpoons X \sim \text{I} + P_i \quad (2)$$

$$X \sim \text{I} + \text{DPN}^+ + \text{succinate} \rightleftharpoons \text{fumarate} + \text{DPNH} + \text{H}^+ + X + \text{I} \quad (3)$$

$$X \sim \text{I} + \text{DPNH} \rightleftharpoons \text{DPNH} \sim \text{I} + X \quad (4)$$

$$X \sim \text{I} \overset{\text{HOH}}{\rightleftharpoons} X + \text{I} \quad (5)$$

This mechanism is formulated in terms of a present view on the mechanism of oxidative phosphorylation[12], but this does not exclude its interpretation in terms of other mechanisms in so far as they can be shown to be consistent with the experimental observations The equations embrace the following ideas

(1) A spontaneous 'hydrolysis' of labile intermediates formed from ATP (equation 5)

(2) A competition for the labile intermediate between 'hydrolysis' and an energy-linked reaction (equations 3 and 4) (Competition between 'dissipative' and 'conservative' pathways[13–15])

(3) Succinate-linked DPN+ reduction is already identified as an energy-requiring reaction and is the most likely one to be involved[1–3], but other carriers can also be affected by this reaction.

(4) The carriers involved in the reaction, particularly DPN+, have the property of binding and thereby expending an intermediate essential to the ATPase activity (equation 4)

The inhibitor sensitivity is consistent with equations (3) and (4), substances which block electron transfer from succinate (malonate, antimycin *A*, hydroxyquinoline oxide)[22] block effects on the ATPase activity In connexion with the effects of antimycin *A* and hydroxyquinoline oxide, we prefer the simple hypothesis that they react by inhibiting electron transfer and thereby prevent the flow of electrons from succinate to DPNH The hypothesis that they activate ATPase[23,24] now appears to be consistent with experimental observations, they activate ATPase by inhibiting equations (3) and (4) The possibility that they activate equation 5 is not eliminated, but does not affect the conclusions drawn here

Lack of appreciable inhibition of the endogenous ATPase activity in the presence of malate, glutamate (and malonate) is in accordance with the observation that these substances reduce only a fraction of the DPN+ reduced by succinate and ATP (ref 1) Thus the concentration of reduced DPN+ may be stoichiometrically insufficient to allow reaction (4) to go to completion and consequently reaction (5) may proceed

Evidence that DPNH is involved in an especial binding in mitochondria is afforded by the greatly enhanced fluorescence of the coenzyme under these conditions[25] Recent data obtained by R W Estabrook of this laboratory and elsewhere[26] suggest that the enhancement factor considerably exceeds that of a binary complex[27] and more closely approaches that of a ternary complex[28] Further evidence also suggests that a protein component may be involved in this binding in view of the ultra-violet peak in the activation spectrum[26]. Thus the experimental evidence speaks in favour of a ternary compound of pyridine nucleotide, a protein component and a third component which, on the basis of these data, is essential to endogenous ATPase activity

A highly active endogenous ATPase (about 25 per cent of the DPN-stimulated value) is characteristic of heart mitochondria, but is not noted in rat liver and kidney mitochondria As an explanation of this basic difference of the two types of preparation we offer the possibility that the stoichiometric ratios of the ATPase intermediate of equations (1–5) relative to that of DPNH and of other carriers which may be involved in the binding varies in the different preparations; in rat liver mitochondria the amount of the ATPase intermediate is sufficiently small that it is stoichiometrically expended by a small extent of reduction of DPN (and other carriers) In the pigeon heart mitochondria it is apparent that the stoichiometric requirements for ATPase inhibition are only met by the high degree of DPN reduction achieved by the energy-linked pathway

Obviously, other factors enter into the control of endogenous ATPase activity, that is, under the conditions used in the measurement of oxidative phosphorylation excess phosphate is present and inhibits equation (2) from proceeding from left to right

Superficially the inhibition of the endogenous ATPase by DPN+ reduction resembles the inhibition of dinitrophenol-stimulated ATPase by anaerobiosis[8] Here, too, the possibility of DPN reduction was considered as an inhibitory factor However, the quantitative nature of the inhibitory effects vastly differ, more than 96 per cent inhibition of the endogenous ATPase is obtained in the aerobic succinate-treated mitochondria In the dinitrophenol-treated mitochondria only about 50 per cent inhibition can be obtained by anaerobiosis and this may be because only a portion of the DPN can be reduced under these conditions[29] Thus mechanisms of the forms of equations (1–5) may be applicable to other systems according to the extent to which DPN+ and other carriers can be reduced in the presence of the uncoupling agents used to activate ATPase

The stoichiometric aspects of the results of these data are of considerable interest in determining the efficiency with which equations (3) and (4) compete with equation (5) under the conditions of our experiment, and furthermore are of value in determining the stoichiometry with which ATP participates in equation (6)

$$n\,\text{ATP} + \text{succinate} + \text{DPN}^+ \rightleftharpoons n\,\text{ADP} + n\,P_i + \text{fumarate} + \text{DPN}^+ + \text{H}^+ \quad (6)$$

However, before the stoichiometry can be determined accurately, the contribution to the *p*H change caused by reduction of intramitochondrial DPN+ must be determined In order to record this change in *p*H, we utilize the fact that addition of glutamate to the succinate-treated mitochondria leads to DPN+ reduction without the need for external energy sources[1] (the reaction under these conditions is oligomycin-insensitive[30]) A determination of the hydrogen ion production caused by glutamate-activated reduction of pyridine nucleotide gives a value of 6 5 mμmoles H+/mgm protein Thus the value for hydrogen ion formation from succinate and ATP (35 mμmoles/mgm protein) can be revised downwards to 28 mμmoles/mgm protein The value from Fig 2 is revised downwards to 24 mμmoles/mgm protein Investigations reported elsewhere[4] indicate that 0 89 H+ is released per mole of ATP broken down at *p*H 7 4 and 25° Using this conversion factor, we obtain for the two determinations mentioned in this article 31 and 27 mμmoles ATP/mgm protein

Since the average value for DPN$^+$ reduction by succinate and ATP in pigeon heart mitochondria is 10 mμmoles/mgm protein (the particular value in Fig 2 is identical to this) the stoichiometric ratio of equation (6) is between 2 7 and 3 1 ATP/DPNH Values determined in this manner are likely to be maximum values for two reasons (1) The effectiveness of the competition with the 'hydrolysis' reaction (equation 5) is not surely known, although the rates are in favour of equations (3) and (4) (2) Carriers other than pyridine nucleotide, although present in smaller concentrations than DPNH, are concomitantly reduced The stoichiometric coefficient obtained by this procedure lies within the range of values determined by other methods[31] although it is significantly larger than the lowest values obtained by spectrophotometric observations of ADP formation[31]

Control of metabolism The results of these experiments are of some interest with respect to present-day theories of metabolic control Theories which propose control of respiration by control of the level of substrate in the mitochondria would seem in the light of these experiments to lead to an activation of endogenous ATPase, a wasteful process Control of metabolism by ADP or phosphate in the presence of excess substrate seems to be more efficient and is, in fact, the type of control that is observed in excised muscles and ascites tumour cells suspensions[32]

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GLUCOSE METABOLISM IN A LOW-GLYCOLYSING TUMOUR, THE MORRIS HEPATOMA 5123

By YU CHEN LIN, J. CLINT ELWOOD*, ADOLFO ROSADO†, HAROLD P. MORRIS and SIDNEY WEINHOUSE

Institute for Cancer Research, and Chemistry Department, Temple University, Philadelphia, and the National Cancer Institute, Bethesda

CONSIDERABLE attention has been focused recently on a new carcinogen-induced, transplantable rat hepatoma, the Morris hepatoma 5123 (ref 1), because it differs markedly from all other transplantable rat liver tumours so far studied in retaining, to a greater extent than has hitherto been observed, many of the morphological and metabolic characteristics of the normal hepatic cell Various properties of this tumour have been described in the recent literature[2–10], and a summary of its enzymatic characteristics may be found in a recent review[11]

Probably the most striking characteristic of this tumour is a very low glycolytic rate Slices of this tumour apparently do not follow the rules of usual behaviour of malignant neoplastic cells in that they produce very small quantities of lactate in the presence of glucose (refs 4 and 10 and unpublished observations of Woods, M , and Burk, D) Although not measured in earlier generations, presumably this characteristic has been retained through 25 serial transfers during the past five years In a study of the glucose metabolism of whole homogenates of these tumours, we have confirmed the low glycolytic rate and we have found that this property is due at least in part to a low glucokinase activity

The tumours used in this work were twenty-third and twenty-fifth generation transplants of sub-lines *C* and *D*, made by subcutaneous, intramuscular or intraperitoneal trocar injection of minced tumour tissue in female rats of the Buffalo strain Approximately 60–80 days after implantation, when the tumours reached a size of 1–2 cm diameter, the rats were decapitated and the tumours carefully but rapidly dissected from surrounding tissue and central necrotic material, if any The tissue was weighed, minced with sharp-pointed scissors, and homogenized Using a Potter–Elvehjem homogenizer with a plastic pestle, the tissue, diluted with twice its volume of distilled water, was dispersed by means of five or six passes of the pestle while cooling in ice Pieces of tissue debris were removed by filtration through glass wool and a portion of the homogenate was taken for dry weight determination

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* This work was done in part during the tenure of a National Institutes of Health Postdoctoral Fellowship Present address Department of Biochemistry, New York State Medical College, Syracuse

† On leave of absence from the Instituto Mexicano del Seguro Social, Mexico, D F

Table 1 EFFECT OF HEXOKINASE* ON LACTATE PRODUCTION AND GLUCOSE OXIDATION IN TUMOUR HOMOGENATES

Tumour	Substrate	Oxygen uptake (μmoles)	Lactate (μmoles)	Glucose carbon in respiratory CO_2 (μatoms)
Hep 5123 subcut and intramusc female	None	18 0	0 54	—
	Glucose-U-^{14}C	17 1	0 68	1 6
	Glucose-U-^{14}C + hexokinase	23 2	18 6	8 9
Hep 7800 subcut male	None	15 8	3 4	—
	Glucose-U-^{14}C	14 9	3 9	2 4
	Glucose-U-^{14}C + hexokinase	17 5	21 0	7 0
Dunning hepatoma	None	2 9	2 6	—
	Glucose-U-^{14}C	2 6	7 0	0 39
	Glucose-U-^{14}C + hexokinase	3 0	22 7	1 02
Primary hepatoma (*DAB*)	None	3 1	2 1	—
	Glucose-U-^{14}C	3 6	4 1	0 11
	Glucose-U-^{14}C + hexokinase	3 7	8 4	0 67
Sarcoma 37† ascites	None	16 7	0 6	
	Glucose-U-^{14}C	12 9	20 0	1 70
	Glucose-U-^{14}C + hexokinase	13 5	19 8	1 67

* Hexokinase was added in an amount of 62 K M units (ref 21)

† Succinate, 0 002 *M* was present in this experiment

Table 2 GLYCOLYSIS FROM GLUCOSE AND GLUCOSE-6-P IN TUMOUR HOMOGENATES

Tumour	Substrate	Oxygen uptake (μmoles)	Lactate (μmoles)
Hep 5123 intraperiton sub-lines *C* and *D*	Glucose	6 6	0 84
	Glucose-6-P	12 5	4 6
Hep 7800 subcut 3rd generation	Glucose	14 8	3 9
	Glucose-6-P	17 6	19 3
Dunning *LC*-18 hepatoma*	Glucose	2 9	7 0
	Glucose-6-P	3 6	22 2
Sarcoma 37	Glucose	10 6	24 6
	Glucose-6-P	9 9	21·9

* Kindly supplied by Dr Pietro M Gullino, Laboratory of Biochemistry, National Cancer Institute

Glucose, uniformly labelled with carbon-14 (glucose-U-^{14}C), was obtained from the Volk Radiochemical Co, Chicago Glucose-6-phosphate and the hexokinase, type V, were obtained from the Sigma Chemical Co

Each Warburg vessel contained 0 5 ml of the tissue suspension, containing 30–35 mgm dry weight in 2 1 ml total volume of medium with the following final composition phosphate buffer, *p*H 7 4, 0 02 *M*, Mg^{++}, 0 002 *M*, potassium chloride, 0 08 *M*, adenosine triphosphate, 0 002 *M*, cytochrome *c*, 1 mgm per vessel, and diphosphopyridine nucleotide, 2 5 mgm per vessel Where added, glucose and glucose-6-P were in a final concentration of 0 005 *M* Incubations were carried out with shaking for 1 hr at 37° with air in the gas phase, and a folded filter paper strip soaked in potassium hydroxide in the centre well The reaction was terminated by tipping in 0 2 ml of 20 per cent trichloroacetic acid from the side-arm, and after 15 min additional shaking, carbon dioxide was recovered from the centre well for radioactivity assay[12] and lactate was determined according to Barker and Summerson[13]

The pertinent results concerning respiration and aerobic glycolysis are summarized in Tables 1 and 2 The endogenous respiration of the fortified whole homogenate of 5123 was very high, corresponding to a Q_{O_2} of 16 The addition of glucose to the homogenate lowered oxygen uptake slightly and the lactate production was quite low, corresponding to a $Q_L^{O_2}$ of only 0 3 Measurement of the radioactivity of the respiratory carbon dioxide revealed that only a very small quantity of glucose was oxidized, accounting for only 8 per cent of the total respiration In the presence of succinate, glucose again lowered respiration somewhat, and both lactate production and glucose oxidation were very low However, addition of yeast hexokinase brought about a remarkable stimulation of glycolysis Under these conditions the aerobic formation of 18 6 μmoles lactate corresponds to a $Q_L^{O_2}$ of 12, a value as high as those displayed by slices of both primary and transplanted hepatomas[14–16] At the same time there was also a three- to five-fold stimulation in the oxidation of glucose carbon to carbon dioxide, raising the proportion of total respiration accountable by glucose oxidation to 38 per cent

Recently another hepatoma, number 7800, was induced in a male rat by the same procedure used for induction of the 5123 tumour in a female rat[1], and has been transplanted into the third generation (The details concerning the induction of hepatoma 7800 and other slowly growing transplantable rat hepatomas will be described in another publication from the National Cancer Institute) The experiment in Table 1 shows that a homogenate of this tumour also displays the same low glycolytic activity, and responsiveness to hexokinase

For comparison, similar data are presented for the Dunning hepatoma *LC*–18, for a primary rat tumour induced by feeding a diet containing 0 06 per cent of dimethylaminoazobenzene, and for a transplantable mouse neoplasm, sarcoma 37, in the ascites form Homogenates of the Dunning and the *DAB* tumours had a lower respiration than the 5123 tumour, and displayed a moderate glycolysis which was enhanced by addition of hexokinase, though the effect was not so marked as that observed with the 5123 and 7800 tumours Glucose oxidation in these particular tumours was very low and responded markedly to hexokinase In contrast, the homogenate of sarcoma 37 had a high respiration and a high glycolysis in the presence of glucose Neither was further stimulated by hexokinase, and glucose oxidation, though low, was also not affected by this enzyme These results leave no doubt that under the conditions of our experiments the 5123 tumour had a low capability for lactate production and glucose oxidation, and that these defects can be repaired by the addition of hexokinase These results also indicate that glucokinase limits glycolysis as well as glucose oxidation in variable degree in other hepatomas

Further evidence for hexokinase as the limiting step in glucose utilization by the 5123, the 7800 and the Dunning *LC*-18 tumours is shown in Table 2 With these tumours lactate production was much higher from glucose-6-P than from glucose, being of the order of that obtained with glucose and hexokinase With sarcoma 37, however, under a variety of conditions, lactate formation was no greater with glucose-6-P than with glucose as substrate

The low glucokinase activity of these tumours may be regarded as yet another characteristic in which they resemble liver Low glucokinase activity has been recognized for many years as a characteristic property of liver[17], and recent studies[18] have also shown that the rat liver enzyme differs markedly from hitherto-described hexokinases of other tissues in having an apparently very low affinity for glucose, so that high concentrations are required for maximal activity Glucokinase assays in the Morris 5123 and 7800 tumours carried out recently in our laboratory, using a sensitive spectrophotometric assay procedure based on triphosphopyridine nucleotide reduction in the presence of excess glucose-6-P dehydrogenase, are in accord with these metabolic studies in displaying a glucokinase activity so much lower than that of normal rat liver that assay values have been at the limit of measurement[19] Whether this is due to a deficiency of enzyme, or to some as yet undetermined inhibitor effect, such as has been proposed by Woods and Hunter[20] for the *S*91 melanoma, remains for further investigation

In contrast with the vast majority of tumours, including both primary and transplantable liver neoplasms, the Morris hepatomas 5123 and 7800 can grow (albeit slowly) and kill their hosts with apparently only minimal use of glucose as a source of glycolytic or respiratory energy A more detailed investigation of the glucose (and fatty acid) metabolism as well as a study of the properties of the hexokinase of these and other liver tumours is now in progress

This work was supported by grants from the National Cancer Institute, the U S National Institutes of Health and the American Cancer Society

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SEPARATION OF STRONTIUM FROM MILK AND PROTEIN SOLUTIONS BY GEL FILTRATION

By W H S. GEORGE*

Department of Experimental Pathology, University of Birmingham

GEL filtration[1] has provided a simple and rapid method for the fractionation of water-soluble substances The process depends on differences in molecular size and the gel can be regenerated after each cycle

Gel filtration on 'Sephadex' (Pharmacia, Uppsala, Sweden) has been used in investigation of the complexes formed between heavy metals and small molecules or ions[2] It was found that heavy metals could be bound to the 'Sephadex' under certain conditions Radioisotope investigations[3] of submicrogramme quantities of inorganic ions have shown that the ions enter the gel phase and can be separated quantitatively from proteins

The factors[4] known to influence the removal of salts from particular proteins, by gel fitration, are flow-rate, particle size of the bed material, volume and viscosity of the sample Although strontium has been separated from milk for analytical purposes by ion exchange[5], it seemed possible that a similar separation might take place by gel filtration

Columns of 'Sephadex *G* 25' and '*G* 50' were prepared by washing the 'Sephadex' three times with 5–10 times its volume of phosphate-buffered saline, 0 5 *M*, *p*H 7 4 Each time the gel was allowed to settle and the fine particles decanted off The gel was then slurried into a vertical burette, the lower end of which was plugged with glass wool and the column allowed to settle Columns were prepared varying in length between 24 and 32 cm and were 0 6 cm diameter Each column was washed through with 100–200 ml of phosphate saline buffer until the eluates showed a similar electrical conductivity, demonstrating that the gel was thoroughly saturated with the eluant.

10 ml of milk were centrifuged at 3,000 r p m for 5 min in a bench centrifuge at room temperature and the fat skimmed off 4 ml of the skimmed milk were thoroughly mixed with 1 ml of strontium chloride containing 0 6 mgm of strontium per ml The 5 ml of milk/strontium mixture were applied to the top of the column, and after they had soaked into the gel were eluted with phosphate saline buffer The eluate was collected in 3-ml aliquots by a mechanical fraction collector At the end of the elution period (120 ml) the column was extruded from the burette and divided into three parts, each part being suspended in phosphate saline buffer

The aliquots of the eluate collected during the equilibration period with buffer, and after addition of the milk/strontium mixture were suitably diluted and their optical density measured at 280 mμ for protein The presence of fat in the eluates was detected by dropwise addition of a sample from each aliquot on to filter paper which, when dried, was stained with a fat stain, sudan black The spot test indicator, *p*-dimethyl amino-benzylidene rhodanine in saturated alcoholic solution, gives a rose pink coloration with strontium The skimmed milk before and after addition of strontium, each aliquot of eluate and the three parts of the column, together with the original 'Sephadex', were all tested for the presence of strontium

A sample of whole milk was also contaminated with a detectable amount of strontium chloride, and after centrifugation the fat and skimmed milk tested for the presence of strontium

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* Present address Pathology Department, Derbyshire Royal Infirmary, Derby

Similar elutions of 1 per cent human γ-globulin in saline, strontium chloride solution and mixtures of both were carried out with phosphate saline buffer from 'Sephadex' columns

Optical density measurements on the eluates from the milk/strontium mixtures revealed those eluates in which protein could be detected and the peak concentration coincided with the eluates containing the maximum amount of fat (Fig 1) The electrical conductivity of the eluates remained unaltered, indicating an absence of any excess ion in them By comparison, the eluates (Fig 2) from γ-globulin strontium mixtures showed a peak conductivity corresponding to the presence of strontium as shown by spot testing

The skimmed milk gave a negative reaction for the presence of strontium before its addition and a positive reaction afterwards All the eluates were negative, but strontium could be detected in the washings from the upper third of the extruded column and also adherent to the gel particles which changed colour on the addition of *p*-di-methyl amino-benzylidene rhodanine 'Sephadex alone showed no reaction with this reagent When strontium was added to whole milk and then centrifuged it could only be detected in the skimmed milk and not in the fat

Although certain metals give colour reactions with *p*-di-methyl amino-benzylidene rhodanine, calcium, sodium and potassium are inert and the milk prior to the addition of strontium was negative This reagent therefore would appear suitable for testing for the presence of strontium and is quicker and easier than group analysis or flame photometry, particularly in the presence of sodium

Unskimmed milk has a viscosity too high for it to be eluted from the columns, and so it was necessary to skim the milk prior to elution from a column Phosphate saline buffer was used to avoid precipitation of protein on the column by variation in *p*H or ionic strength

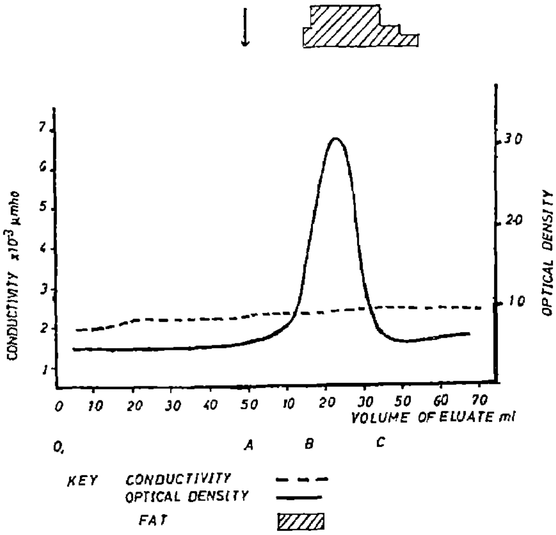

Fig 1 The elution diagram for a sample of skimmed milk to which strontium had been added The column 28 cm long × 0 6 cm diameter was of 'Sephadex *G* 50', and the eluant phosphate saline buffer The optical density of the eluates has been measured at 280 mμ for proteins and the conductivities in μmho The eluates from 0 to *A* were collected during the period of equilibration with the eluant and show constant conductivity and optical density At the point *A*, marked with an arrow, 5 ml of milk/strontium mixture were added At the point *B* to *C* the rise in optical density demonstrates the presence of protein while the shaded area demonstrates the eluates containing fat Despite continued elution, no eluates demonstrate the presence of strontium when tested with the spot test indicator, but the upper third of the 'Sephadex' column when extruded contained strontium This demonstrates the complete separation of strontium from skimmed milk

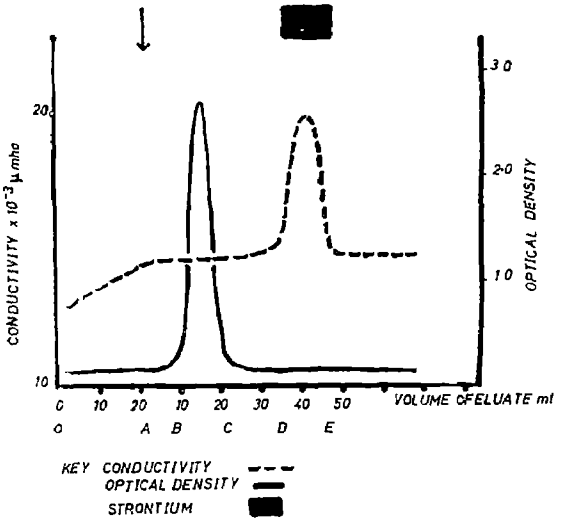

Fig 2 The elution diagram for a solution of human γ-globulin in phosphate saline to which strontium has been added to a concentration of 0 12 mgm /ml of strontium The column of 'Sephadex *G* 25' was 30 cm long × 0 6 cm diameter, and the eluant phosphate buffer *p*H 7 4 The period 0 to *A* represents the period of equilibration of the column with phosphate saline when equilibrium conductivity and optical density were attained At point *A* 9 ml of γ-globulin strontium solution were added to the column and eluted The period *B* to *C* represents the eluates containing protein as demonstrated by the optical density *C* to *D* represents the volume of eluant separating the eluates containing protein from those containing strontium This volume was equivalent to 1 column volume *D* to *E* represents the eluates containing strontium, reacting positively with the spot test and demonstrating the rise in conductivity Absence of positive spot test and equilibrium conductivity over eluates *B* to *C* demonstrate absence of strontium in these eluates

Since no strontium was detected in the eluates after elution of the milk/strontium mixtures, but was found on the upper third of the column, it would appear that gel filtration is a suitable procedure for the removal of strontium from milk However, the sensitivity of the spot test may not be sufficiently great to detect very small quantities of strontium passing into the eluates Radioisotope investigations are necessary to clarify this point since they are more sensitive The apparently complete removal of artificially added strontium in a concentration of 0 12 mgm /ml from milk is not strictly comparable to the removal of strontium which has been added to milk during its secretion by the cow, since in the latter case the amount is smaller and may be more difficult to remove

No assessment of the amount of protein-bound strontium was made, but this is likely to be low when the milk contains an excess of ionized and bound calcium If the protein-bound strontium, when present in cows' milk, is in equilibrium with ionic strontium, then removal of the ionic strontium on the column should successively displace the strontium from its protein conjugate, particularly in the presence of excess calcium It would be possible to wash the columns with calcium salts to ensure saturation prior to elution of the milk and thus aid removal of strontium

The observation that strontium is not associated with the fat after skimming but remains in the skimmed milk implies that all the strontium present in milk is potentially available for absorption on a 'Sephadex' column Had any strontium been associated with the fatty fraction skimmed off the milk it would be unavailable for absorption by a column since the fat was too viscous to traverse a column as we prepared it.

The importance of this method of removal of strontium from milk lies in the ease of handling small samples of milk to which strontium has been added and the speed with which all the strontium detectable chemically can be removed from the milk. Radiostrontium absorbed on to such a column would be readily estimated after ashing the column.

The use of this method for removing strontium and possibly similar ions from protein mixtures is demonstrated by Fig. 2, in which there was a column volume difference in the eluates between the protein and the strontium, sufficient to yield clean separation. The observation that strontium came off the column in the case of γ-globulin/strontium mixture but remained associated with the column when milk/strontium mixture was eluted may be explained by differences in the composition of the samples. The γ-globulin/strontium chloride mixtures and the milk/strontium mixtures were all eluted by phosphate saline buffer but the presence of other ions, carbohydrate and fat in milk may have caused binding of the strontium to the gel bed.

The possible benefits of gel filtration over ion exchange procedures in the analysis of the strontium present in milk are that gel filtration appears to need only one column procedure and ion exchange requires at least two. The strontium is retained on a column which is readily ashed in preparation for counting procedures in the case of gel filtration, and no preliminary separation of proteins is required.

Quantitative radioisotope investigations comparing the ion exchange and gel filtration procedures need to be carried out.

The largest volume of milk put through a column has been 5 ml., which required about 1 hr. of elution time; the factors involved in using larger volumes and the potability of the product have not been investigated.

I thank Messrs. Pharmacia, Uppsala, Sweden, for gifts of 'Sephadex', and the Endowment Fund of the United Birmingham Hospitals for a grant.

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FRACTURE REPAIR AFTER TREATMENT WITH DEOXYRIBONUCLEIC ACID FROM FISH

By Dr. E. A. TONNA and Dr. E. P. CRONKITE
Medical Research Center, Brookhaven National Laboratory, Upton, New York
AND
Dr. F. J. SANEN
Fougera and Co., Inc., Hicksville, New York

THIS investigation was stimulated by reports of Jacob[1] and Fauchet[2] claiming that marked differences were noted in resorption of the fracture clot, the osseous transformation of the cartilaginous masses, and the spreading of the hæmatopoietic tissue in the intertrabecular spaces of the new-formed bone in human and rat, in comparison with their controls, when deoxyribonucleic acid (DNA) from fish was administered. Meek and Hewer[3] reported the occurrence of duodenal adenocarcinoma in mice following treatment with fish milt-DNA. This was also reported in tropical fish (Cichlids) by Stolk[4].

In view of the importance of enhanced fracture repair in aged individuals and in cases with difficulties in bone healing, it was felt necessary to repeat the histological experiments of Jacob and Fauchet using a more precise method for evaluating enhanced fracture repair. Tritiated thymidine autoradiography offers a simple and efficient method for estimating in any given cell population the number of cells which are capable of synthesizing DNA at a given time and of undergoing mitosis afterwards[5,6].

Sixty, 6-months-old, female mice of a Brookhaven National Laboratory Swiss albino strain, having an average weight of 30 gm., were used in the experiments. The mice were divided into three groups, namely:

Group A. (12 mice.) The left femur was fractured at mid-shaft by digital pressure while the animal was under ether anæsthesia. Six mice were killed 1 week after fracture and another six at 2 weeks. These animals served as normal controls.

Group B. (12 mice.) The left femur was fractured as in Group *A*. This group received a daily subcutaneous injection of 0·25 ml. of herring-milt DNA (20 mgm./ml. diluted 1 : 10 with sterile physiological saline). A total dose of 0·5 mgm. DNA/day was administered for 7 days and then the animals were killed, 35 days after the first injection. This group served as a control against the possibility of the formation of duodenal adenocarcinoma. Immediately after death, the entire gastrointestinal tract was removed, opened, and examined along its entire length with a stereoscopic microscope.

Group C. Consisted of 36 mice. The left femur of each was fractured at mid-shaft and the mice were then subdivided into three groups, receiving 5, 10 and 20 mgm. of DNA/day in a single dose, respectively. One group of 6 mice was given 5 daily doses and killed at the seventh day. Another group of 6 was given 10 daily doses and killed after 14 days. (The DNA preparation was supplied by E. Fougera and Co., Inc., Hicksville, New York.)

The animals in Groups *A* and *C* received 0·5 μc. tritiated thymidine subcutaneously 1 hr. prior to death. The femora were fixed in cold, buffered formalin for 24 hr., washed in running tap-water for an additional 24 hr., and decalcified in a 10 per cent solution of disodium versenate. Paraffin blocks were prepared and sections cut at 5μ. One set of sections was stained routinely with hæmatoxylin and eosin, and two sets were used for autoradiographic preparations. These sections were covered with Kodak NTB_3 liquid emulsion and exposed for 16 days in a cold, dry atmosphere. The emulsions were developed then stained with hæmatoxylin.

Group A. Histological sections of this group revealed the classical picture of fracture repair. One week after fracture, the callus was essentially made up of chondrogenic cells. Some cartilage formation and early bone formation was seen at the periphery of the callus. At the edge of the fragments, early bone formation was taking place. Two weeks following the fracture, the large callus consisted essentially of cartilage, and new trabecular bone formation was seen at its periphery. Extensive bone formation was also seen at the edges of the fragments as the cartilaginous mass was being replaced.

Autoradiographs revealed extensive labelling of periosteal osteogenic cells about the fracture site. Osteoblasts, lining the newly formed trabeculæ and those present in regions where cartilage cells were being replaced by trabecular bone, were highly labelled. Fibroblasts and chondrogenic cells comprising the population of the fracture callus were frequently labelled.

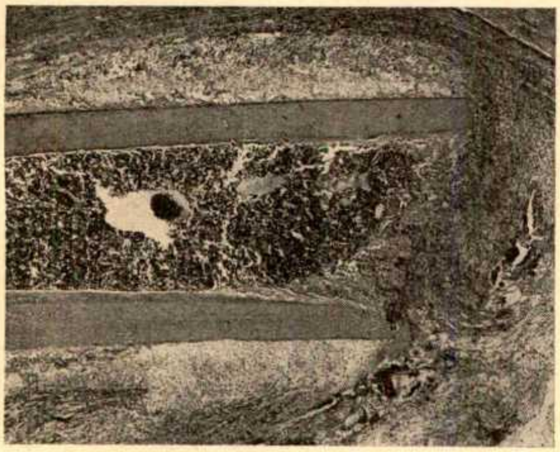

Fig. 1. A fractured femur 7 days after trauma and daily treatment with 10 mgm. herring-milt DNA. Hæmatoxylin and eosin stain. (×22·5)

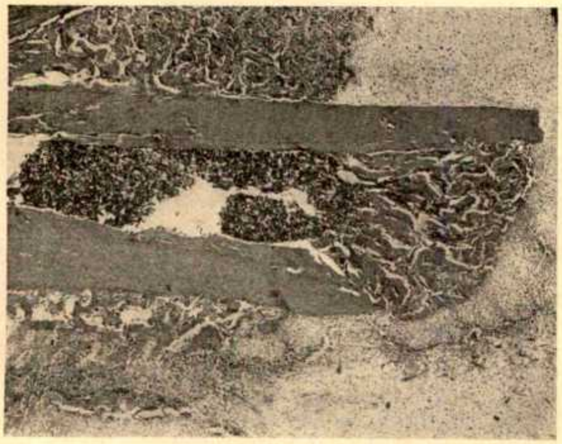

Fig. 2. A fractured femur 14 days after trauma and daily treatment with 10 mgm. herring-milt DNA. Hæmatoxylin and eosin stain. (×22·5)

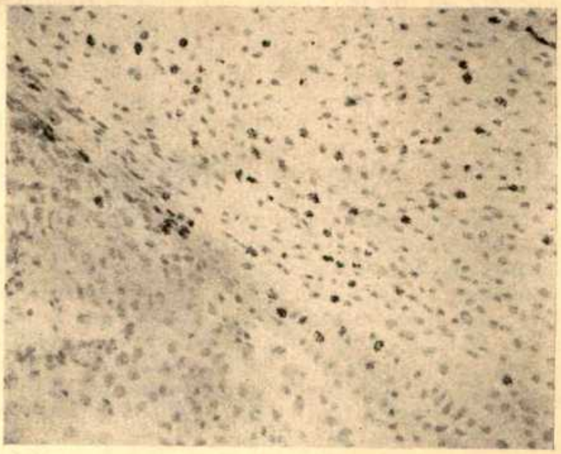

Fig. 3. An autoradiograph of a 7-day fractured femur treated daily with 10 mgm. herring-milt DNA. The photomicrograph was taken from a region of active cell proliferation and osteogenic cell transformation into cartilage cells. Although labelling is extensive, it does not represent an increase over the non-treated controls when similar areas are evaluated. Hæmatoxylin stain, ×150

Group B. Examination of the entire gut along its length with a stereomicroscope revealed neither pathology nor local hyperæmia. The gut appeared grossly normal. No pathology was seen in any of the groups.

Group C. The histological sections in this experimental group which were treated with herring DNA revealed a similar picture at 1 and 2 weeks after fracture as that found in the control Group *A* (Figs. 1 and 2). Cartilaginous callus was abundant at 2 weeks and the replacement of the callus by bone was not increased over the controls.

Autoradiographs exhibited an extensively labelled periosteum, especially at the fracture site. Numerous labelled fibroblasts and mesenchymal cells were observed. The results were similar to the controls (Fig. 3).

It has long been known that most animals do not require exogenous sources of purines and pyrimidines to meet the needs of the organism in synthesizing DNA. Dietary ammonia nitrogen was shown by Barnes and Schoenheimer[7] to be readily utilized by rat tissues in the synthesis of the purines and pyrimidines of nucleic acids. Subsequent experiments revealed that simple carbon compounds (for example, carbon dioxide, glycine, formate), served to supply the carbon atoms necessary for the synthesis of nitrogenous bases. It appears that the animal body can synthesize the necessary heterocyclic compounds at a rate which meets the needs of the organism. It is obvious that during the process of skeletal repair, an abundant supply of DNA precursors is necessary. Perhaps an adequate supply of precursor material is not available during fracture repair in older animals resulting in prolonged repair. DNA or its breakdown products which are not normally essential components for nucleic acid synthesis might under such conditions be utilized and thus result in enhanced fracture repair. Contrary to the findings of Jacob and Fauchet, our results did not show, in any of our animals, gross or microscopic evidence of enhanced fracture repair after parenteral administration of 5, 10 and 20 mgm. of herring-milt DNA and observing fractures for 1 and 2 weeks after trauma. The autoradiographs also failed to show stimulation of cell proliferation following treatment. Moreover, prolonged DNA treatment did not result in the production of adenocarcinoma of the gut or other pathology.

This work was supported by the U.S. Atomic Energy Commission.

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LINKAGE OF SIALIC ACID IN THE HUMAN ERYTHROCYTE ULTRA-STRUCTURE

By G. M. W. COOK

Department of Radiotherapeutics, University of Cambridge

THE sialic acids (*N*-acylated neuraminic acids) have been shown to be present in many mammalian tissues[1] and in bacteria[2]. In the human being, sialic acid is present in serum proteins, seminal fluid, cerebrospinal fluid and in red cells. In human red cell stroma Bial's colour reaction suggests the presence of about 1 per cent of neuraminic acid[3], and it has been found that the sialic acid liberated from human erythrocyte stroma following receptor-destroying enzyme (RDE) treatment was *N*-acetyl neuraminic acid (NANA) (ref. 4). Recently it has been shown that bound sialic acid is liberated as a sialomucopeptide from the human erythrocyte[5] following treatment with trypsin[6,7] and, using RDE (ref. 8), that the carboxyl group of a sialic acid is the dominant ionogenic group present at the surface of the human erythrocyte.

Besides contributing to the negative charge of the human red blood cell membrane, sialic acid has been shown to be associated with the M and N receptors of this cell[9]. Recently, Romanowska[10] has studied the products of mild acid and alkaline hydrolysis of M and N blood group preparations, obtained by phenol extraction of human erythrocyte stroma, and concluded that *N*-acetyl neuraminic acid may be combined with galactose or *N*-acetyl hexosamine in these biologically active materials. It is thus possible that these disaccharides form an essential part of the prosthetic group of M and N mucoids.

Since trypsin treatment destroys M and N antigenic characteristics of erythrocytes[9,11], it seems very likely that the released sialomucopeptide is closely associated with the M and/or N antigenic configuration, especially as sialic acids have been shown to have inhibitory properties towards anti-M and anti-N agglutinins[11]. In view of this and the interest in sialic acid as an important constituent of other animal cell membranes[12,13] it was decided to investigate further the nature of the carbohydrate moiety of this sialomucopeptide. Mild acid hydrolysis (0·5 *N* hydrochloric acid at 105° C. for 16 hr.) failed to show the presence of neutral sugars. However, more vigorous hydrolysis (6 *N* hydrochloric acid at 105° C. for 15 hr.) liberated large quantities of galactosamine in addition to eight different amino-acids (leucine (or isoleucine) threonine, alanine, glycine, arginine and glutamic acid and as yet two unidentified amino-acids in trace quantities). The sialosubstance would therefore appear to consist of sialic acid, galactosamine (or an acyl derivative) and an aliphatic peptide chain. Sulphur-containing amino-acids were not detected in hydrolysates of this mucosubstance.

Work on the sialosubstances has shown that sialic acid regularly occurs together with hexosamine[14] and the structures of two sialic acid-containing compounds have been elucidated, namely, milk oligosaccharide (sialyl-lactose (α-D-sialyl (2–3) β-D-galactopyranosyl (1–4) D-glucopyranose)) and the prosthetic group of bovine salivary mucoprotein (BSM), (6-α-D-sialyl-*N*-acetyl galactosamine)[15]. In view of the general analysis of the sialomucopeptide under investigation and the presence of sialic acid in BSM and associated with the disaccharides described by Romanowska, a reasonable structure for the carbohydrate moiety of this sialomucopeptide would be 6-α-D-sialyl-*N*-acetyl galactosamine. This possibility was investigated using RDE and mild alkali (treatments which degrade the carbohydrate in preference to the protein) on samples of the sialomucopeptide and a sample of purified BSM (refs. 16 and 17) used as a model. BSM is believed to consist of a protein core surrounded with *N*-acetylgalactosamine units with external end groups of sialic acid[15].

Samples of sialomucopeptide[5] were examined by paper electrophoresis[18] and those which contained only trace contaminants (di- and tri-peptides) were freeze-dried and stored under nitrogen at –25° C.

Alkaline hydrolysis of sialomucopeptide. Mucosubstance (1–2 mgm.) was treated with saturated barium hydroxide solution. The mixture was kept at 80° C. for 15 min., cooled under tap-water and acidified with *N* sulphuric acid to *p*H 6. The mixture was then centrifuged, the clear supernatant reduced to a small volume at room temperature on a watch glass in a vacuum desiccator over potassium hydroxide, and examined by paper chromatography.

Alkaline hydrolysis of bovine salivary mucoprotein. BSM (27·0 mgm.) dissolved in distilled water (5 ml.) was treated with saturated barium hydroxide solution (2 ml.) using the same procedure as already outlined except that the hydrolysate was dialysed against twice distilled water at 4° C. and the dialysate then lyophilized. The residue thus obtained was taken up in a few drops of distilled water for examination by paper chromatography.

Preparation of chromogen I and chromogen III. *N*-acetyl hexosamine (200 mgm.) was treated with 0·02 *N* barium hydroxide solution using the hydrolysis technique already described. The lyophilized dialysate was examined by paper chromatography (Whatman No. 1 paper, butan-1-ol/pyridine/water 6 : 4 : 3 v/v descending solvent[15]). Development with Ehrlich reagent showed the presence of chromogen I (anhydro-*N*-acetyl hexosamine). A quantity of chromogen I (6 mgm.) was treated with 0·1 *N* sodium carbonate solution (1 ml.) at 80° C. for 30 min. and then cooled and brought to *p*H 6 with 0·1 *N* hydrochloric acid solution. After electrolytic desalting and reduction of volume under reduced pressure paper chromatography showed the presence of chromogen I and chromogen III (5-dihydroxyethyl-3-acetamidofuran) in the solution This mixture, which was stored at $-25°$ C, was used as a standard marker mixture in the subsequent paper chromatography[15]

The various components present on the chromatograms were detected using characteristic reactions Where necessary the paper was cut into various strips if more than one reagent was to be used *N*-acetyl neuraminic acid was detected using orcinol–trichloroacetic reagent[8] Chromogens I and III were detected using Ehrlich reagent (*p*-dimethylamino benzaldehyde (500 mgm) dissolved in a mixture of ethanol (15 ml) hydrochloric acid (15 ml conc), butan-1-ol (90 ml) in the cold. Other components (hexosamines) were detected using indirect Ehrlich reagent This involved pre-treating the strip with alkaline acetyl-acetone reagent (reagent I· acetyl acetone (b p 138°–140° C (0 5 ml) in butan-1-ol (50 ml), reagent II aqueous potassium hydroxide (5 ml. 50 per cent v/v) in ethanol (20 ml). Reagent II (1 ml) was mixed with reagent I (20 ml) a few minutes before use and then the strip heated at 105° C for 5 min before treating with Ehrlich reagent at 90° C for 5 min

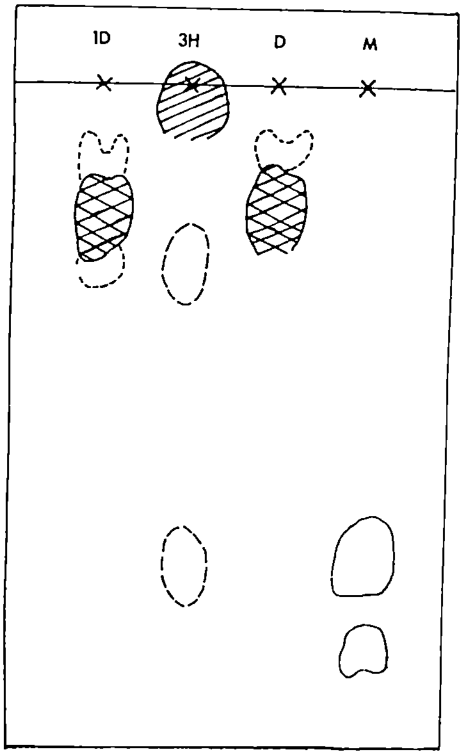

Fig 1 Tracing of chromatogram of products of alkaline hydrolysis of erythrocyte sialomucopeptide

1*D*, commercial sample of NANA, developed with orcinol reagent, sample shows traces of *N*-glycolyl and methoxy derivatives, 3*H*, alkaline hydrolysate of sialomucopeptide developed with indirect Ehrlich reagent and showing presence of sialyl-*N*-acetyl galactosamine, galactosamine and chromogen I, *D*, sample of NANA containing *N*-glycolyl neuraminic acid developed with orcinol reagent, *M*, marker mixture of chromogens I and III developed with Ehrlich reagent in the cold

Paper Whatman No 1 Solvent, butan-1-ol/pyridine/water (6 4 · 3 v/v)

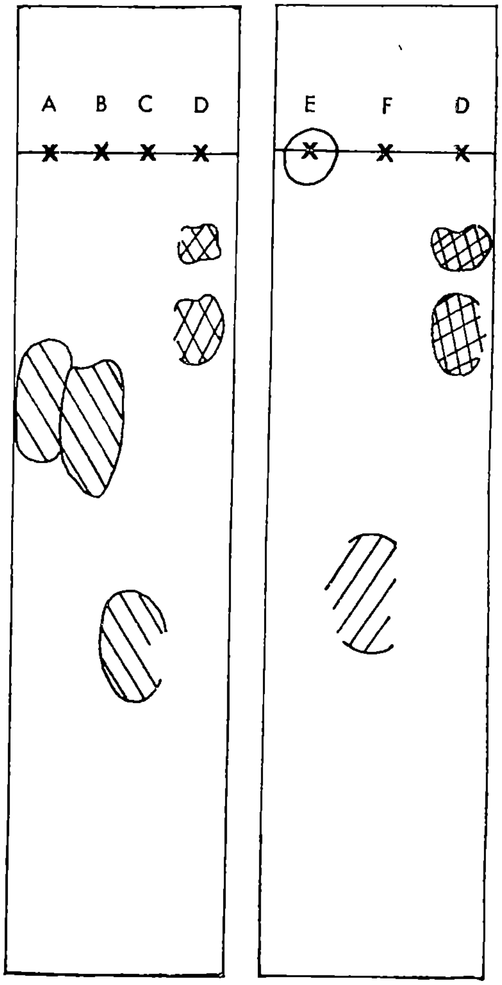

Fig 2 Tracing of chromatogram of standard amino sugars and sialic acids

A, Galactosamine, *B*, glucosamine, *C*, *N*-acetyl glucosamine, *F*, *N*-acetyl galactosamine, all developed with indirect Ehrlich reagent, *D*, sample of NANA containing *N*-glycolyl neuraminic acid developed with orcinol reagent, *E*, sample of sialomucopeptide after alkaline hydrolysis showing slow-moving peptide Developed with ninhydrin (5)

Paper Whatman No 1 Solvent, butan-1-ol/pyridine/water (6 4 3 v/v)

Paper chromatography of the alkali-treated mucopeptide showed the presence of a compound of low R_F value giving a reddish purple colour with indirect Ehrlich reagent, typical of sialyl-*N*-acetylgalactosamine[15] (Fig 1) contaminated with peptide which, being immobile in the solvent (Fig 2), caused a slight brown discoloration on the lower edge Spots corresponding to galactosamine and chromogen I were also detected Similar results were obtained after alkaline treatment of BSM (Fig 3) Free *N*-acetyl neuraminic acid was detected[19] in acid hydrolysates (0 1 *N* sulphuric acid at 80° for 1 hr) of both erythrocyte mucopeptide and from sialyl *N*-acetylgalactosamine (prepared as an eluate from chromatogram of alkali-treated BSM (Fig 3) and also following RDE treatment, thereby indicating that the sialic acid in the sialomucopeptide was linked terminally and α-ketosidically Direct quantitative determination of hexosamine[20,21] after acid treatment was not possible in view of the small amounts available Since hexosamines and their *N*-acetyl derivatives have been shown not to interfere with the estimation of sialic acid by Warren's method[19] one can calculate the amount of hexosamine, assuming that the ratio of sialic acid to hexosamine in erythrocyte sialomucopeptide is the same as that in sialyl-*N*-

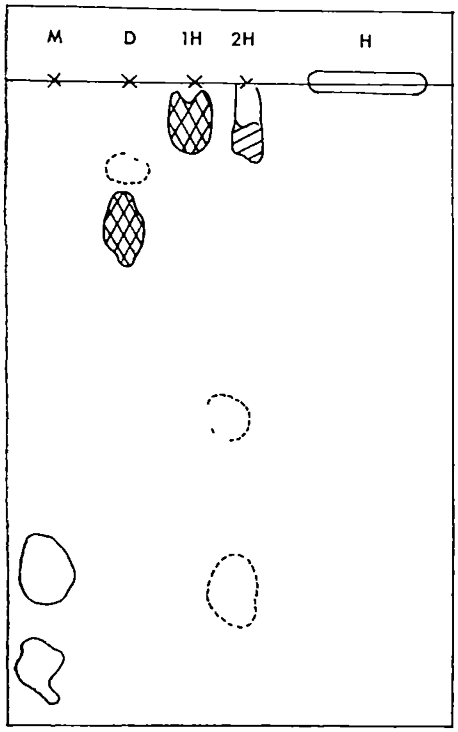

Fig 8 Tracing of chromatogram of products of alkaline hydrolysis of BSM

M, marker mixture of chromogens I and III developed with Ehrlich reagent in the cold, *D*, sample of NANA containing *N*-glycolyl neuraminic acid developed with orcinol reagent, 1*H*, alkaline hydrolysate of BSM developed with orcinol reagent and showing presence of sialyl-*N*-acetyl galactosamine, 2*H*, alkaline hydrolysate of BSM developed with indirect Ehrlich reagent and showing presence of sialyl-*N*-acetvl galactosamine, very slight trace of *N*-acetyl galactosamine, and a trace of chromogen I, *H*, alkaline hydrolysate of BSM, untreated Region corresponding to sialvl-*N*-acetyl galactosamine eluted for further experiments

Paper Whatman No 1 Solvent, butan-1-ol/pyridine/water (6 4 3 v/v)

acetyl galactosamine Mixtures of sialic acid (*N*-acetyl neuraminic acid) and galactosamine were made up, the amounts present being calculated as already explained The mixtures were then subjected to the same acid treatments as the sialomucopeptide before evaluating optically the chromophore produced by such a mixture in the Rondle Morgan hexosamine determination The same type of experiment was performed using the eluate of sialyl-*N*-acetyl galactosamine In all these experiments there was good agreement between the actual optical density reading in the experiment and the predicted optical density reading obtained from standard mixtures

The foregoing experiments suggest that sialic acid present in a sialomucopeptide liberated by the action of trypsin on the human red blood cell ultrastructure is linked α-ketosidically to a galactosamine (most probably the *N*-acetyl derivative judging from the presence of chromogen I in the hydrolysate) residue in an alkaline labile saccharide moiety Comparing the behaviour in the above experiments of erythrocyte sialomucopeptide with that of BSM, a known source of 6-α-D-sialyl-*N*-acetyl galactosamine[15], it would appear that the saccharide moiety (or moieties) in this mucopeptide is of similar structure to the prosthetic group of BSM From Romanowska's work[10], it is evident that such a chemical structure is of possible importance in the M and N blood group substances as well as *N*-acetylneuraminyl-galactose It is therefore interesting that a mucosubstance liberated from the native red cell by trypsin, which results in the concomitant loss of some of this cell's M and N character[11]), contains a chemical structure similar to that in the M and N mucoids obtained by phenol extraction of human erythrocyte stroma Further, this result lends weight to the suggestion[5] that structures analogous to those present in ovine submaxillary gland mucoprotein[22] are an important feature of the architecture of the human red blood cell ultrastructure

I thank the Medical Research Council for financial assistance, Dr. D H Marrian for his advice on the preparation of the manuscript, Prof J S Mitchell, Dr D. H. Heard and Dr G V F Seaman for their interest in this work I also thank Prof Ward Pigman for a gift of purified BSM and Mr E. W. Mitchell and Miss C A Cavanagh for preparing the chromatograms for photography.

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AEROBIC RESPIRATION IN SOIL CRUMBS

By Dr D J. GREENWOOD and G. BERRY

National Vegetable Research Station, Wellesbourne

In previous work[1] a theory was developed to relate the aerobic respiration-rates of spherical soil crumbs to their size and the oxygen partial pressure surrounding them The object of this communication is to correct an error in the formulation of the theory and to extend it to describe the respiration-rates of irregular non-spherical crumbs

Consider a sphere of soil saturated with water and containing a uniform distribution of micro-organisms Let M be the aerobic respiration-rate Then M can be taken[1] as constant and independent of oxygen concentration for $C \geqslant C_0$ and zero for $C < C_0$ Suppose the oxygen concentration is C at a distance r from the centre of a sphere of radius R_1 and D is the diffusion constant of oxygen through the sphere Then for the stationary state

$$\frac{D}{r}\frac{\partial^2(rC)}{\partial r^2} = M \qquad (1)$$

The boundary conditions are

$$C = C_1 \text{ for } r = R_1$$

$$\frac{\partial C}{\partial r} = 0 \text{ for } r = R_0$$

where the sphere is anaerobic for $r < R_0$ so that $C = C_0$ for $r = R_0$

On solving (1) and putting $r = R_1$ we have

$$6D(C_1 - C_0) = M(R_1^2 + 2R_0^3/R_1 - 3R_0^2) \qquad (2)$$

By polarographic techniques similar to those used by Longmuir[2] we found that the respiration-rate of soil and yeast organisms did not decrease by lowering of the oxygen concentration until very low values were reached The oxygen concentration causing a 50 per cent inhibition of respiration was about 5×10^{-6} M Hence, as a close approximation we have

$$6DC_1 = M(R_1^2 + 2R_0^3/R_1 - 3R_0^2) \qquad (3)$$

By taking K as the respiration-rate expressed per unit of total volume including the part in which $C < C_0$

$$6DC_1 = MR_1^2(3 - 3(1 - K/M)^{2/3} - 2K/M) = MR_1^2 f(K/M) \text{ say} \qquad (4)$$

this equation replaces equation (4) of the previous paper[1]

If Penman's[3] formula $D = 0\ 66D_0S$ (where S is pore space and D_0 is the diffusion constant of the material in the pore space) holds for liquids as well as for gases equation (4) becomes

$$3\ 96D_0SC_1 = MR_1^2 f(K/M) \qquad (5)$$

Since other work[4] suggests that the interfacial resistance between the liquid and the gas phase is small, C_1 can be taken as the oxygen concentration in water which is in equilibrium with the oxygen partial pressure surrounding the crumbs

Equation (5) was tested by measuring the respiration-rates of moist moulded spheres of soil (radius 0 3–0 5 cm) when they were surrounded with oxygen partial pressures between 0 and 16 cm of mercury The results are shown in Fig 1 C is in cm of mercury, S and R^2 were calculated from weight, specific gravity, and moisture content determinations by assuming that the pore space was completely filled with water and that there were no gas-filled pores For equation (5) to hold the points in the figure should fit on a straight line passing through the origin Since the diffusion constant of oxygen through water calculated from the gradient of the line is $2\ 6 \times 10^{-5}$ cm ^{2} sec $^{-1}$ and that quoted by Millington[5] is $2\ 38 \times 10^{-5}$ cm ^{2} sec $^{-1}$ it appears that equation (5) is approximately valid

Surface areas of different-sized crumbs obtained from sieved soil were determined by measuring the mean chord-lengths on random transects through the crumbs If t is the mean chord length, A the surface area and V the volume of the crumb, which is assumed to be approximately convex, then t is equal to $4V/A$ (ref 6) The mean value obtained for the shape factor $A/V^{2/3}$ was 6 06 compared with 4 84 for a sphere

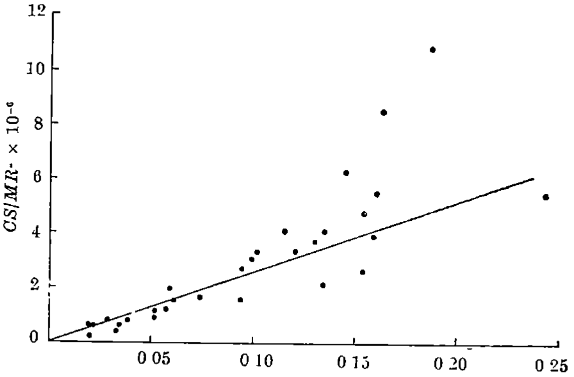

Fig 1

Soil aggregates of irregular shape and spheres having the same A/V ratios might be expected to have similar diffusion characteristics Thus, for any irregular shape we can define an equivalent spherical radius given by $R_1 = 3V/A$ Substituting in equation (4) we have

$$2DC_1A^2 = 3MV^2 f(K/M) \qquad (6)$$

It was hoped to test the validity of this equation by cutting or moulding shapes of soil to known A/V ratios and then finding the effect of oxygen partial pressures on their respiration-rates However, with such a technique there were large variations in replicate determinations of K/M Because we found that the effect of oxygen concentration on the respiration of yeast was similar to its effect on the respiration of soil organisms, errors were minimized by using shapes prepared from agar, yeast and nutrient solution (2 per cent glucose, 0 1 M phosphate buffer pH 6 8) in place of shapes prepared from soil For each of the different shapes, the points obtained by plotting $f(K/M)$ against C gave a good fit to a straight line passing through the origin Values for D were calculated from the gradient of the straight lines, the A/V ratios, and equation (6) by taking the solubility of oxygen in agar to be the same as in water If equation (6) were correct the values for D obtained with the different shapes would be the same irrespective of their different shape factors Table 1 shows that for a limited number of shapes the diffusion constants were roughly independent of shape factor Thus the table, when taken in conjunction with the shape factor determinations on soil crumbs, provides limited evidence that the equation applies to the shapes found in soil

Table 1. Estimated Diffusion Constants of Agar Shapes

Material	$A/V^{2/3}$	$D \times 10^5$
Agar shapes	6 47	1 20
,, ,,	5 70	1 07
,, ,,	5 69	1 02
,, ,,	5 54	0 99
,, ,,	6 57	1 08
Soil crumbs	6 06	—
,, spheres	4 84	—

Previously reported measurements[1] of the aerobic respiration-rates of soil crumbs of 2–4 mm diameter when moistened in Warburg flasks and maintained under different oxygen partial pressures were lower than those obtained theoretically from equation (6) It may be that in the theoretical determinations, since no account was taken of any reduction in the surface area of the crumbs by contact of the crumbs with each other and the bottom of the flasks, the values that were taken for the surface areas were too high, and, in consequence, the values for K/M were also too high

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FLAVONOID PRODUCTION BY MATURE CITRUS FRUIT TISSUE PROLIFERATING *IN VITRO*

By Dr. HERBERT A KORDAN and Dr L MORGENSTERN

Department of Surgery, Cedars of Lebanon Hospital,
Los Angeles

FLAVONOID compounds of higher plants have received considerable attention from diverse fields of study[1-9] Their biological role, however, is for the most part unknown[2,7,10] with the possible exception of lignin formation[7] and attracting insects for pollination[2,7,10]

Vesicle stalks from mature lemon fruits (*Citrus limon* (L) Burmann, var Eureka) proliferating *in vitro*[11] frequently release a white water-insoluble material to the external medium Sub-cultures and straight transfers of proliferating tissue invariably release this material in noticeable quantities This article presents evidence that the water-insoluble material contains flavonoids which are continually synthesized by the proliferating tissue.

Mature lemon fruits contain the flavanone glycoside hesperidin[12] which behaves like a true ketone forming oximes, phenylhydrazones, dinitrophenylhydrazones, ketazines and semicarbazones[13] On the basis of this information, ethyl alcohol (95 per cent) extracts of normal non-proliferating vesicle stalks, senescing non-proliferating stalks, proliferating stalks and alcoholic solutions of nutrient medium containing the water-insoluble material were subjected to a 2,4-dinitrophenylhydrazine test for carbonyl compounds (Table 1)[14] The results in Table 1 indicate that the water-insoluble material contains a carbonyl group(s) and that it was derived solely from the proliferating tissue Autoclaved freshly prepared nutrient solution gave a negative test with the carbonyl reagent as did ethyl alcohol alone (controls) The largest quantity of extractable material was obtained from the proliferating tissue whereas the largest amount of dinitrophenylhydrazone derivative was obtained with the water-insoluble material present in the nutrient medium which had supported the growth of sub-cultures and transfers of proliferating tissue The quantitative increase in the carbonyl compound(s) appear to be the result of growth of tissue rather than senescence

Living non-proliferating vesicle stalks are translucent in appearance When exposed to ammonia vapour from concentrated aqueous ammonium hydroxide solutions, the tissue becomes a bright yellow in colour Proliferating tissue also turns bright yellow when exposed to ammonia fumes This test is sensitive to polyphenolic carbonyl compounds represented by flavones, flavanones, chalcones and xanthones[2] The addition of concentrated ammonium hydroxide to nutrient solution containing the water-insoluble material causes the immediate production of a yellow solution which fluoresces markedly under short-wave ultra-violet light (253 mμ) The addition of 1 0 N sodium hydroxide to the medium results in the same effect as obtained with ammonium hydroxide The appearance of a yellow coloration on the addition of alkali can serve as a substitute for the ammonia test described here[2] The positive test with ammonia on proliferating tissue demonstrates that a flavonoid compound(s) is continually synthesized by the growing tissue The positive alkali test indicates that the water-insoluble material contains a flavonoid(s) the same or similar to those present in the normal and proliferating tissue

The addition of 0 5 ml of 95 per cent ethyl alcohol to each vial containing nutrient solution plus the water-insoluble material results in the complete solution of the latter A number of qualitative colour reactions for flavonoid compounds[2] were performed on the ethyl alcohol solutions containing the unknown(s) In all cases, ethyl alcohol solutions of freshly prepared and autoclaved nutrient medium

Table 1 Results of the 2,4-Dinitrophenylhydrazine Test for Carbonyl Compounds

Alcohol extract	Reaction
Normal stalks	+
Senescing stalks	+
Proliferating stalks	+3
Medium supporting tissue growth	+5
Alcohol (95 per cent)*	—
Autoclaved freshly prepared medium*	—

* Controls

Note —=Negative reaction, +=positive reaction +=quantity present in normal tissue +3=three times the normal, +5=five times the normal

Table 2 Colour Tests for Flavonoid Compounds

Test solution	Reagent	Reaction	Colour
Alcohol + unknown	HNO_3 (con)	+	Blue-green
,, + medium*	,,	−	Colourless
,, + unknown	NH_4OH (con)	+	Yellow
,, + medium*	,,	−	Colourless
,, + unknown	NaOH (1 0 N)	+	Yellow
,, + medium*	,,	−	Colourless

* Controls consist of 95 per cent ethyl alcohol plus freshly prepared autoclaved nutrient medium

Con , concentrated acid or base , − =negative reaction , + =positive reaction

plus the test reagent served as controls Three tests were always positive for flavonoids (Table 2)

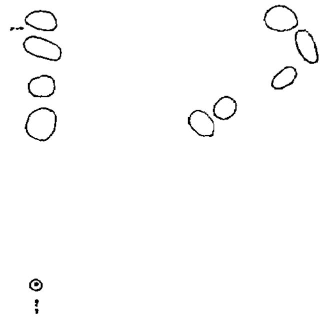
A spot of ethyl alcohol solution of the unknown placed on Whatman No 1 filter paper sheets (10 in × 10 in square) fluoresces strongly under short-wave ultra-violet Exposure of the spotted paper to ammonia fumes markedly increases the fluorescence of the spot. Two-dimensional ascending paper chromatography[15] of the ethyl alcohol solution, using water saturated with *n*-butanol plus ammonium hydroxide to *p*H 11 as the solvent for the first run and water saturated with *n*-butanol as the solvent for the second run, reveals five fluorescent spots (Fig 1) Fluorescence of the spots is increased by exposing the chromatogram to ammonia fumes An alcohol extract of normal non-proliferating stalks spotted on chromatographic paper is non-fluorescent. The addition of alkali to the alcohol solution results in the immediate production of a yellow solution which is strongly fluorescent Autoclaved freshly prepared nutrient solution (control) is non-fluorescent

Living normal non-proliferating vesicle stalks as well as other tissues of the mature lemon fruit do not exhibit fluorescence under short-wave ultra-violet The living proliferating tissue *in vitro*, however, manifests marked spontaneous fluorescence This capacity for spontaneous fluorescence appears to be restricted to the dividing and/or growing cells Living non-dividing regions of a proliferating stalk appear as non-fluorescent material Fluorescence of the proliferating tissue demonstrates that the material released to the medium is produced by the dividing and/or growing cells

Stems and roots of germinating seeds of lemon, citron (*Citrus medica* (L)) and sour orange (*Citrus aurantium* (L)) are spontaneously fluorescent under short-wave ultra-violet light Two-dimensional chromatography of alcoholic extracts of the intact stems of citron and sour orange seedlings using the solvent systems described here reveal fluorescent spots, some of which appear to be the same or similar to those from the proliferating fruit tissue (Figs 1, 2 and 3)

The relationship between tissue fluorescence and cell division and growth in the present *in vitro* system is not known It is interesting to note, however, that synthetic carcinogens such as 3,4-benzpyrene, 3-methylcholanthrene and other chemically related compounds and a number of plant growth regulators[9] are fluorescent compounds. Further studies on the nature of this water-insoluble material and the flavonoids and their relationship to the spontaneous growth of mature citrus fruit tissue *in vitro* are in progress

This work was supported by a grant from the National Institutes of Health and Cedars of Lebanon Hospital Surgical Research Funds



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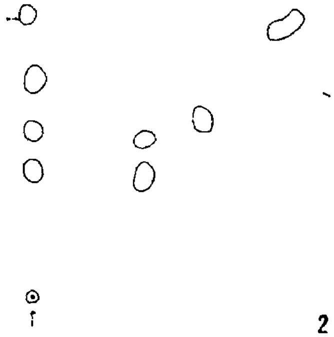

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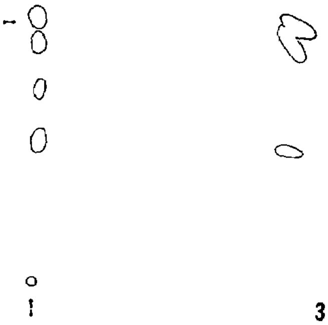

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Fig 1 Two-dimensional chromatogram of an alcoholic solution of the white water-insoluble material released by proliferating lemon fruit tissue *in vitro*

Fig 2 Two-dimensional chromatogram of an alcoholic extract of intact stem tips from germinating citron seeds

Fig 3 Two-dimensional chromatogram of an alcoholic extract of intact stem tips from germinating sour orange seeds

All spots shown on these chromatograms fluoresce under short-wave ultra-violet light (253 mμ)

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LETTERS TO THE EDITOR

RADIOPHYSICS

Brightness Distribution of the Radio Source 14*N*5*A*

MANY radio sources are now known to have brightness distributions differing markedly from a radially symmetrical gaussian distribution[1,2] Consequently the detailed investigation of individual radio sources by long baseline measurements has become particularly significant The normal techniques which involve the moving of an outstation aerial to many sites at distances comparable with the maximum length of the baseline are difficult to apply with baselines of the order of 100 km A further extension has therefore been made of the tracking technique[3,4] in which the motion of the source across the sky is used to change the effective length of the baseline and its position angle relative to the source This communication describes the investigation of the radio source 14*N*5*A* by this method This source is of particular interest since it is the most distant source to be identified with an optical object

The observations were made with the 250-ft radio telescope at Jodrell Bank together with a small steerable broadside array having a collecting area of 36 m.² at the distant station These were used in conjunction with equipment which continuously adjusted phase and compensating delay and so enabled fringes to be obtained without interruption for a period of several hours The results of the observations on the radio source 14*N*5*A* are shown plotted against hour angle in Fig 1, where the flux scale has been derived on the assumption that the source 3*C* 48 is unresolved at 61,100λ and has a flux density of 50×10^{-26} W m.$^{-2}$(c/s)$^{-1}$ at the observing frequency of 158·6 Mc/s

When a radio source is observed for a period of many hours using an interferometer with a baseline which is nearly east–west and horizontal, it can be shown that the results are equivalent to observations of the source in the zenith with an interferometer, one aerial of which is fixed while the other is moved continuously along an elliptical path The particular ellipse obtained for the radio source 14*N*5*A* with the present baseline is shown in Fig 2 in which the dimensions are in wave-lengths and the fixed aerial is at the origin If results were available from many different baselines a contour map of fringe amplitude could be plotted on this diagram and then the results in Fig 1 would represent a section along the ellipse through these contours It is found that the contour diagrams calculated for any source model consisting of a single component, even if this is an elliptical ring, cannot lead to the results shown in Fig 1, and it is necessary to resort to a two-component model If the components are of equal intensity and of negligible size the contour diagram which could be plotted on Fig 2 consists of parallel 'ridges' of high fringe amplitude which are separated by 'valleys' of zero fringe amplitude One of the ridges must pass through the origin and if this ridge can be identified at its crossing of the ellipse, the major axis of the source is defined approximately The maximum at an hour angle of 20 hr cannot be the origin ridge because the maximum at 3 hr would then be closer to a valley than its observed amplitude would allow The maximum at 3 hr must therefore come from the origin ridge and, since the zeros are symmetrical about the origin they must lie along the dotted lines shown in Fig 2 Thus the two components must be 4″ of arc apart in position angle 135° Then to account for the large fringe amplitude at 20 hr compared with the smaller one at 3 hr the components must be less than 1″ of arc along a line joining them but 1·7″ of arc in length perpendicular to this line

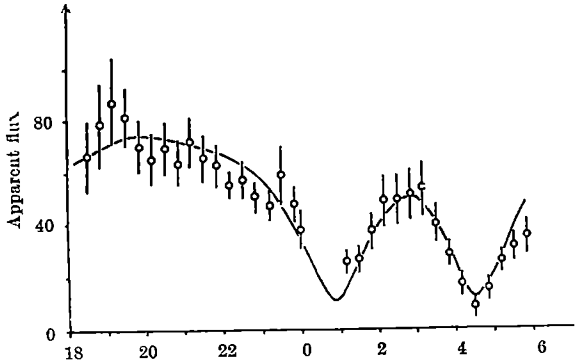

Fig 1. Observations of the radio source 14*N*5*A* at different hour angles, with an interferometer of aerial separation 61,100λ Each point is the mean value over 20 min with its standard deviation

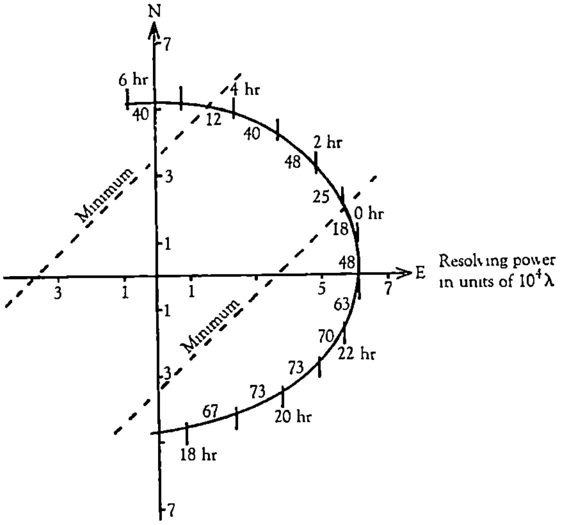

Fig 2 A diagram showing the resolving power, at different hour angles of observation, of an interferometer with aerials 61,100λ apart The elliptical line is the path followed by the tip of a vector from the origin, the direction of which gives the position angle of the base-line relative to the source 14*N*5*A* The length of this vector is proportional to the effective resolving power in that direction The figures outside the elliptical line give the hour angle of observation, while the figures within the line are computed values of fringe amplitude for the double source model proposed

The curve shown in Fig 1 is the calculated fringe amplitude for this model with the major axes of the components perpendicular to the line joining them and the flux from one component equal to two-thirds of the flux from the other

The radio source 14*N*5*A* has been identified with the brightest member of a very distant cluster of galaxies[5] with a red-shift ($d\lambda/\lambda$) of 0 47 Thus, assuming Hubble's constant to be 100 km /sec /Mps , and assuming a cosmological model, the projected component sizes can be calculated[6] and are given in Table 1

Table 1 PROJECTED DIMENSIONS OF 3C295 FOR TWO COSMOLOGICAL MODELS

	Steady state	Einstein—de-Sitter
Component separation	20 kps	16 kps
Major axis of component	8 kps	7 kps

The corresponding calculations for the Cygnus-*A* source give 69 kps for the component separation and 35 kps for the length of the major axes of the components, these values being nearly independent of the cosmological model assumed Thus it is seen that the projected size of the Cygnus-*A* source is between 3 5 and 4 5 times the projected size of the source 14*N*5*A* There is also the further difference that the major axes of the components of the Cygnus source are parallel to a line joining the two components It is possible to construct many three-dimensional source models each of which, when observed in different orientations, will appear similar both to Cygnus-*A* and to 14*N*5*A* For example, two parallel cigar-shaped components each 37 kps long inclined at an angle of 20° to the line joining their centres would resemble Cygnus-*A* when observed along a direction in the plane of the source, and 14*N*5*A* from a direction above this plane when observed at a small angle to the line joining the components The appearance of the source changes rapidly as the direction is altered in this latter case, and hence there is a low probability that this interpretation is correct It seems more likely that there are real differences in the structure of these two sources and these would appear to be large enough to mask cosmological effects, even with recessional velocities up to half the velocity of light

We thank L R Allen for help with these observations and the maintenance of the equipment for the baseline of 61,100λ We wish also to acknowledge the award of a Leverhulme fellowship (B R) and a Department of Scientific and Industrial Research maintenance grant (B A)

B ANDERSON
H P PALMER
B ROWSON

Nuffield Radio Astronomy Laboratories
(University of Manchester),
Jodrell Bank, Cheshire

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PHYSICS

Charge Ratio of High-Energy Cosmic Ray Muons

RESULTS are given in this communication of the ratio of the number of positively charged to negatively charged muons in the vertical cosmic radiation at Durham (200 ft above sea-level), in the momentum range 5–1,000 GeV /c The measurements were made with the Durham cosmic ray spectrograph which consists of a large electromagnet with layers of Geiger counters and neon flash-tubes as the detecting elements[1,2] The measurements used in the present analysis were made by us as part of our work on the determination of the high-energy spectrum[2], the results refer to the period November 1959–March 1960 Instrumental bias was reduced by operating with frequent reversals of the magnetic field

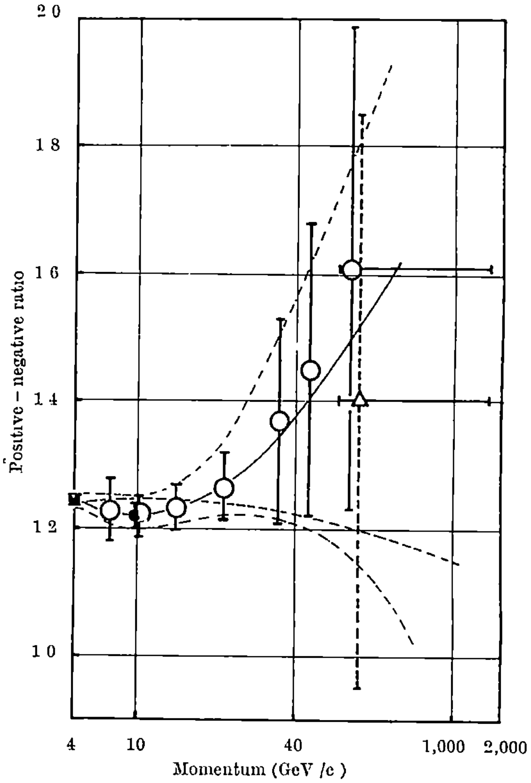

Fig 1 The measured charge ratio of muons ——, best line, – – –, confidence limits, – – –, pion fluctuation model, ●, Geiger counter data, ○, △, flash-tube data

The results on the charge ratio are given in Fig 1 and Table 1 Those in Table 1 and most of the results in Fig 1 refer to observations with the flash-tube arrays With the exception of the two ratios at the highest momentum the values are all independent The ratio shown with a dotted error line is derived from a restricted sample of the particles in the highest momentum band, selected so as to give the highest attainable accuracy in momentum determination For these particles the maximum detectable momentum is 657 ± 112 GeV /c

Small corrections have been applied for the effect of protons using the results of Brooke *et al* [3], the maximum correction to the ratio is 1 2 per cent at 6 7 GeV /c Further corrections, allowing for random errors of measurement, are significant only at the highest momentum where they amount to increases of

Table 1 THE MEASURED CHARGE RATIO OF MUONS

Median momentum (GeV /c)	Charge ratio	Statistical error (*S D*)
6 7	1 229	0 049
10 4	1 223	0 038
17 5	1 233	0 037
35	1 268	0 051
77	1 37	0 16
120	1 45	0 23
240 *	1 61	0 38
240 *	1 40	0 45

* The measurements made at this momentum have been made by two different methods and are not statistically independent

11 and 6 per cent in the ratio measured by the two methods

Also shown in Fig 1 are the ratios for the two highest momentum categories for which the Geiger counter data were used Since these results come from a previous experiment carried out by Gardener *et al* [4], in the period March–September 1958 they can be regarded as largely independent of the present results There is good agreement over the common momentum-range

An attempt is made in Fig 1 to show confidence limits, based on the statistical standard deviation, outside which it would appear that the ratio is unlikely to lie The line giving best fit to the data is also shown, and this indicates a continuous increase in the ratio with increasing momentum

Comparison of the present work with other results (for example, the review of Holmes *et al* [5]) shows that there is general agreement up to about 20 GeV /c Above this momentum most other measurements are of lower statistical accuracy, having come from instruments of lower momentum resolution The measurement at the highest momentum reported previously is that of Holmes *et al* [5], in which a ratio 1 02 ± 0 14 at a mean momentum of 98 GeV /c was found, suggesting a much more rapid fall off than found here

The measured charge ratio can be used to give information on the nature of the interactions in which the parents of the muons are produced Two cases will be considered first, that the majority of muons come from pions which are generated directly in the interactions of the primary cosmic rays (or their secondaries), and secondly following a recent suggestion by Peters[6], that an intermediate hyperon plays a predominant part at high momenta

In the first case, an approximate analysis is made in which the effects of secondary collisions are neglected Assuming that the multiplicity, M, of pion production varies with the energy of the primary particle, E_p, as $M = BE_p^{\alpha}$, and, using mean values of B and α taken from direct measurement with nuclear emulsions, the predicted charge ratio of muons is derived This proves to be much nearer unity than observed The importance of fluctuations in multiplicity from one interaction to another has been noted by Pine *et al* [7], and detailed calculations have been made by Hayman[8] The calculations show that when allowance is made for the fluctuations, the predicted ratio is closer to the ratio observed in the range 5–10 GeV /c , although the predicted decrease in ratio with increasing momentum is still too rapid It is possible to obtain the variation shown in Fig 1 as the dotted line, which is just consistent with the observations, if it is assumed that the fluctuations in multiplicity become greater as the primary energy increases, there is in fact some evidence for such an increase in fluctuations from the emulsion results reviewed recently by Perkins[9]

The second case has been examined by Babu and Yash Pal[10], who have shown that the hyperon model predicts a charge ratio of 0 54 at 100 GeV /c and still lower values at higher momenta, that is, there is a negative charge excess Such predictions are inconsistent with the present data The same authors also point out that the negative excess probably could be removed if the probability of a Σ^+-hyperon emerging from a high-energy interaction were much greater than that for other hyperons

It is concluded that the present measurements of the charge ratio of muons at sea-level are consistent with a pion source in which the fluctuations increase with energy The results are inconsistent with the predictions of the hyperon model as originally proposed by Peters

We thank Prof G D Rochester for his interest in the work

Acknowledgment is made to the Department of Scientific and Industrial Research for the award of special research grants and the award of a research studentship and research fellowship to one of us (P J H) We thank our colleagues in the spectrograph group, in particular Messrs G Brooke and F E Taylor, for their help

P J HAYMAN
A W WOLFENDALE

Department of Physics,
Durham Colleges,
University of Durham

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Foundations of Relativistic Quantum Mechanics

THE fundamental role of Dirac's equation is well known, by demanding relativistic invariance of a first-order equation you get two of the most essential properties of a particle, namely, spin and the existence of an anti-particle So long as one thinks of groups acting on co-ordinate space no significant generalization of this formalism is possible in existing quantum theory

However if one examines the proof of invariance[1] the essential step consists in assuming that the infinitesimal transformation

$$x' = AxA^{-1}$$

on E_4, of the Lorenz group B_2, induces a transformation

$$\psi' = A\,\psi$$

on S_4, the space of spinors But this assumption is not necessary and even has the appearance of arguing in circles That is one could have just as well a larger group G acting on the combined space $E_4 + S_4$, leaving invariant a function $f(x, \psi)$ which could be considered the Lagrangian of a wave equation

In fact, it follows from a geometrical study of E Cartan[2], there is just one possibility for G, namely, the exceptional simple Lie group F_4 which acts as an orthogonal group on E_9 and also on its space of spinors S_{16} leaving invariant a trilinear form[3] $f(\chi, z, \psi)$

One may think that the 8 + 1 dimension is an embarrassment, but this is not at all the case since it is known that quantum mechanics admits a consistent interpretation as a group over classical phase space[4,5] in which some of the co-ordinates z have character of hidden variables As a consequence we get two additional spin characters for a particle (since F_4 is of rank 4). To see what they may be we have to note the property of F_4 which makes it unique.

The space S_{16} is the non-Arguesian plane over the hypercomplex number field of octonions[3]. The non-Arguesian property which makes it impossible to increase the number of dimensions[6] is a consequence of the non-associativity of octonions. In view of this we may expect the wave equation derived from $f(\chi, z, \psi)$ not to be self-adjoint, that is

$$(\chi H)\psi - \chi(H\psi) = k_0 \neq 0 \qquad (3)$$

which states that the probability of creation of matter is greater than that of anti-matter. This with k_0 cosmologically small (say $k_0/k = 10^{-40}$, $k = m_p c/h$) is the basic postulate of the new cosmology[7]. If we assume, as we must, that change is conserved this is the same as admitting the pair lepton-baryon as a new spin state. It is worth noting that several phenomenological theories[8,9] of interaction involve the subgroup G_2 of F_4 as gauge group and this may give a clue as to the fourth spin state.

Finally, non-associativity expresses something about the nature of observation which is not given by non-commutativity alone: namely, that pure states are unobservable. The idea that repeated observation should give certainty may seem essential to existing theory[10], but it is not difficult to see that it is the source of all the logical difficulties. What is involved here was known to C. S. Peirce[11] as the principle of triality and is this: observation requires not only an observer O and a universe U, but also signs S to pass between them. Now, while this situation is quite familiar in physics, there is the tacit assumption that the detachment of S from U leaves it unchanged. Just how near this is to being true is shown by the smallness of k_0/k: but when the existence of matter is in question the assumption is fatal. What is remarkable, however, is that when this triality is expressed in the variables (χ, z, ψ) there should exist the fundamental invariant form $f(\chi, z, \psi)$.

I thank the Leverhulme Foundation for the award of a fellowship (1949) during which this problem was investigated.

M. C. GOODALL

Massachusetts Institute of Technology,
Cambridge, Mass.

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An Economical Prism System for Fast Spectrographs

FAST spectrographs for studying weak light sources, such as the airglow and the aurora, commonly use cameras of large aperture and short focal length—5 in. and 4 in. respectively in a typical case. A prism or grating sufficiently large to fill the aperture of the camera with light, and with sufficient angular dispersion to give a usable linear dispersion in the final spectrum in spite of the short focal length of the camera, necessarily has a high resolving power. On the other hand, the fastest available photographic emulsions are commonly used in these instruments, and spectra recorded on such emulsions show a resolving power only about one-twentieth of that given by the optical components. This large discrepancy led me to seek dispersing systems that would equal the usual systems in aperture and angular dispersion, but which would be less costly or have other advantages, though with resolving power sacrificed by up to a factor of ten.

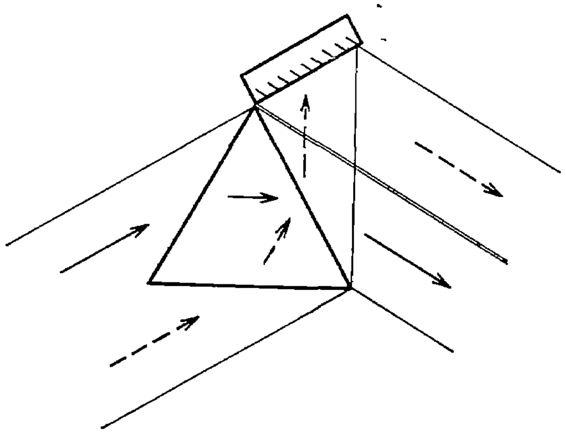

Fig. 1

I could not find a solution to my problem of cost reduction using diffraction gratings of types at present available. However, there are several advantageous prism systems. The most easily constructed is shown in Fig. 1. Half the light from the collimator is allowed to fall on the base of the prism, which becomes a second entrance face. The two angles of incidence must be equal. The second beam of light emerges in an unhelpful direction, but a plane mirror normal to the exit face redirects this beam parallel to the first, and the dispersions are then also in the same direction. The volume of glass in the prism of this economical system is only one-quarter that of the large simple prism which it replaces, since the height is unaltered but the widths of the faces are halved. For large prisms this means a reduction in cost of the raw glass by a factor considerably exceeding four. (It is true that there is now the extra cost and weight of an auxiliary mirror and its support, but there is still a considerable overall saving in both respects.) The reduced volume of glass also makes it easier to secure a piece free from striæ and other imperfections, and to achieve close temperature control in use.

At many wave-lengths the two emergent wave-fronts arrive at the camera in phase. (The two optical paths differ in length by a large whole number of wave-lengths.) The resolving power is then not significantly different from that given by the large simple prism. At those intermediate wave-lengths where the wave-fronts are out of phase the resolving power is reduced to a little less than half that given by the simple prism. These alternating wave-lengths are closely spaced through the spectrum. In a typical case for green light a change in wave-length of 0.01 Å produces the change from the in-phase to the out-of-phase condition.

Because the longer optical paths near the base of the large simple prism have been replaced here by short paths, the economical prism system is noticeably the more transparent at those wave-lengths where absorption is significant. A simple prism needs a face width nearly twice the face height if it is to deliver a beam of light of square or circular cross-section. To do the same, an economical prism requires faces that are square, or nearly so, and this reduces the difficulty of achieving a good optical figure on the faces. On the other hand, all three faces of the economical prism must have a good figure instead of only two. When the economical prism system is used, the prism is placed symmetrically on the axis of the collimator and the camera is much nearer to this axis than in the normal prism spectrograph. This leads to a less-inconvenient shape for the instrument and to greater compactness.

An economical prism system similar to that of Fig. 1 is in use in a spectrograph at the Dominion Physical Laboratory's Auroral Station at Lauder, Central Otago. It replaces an unsatisfactory diffraction grating which had a poor blaze and which was too small to fill the aperture of the camera with light. Exposure times for infra-red plates have been reduced by a factor of four. The prism is a liquid one filled with cinnamic aldehyde, chosen for its exceptionally high dispersion but having a high temperature coefficient of refractive index which demands control to ± 0·2° C. in our case.

N. J. Rumsey

Dominion Physical Laboratory,
Department of Scientific and Industrial Research,
Lower Hutt, New Zealand.

METALLURGY

Point Defects in Niobium, Molybdenum and Tantalum

Experiments in which the thermal recovery of neutron irradiated and plastically deformed molybdenum[1-3] and tungsten[1,4-6] has been followed by measuring changes in electrical resistivity have revealed that in both metals one species of point defect, probably the lattice vacancy, becomes mobile above room temperature. The purpose of this communication is to give a preliminary account of similar experiments on neutron-irradiated niobium and molybdenum and plastically deformed niobium and tantalum.

Recrystallized niobium and molybdenum wires were irradiated for periods of 3, 6, 9 and 12 months at 40° C. in the reactor *BEPO* at the Atomic Energy Research Establishment, Harwell. The irradiations were carried out in a vertical hole in the reactor where the epithermal neutron flux was about $1{\cdot}2 \times 10^{12}$ n./cm.^{2}/sec.[1] and where about 1 epithermal neutron in 10 had an energy > 1 MeV. Isochronal and isothermal recovery curves were obtained for each metal and neutron dose. These revealed one recovery peak for each metal. That for niobium occurred at 120° C. and had an associated activation energy of 1·22 ± 0·02 eV. while that for molybdenum was at 160° C. and had an associated activation energy of 1·25 ± 0·08 eV. For both metals the magnitude of the recovery, $\Delta\rho$, passed through a maximum with increasing neutron dose. The temperature of the peak and the activation energy found for molybdenum agree closely with those reported earlier[1].

Recrystallized niobium and tantalum wires were deformed in tension at room temperature at a strain-rate of about $1{\cdot}3 \times 10^{-5}$ sec.$^{-1}$. Isochronal and isothermal recovery curves were obtained for specimens given elongations of up to 12 per cent for niobium and 16 per cent for tantalum. The niobium gave a recovery stage and an activation energy similar to those found for irradiated material. The relationship between the resistivity recovery, $\Delta\rho$, and the fractional tensile strain, ε, was found to be:

$$\Delta\rho = 0{\cdot}77\,\varepsilon^{1{\cdot}46}\,\mu\Omega\,.\,\text{cm.} \qquad (1)$$

Specimens with a higher initial dislocation density produced by annealing cold-drawn wire at 700° C., a temperature insufficient to cause recrystallization, behaved rather differently. The peak temperature and activation energy were the same but the relationship between $\Delta\rho$ and ε was found to be :

$$\rho\Delta = 0{\cdot}45\,\varepsilon^{0{\cdot}66}\,\mu\Omega\,.\,\text{cm.} \qquad (2)$$

In the case of tantalum a recovery peak was found at 120° C. with an associated activation of 1·25 ± 0·02 eV. The relationship between $\Delta\rho$ and ε was found to be:

$$\Delta\rho = 2{\cdot}01\,\varepsilon^{1{\cdot}41}\,\mu\Omega\,.\,\text{cm.} \qquad (3)$$

This work was supported financially by the United Kingdom Atomic Energy Authority and one of us (D. E. P.) is indebted to the Department of Scientific and Industrial Research for the award of a research studentship.

D. E. Peacock *
A. A. Johnson

Department of Metallurgy,
Imperial College, London, S.W.7.

* Present address: Advanced Materials R. and D. Lab., Pratt and Whitney Aircraft, North Haven, Conn.

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Aggregate Structure in Thin Nickel Films

It is recognized that in the initial stages of metal film growth by vacuum deposition the incident atoms may possess sufficient mobility on the substrate to form aggregates (clusters of crystallites)[1,2]. The degree of aggregation is chiefly a function of the substrate temperature and rate of deposition, and is conveniently thought of as a film thickness above which the films have a continuous structure. Various investigators have obtained electron microscopic evidence of the aggregated structure in thin films of a number of metals prepared under various conditions[3-6]. In addition, precise density measurements have indicated that aggregation in the initial portion of the film formation may result in a decrease in the observed density[7]. Severe aggregation may also be detected by electrical resistivity measurements. In contrast to the foregoing procedures, a simple chemical etching technique has been successfully used to observe metallographically the aggregate structure in thin nickel films. Despite the difference in magnification, such photomicrographs bear a striking resemblance to the published electron micrographs indicating aggregated structures in thin metal films.

The films used in this work were deposited on to glass substrates (40°–60° C.) in a vacuum of 2·8–10·0 × 10^{-6} torr at a rate of 8–14 Å./sec. The source consisted of nickel wire wound around three-braid tungsten wire filaments at a distance of 6–8 in. from the substrate, and arranged so that the vapour beam was at normal incidence to the substrate. Spectrographic analysis indicated a film purity of 99·9 per cent. X-ray diffraction examination indicated that the films had the normal face-centred cubic structure. Film thickness was determined by the use of multiple-beam interferometry.

The etching of the nickel films was accomplished by placing the films at normal incidence to the vapours of a heated (80° C.) solution of 50 per cent concentrated nitric : 50 per cent glacial acetic acid. The films were exposed for 3–5 sec., rinsed with water and examined metallographically; the procedure was repeated until a structure was evident. No apparent structure was noted until the film thickness was reduced to about 300–400 Å. Previous investigators using electron microscopy found no aggregation above about 300 Å. in nickel films prepared under similar conditions[5].

Representative photomicrographs of the various structures observed are shown in Figs. 1 and 2. It may be noted that in the thinner film (Fig. 1) the lateral size of the aggregates is smaller than in the thicker film (Fig. 2), which is to be expected. It is interesting to note the difference in the mode of etching in these films. In the thinner film the interior regions of the aggregates appear to have been preferentially attacked, while in the thicker film the boundaries are attacked. In this case, boundary refers to the material which is deposited in the channels between the aggregates already formed on the substrate.

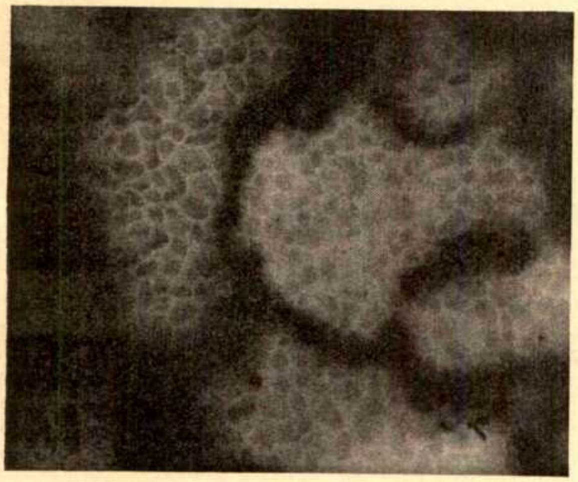

Fig. 1. Aggregate structure in thin nickel film approximately 100 Å. thick (original thickness, 1170 Å.). Wide dark channels indicate complete removal of film from substrate. (× 200)

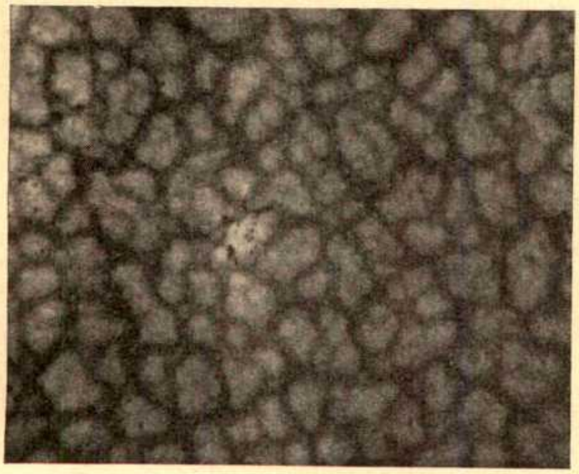

Fig. 2. Aggregate structure in thin nickel film approximately 300 Å. thick (original thickness, 1150 Å.) (× 200)

This chemical etching appears to be a fast convenient method to determine the gross structure of thin films so that adjustment of the deposition parameters may be carried out prior to examination of electrical, magnetic, optical or mechanical properties. Extension of this technique to other metals is under investigation.

JOEL S. HIRSCHHORN
ROBERT J. MACIAG

Metallurgical Engineering Division,
Polytechnic Institute of Brooklyn,
Brooklyn 1, New York.

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ENGINEERING

Conducting Coatings for High-Voltage Insulator Stabilization

SINCE the pioneering work of Marshall and Forrest[1], many further attempts have been made to control the voltage distribution over the surface of ceramic insulators by incorporating a conducting system in the glaze layer to allow a small leakage current to flow through it; recent progress in this field has been reviewed in papers by Forrest *et al.*[2,3]. Lucas[4] and Smith[5], of this laboratory, have shown that the major disadvantage of the semiconducting compounds (commonly metal oxides) used to achieve the preferred value of resistivity, between 7 and 17 MΩ/sq., is their susceptibility to damage by electrolytic corrosion in polluted atmospheres. The purpose of this communication is to describe two new conducting coating systems which show some promise of possessing increased resistance to corrosion. They are glazes containing dispersions of carbon and glazes carrying a film deposited by vacuum evaporation of silicon carbide.

(1) Carbon powder of 99·5 per cent purity and mean particle size of 400 Å. was incorporated into a commercial feldspathic glaze normally used for covering high-tension insulators. When carbon/glaze mixtures were fired in dry nitrogen or argon, 50–75 per cent of the carbon was lost due to oxidation by the molten glaze. A glaze did not form if the mixture was fired in hydrogen. Experiments with firing atmospheres consisting of mixtures of nitrogen and hydrogen indicated that 5–20 per cent of hydrogen was necessary for the formation of a satisfactory glaze with few bubbles.

The glazes studied contained 1–10 per cent by weight of carbon. Surface resistivities ranging from 0·01 MΩ/sq. for 3 per cent carbon to 500 MΩ/sq. for 0·5 per cent carbon were measured, using fired-on silver contacts. Some spread of resistance values for a given composition was observed, typically 0·1–0·6 MΩ for 1·5 per cent carbon. Devitrification of the glaze has not been observed in thin sections cut from the glaze.

Electrolytic corrosion tests using the method described by Lucas[4] have been carried out on some specimens. Some electrolytic attack occurred, the carbon being removed, presumably as carbon dioxide. Carbon particles appeared in the electrolyte during the first hour of a test on one specimen which had a low resistance (0·08 MΩ). The particles were removed and the electrolyte changed; no more particles appeared during the remainder of the test. The damage observed in all the tests was slight, and there did not appear to be any increase in corrosion after the first few hours.

(2) Silicon carbide has been evaporated on to the surface of glazed and unglazed porcelain samples. This was carried out in vacuum, the pressure initially being 10^{-4} torr. An arc was struck between two rods of silicon carbide and maintained for about 15 sec., sufficient to give a film thickness of about 1μ. The porcelain pieces were heated by a molybdenum wire heating element to the temperatures given in Table 1. After removal from the vacuum deposition apparatus, silver contacts were fired on the film and the resistances measured at room temperature in vacuum (to avoid difficulties due to water vapour absorption). The surface resistivities are given in Table 1.

Table 1

Approximate substrate temperature	Glazed porcelain	Unglazed porcelain
20° C.	> 100 MΩ/sq.	> 100 MΩ/sq.
400° C.	1·1 ,,	5·3 ,,
800° C.	0·3 ,,	1·6 ,,
1,000° C.	41·0 ,,	27·8 ,,

Films that formed on the substrate at 20° C. were weak and easily removed from the surface. All the others were strong, coherent films which adhered well to the glaze and porcelain surfaces. The films had good resistance to abrasion and almost a mirror finish. The identification of the film material by X-ray and electron diffraction has proved difficult because of its thinness, but the evidence obtained so far is consistent with it being a mixture of silicon carbide, silicon and carbon with the possibility of other unknown constituents. The decomposition into silicon and carbon is in agreement with the observation of Drowart and de Maria[6] that these two species are formed when silicon carbide is vaporized. The minimum which appears to exist in the variation of resistance with deposition temperature should make control of resistivity simpler than with other systems. Electrolytic corrosion tests have been started and after 300 hr. there is no damage visible.

Work is continuing on both systems and corrosion-rates are being determined for comparison with figures reported for other conducting systems. The reproducibility of the resistivity values is also being examined.

We thank Mr. G. Siddall, of Edwards High Vacuum, Ltd., for the preparation of the silicon carbide films.

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R. W. Sanders
D. R. Holmes

Central Electricity Research Laboratories,
Leatherhead, Surrey.

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CHEMISTRY

Nitrogen-14 in Natural Diamonds

Kaiser and Bond[1] showed, by mass-spectrographic analysis, that many type I diamonds contain up to 0·2 per cent of nitrogen-14 as impurity, and quantitatively correlated this fact with certain features of the type I infra-red and ultra-violet spectra. Smith *et al.*[2] also found by electro-spin resonance measurements that there is a C—N bond in about one half of the diamonds examined. A correlation has now been established between spike intensity and ultra-violet transparency in natural diamonds[3], and dislocation loops and segregated platelets, presumed to be nitrogen, have been observed in etched diamonds by transmission electron microscopy[4]. Kaiser and Bond deduced that the nitrogen is substitutional and not interstitial, on the basis of density measurements on eight diamonds of gem quality.

Without a careful study of the other impurities normally present even in gem diamonds it seems doubtful whether density or lattice constant measurements (of the accuracy involved, which is better than 1 in 10,000) can prove much. Lonsdale[5] found a good type I diamond having a lattice constant smaller than any type II reported, including those of Kaiser and Bond; and fast neutron irradiation can reduce the diamond density by more than 4 per cent, so that small amounts of disorder, however produced, could easily account for the density changes found by Kaiser and Bond.

One most interesting question that seems so far to have excited no comment is that of the origin of the nitrogen, if and when it occurs. The suggestion that there is nitrogen at great depths in the Earth's crust is not favoured by most geological theory. It is important, therefore, to know whether the nitrogen might have diffused into the diamonds after they had been formed (in which case it might be expected to be interstitial).

It would be interesting, for example, to know whether a type II diamond could be converted into a type I by forcing nitrogen into it at high pressures. One might suppose that interstitial nitrogen could be driven off by heating *in vacuo*. If this nitrogen were responsible for the 'extra spikes', then these should disappear concurrently. This certainly does not happen. Rapid heating of type I diamonds to ~2,000° C., which produced fragmentation and incipient graphite formation, did not eliminate the extra spikes[6]. The extra spikes are also very strong in some graphite-coated diamonds from Premier Mine.

Examination of coated diamonds from the Belgian Congo showed that some core gave good extra spikes; the coat showed none. On heating pieces of core and coat simultaneously to 1,650° C., the coat graphitized, the core did not. These observations certainly imply that it is unlikely that nitrogen could have diffused into the diamond after crystallization. It seems most likely that if it is there at all, it was present as a substitutional impurity from an early stage of growth, and then or later segregated into platelets.

We have examined synthetic diamonds kindly given to us by Dr. R. H. Wentorf, jun., of the General Electric Research Laboratories, Schenectady, New York, which are expected, from their work in this field, to contain about 0·1–0·2 per cent of nitrogen. All synthetic diamonds when set near to Bragg positions show irregular diffraction spots and streaks which indicate imperfections, but these are distinguishable from the type I extra spikes, which are not present in these nitrogen-containing diamonds. This does not prove that the extra spikes found in natural diamonds cannot be due to nitrogen incorporated in some other way; but we regard it as significant that boron-containing synthetic diamonds do show evidence of extra spikes although these are not identical in appearance with those found for natural diamonds. If the extra spikes found for natural diamonds are due to the platelets observed by Evans, then the following questions still remain unanswered: (1) When, and how completely, did the impurity segregate into platelets? (2) If they do consist of nitrogen, and not some other impurity of mass number 14, where did the nitrogen come from?

H. J. MILLEDGE
H. O. A. MEYER

Department of Chemistry,
University College,
London, W.C.1.

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Liquid-induced Crystallization in Polymers

IT has been reported that a number of organic liquids will induce crystallization in amorphous polyethylene terephthalate[1]. This occurs readily at ambient temperatures, that is, at much lower temperatures than those at which the normal 'thermal' process is observed. It has been further suggested that the phenomenon of liquid-induced crystallization is probably common to other polymers which are crystallizable but which may be obtained in the amorphous state[2].

The presence of a crystalline phase has so far been inferred from a development of opacity, generally associated with a spherulitic structure in unoriented polymer, from a visual change in mechanical properties and from an increase in polymer density, due allowance being made for any imbibed liquid. This communication describes work which has been carried out to confirm this assumption by means of X-ray diffraction techniques and at the same time to extend the study to a bisphenol *A*-type polycarbonate.

A weighed sample of essentially amorphous polyethylene terephthalate film (approx. 1 × 1 × 0·025 cm.) was immersed for one day in acetone at 25° C. It was then removed and vacuum-dried at 60° C. for three days to remove most of the imbibed acetone, and re-weighed. A similar but unweighed sample was heated 30 min. in air at 180° C., that is, at a temperature at which normal crystallization proceeds rapidly. A third sample was left untreated. The X-ray diffraction photograph of the third sample is shown in Fig. 1*A*. In the case of the first and second samples, in order that their X-ray diffraction photographs could be readily compared a quadrant type cassette was used in conjunction with the flat plate camera (Fig. 1*B*).

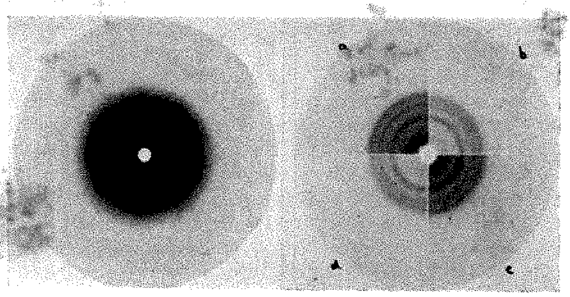
A *B*

Fig. 1. X-ray diffraction photographs of polyethylene terephthalate. *A*, Untreated; *B*, *a* and *c*, heat-treated; *B*, *b* and *d*, acetone-treated

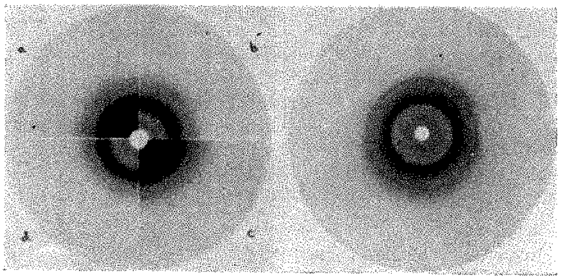
A *B*

Fig. 2. X-ray diffraction photographs of polycarbonate. *A*, *a* and *c*, untreated; *A*, *b* and *d*, heat-treated; *B*, acetone-treated

A similar procedure was carried out with samples of the polycarbonate which had been previously conditioned by heating rapidly to a molten condition at 360° C. and then plunged into 'dry-ice' in an attempt to freeze an amorphous state. The only difference in subsequent experimental procedure was in that one sample was heated at 200° C. instead of 180° C., that is, at a temperature approximately mid-way between the 'glass-temperature' and the melting-point[3,4] where for many crystallizable polymers the rate of crystallization is most rapid. In contradistinction to the acetone-treated sample no opacity was observed. Quadrant photographs of the quenched and heat-treated samples are shown in Fig. 2*A*. Fig. 2*B* shows the picture of the acetone-treated sample.

Density determinations were carried out by means of a flotation method[1], prior studies having indicated that, within the experimental time-scale, the method was also applicable to the polycarbonate. The results are given in Table 1.

Table 1

Polymer	Density (gm./c.c.) Untreated	Acetone-treated	Heat-treated
Polyethylene terephthalate	1·340	1·384	1·384
Polycarbonate	1·207	1·222	1·207

We conclude from these studies that crystallization has taken place in the polyethylene terephthalate, but reliable estimates of the crystalline-amorphous ratio were not attempted since the imbibed liquid is believed to interfere with the general X-ray scattering. From the position of the rings characteristic of crystalline material it appears that the acetone has no influence on the crystal structure, but some broadening suggests a smaller crystallite size in the case of the liquid-treated sample. It would be difficult to express an opinion, therefore, on the implication of the density results, that the degree of crystallinity is the same in both samples. It might be mentioned that the density values agree with that of previous workers[5]. In the case of the polycarbonate it appears that heat treatment has had little effect on the initial state, nor indeed does similar treatment at higher temperatures, but with acetone further crystallization is apparent. It is not easy to explain this behaviour. It may be that organic liquids (of the correct solubility parameter[1]) will induce crystallinity in those polymers which normally only crystallize with difficulty. It should be pointed out that the physical change and the development of opacity occur during immersion, thus repudiating any suggestion of a post-immersion and crystallization process.

We thank the Plastics Engineering Research Committee of this Institute for arranging facilities for part of this work, with the Department of Textile Industries.

R. P. SHELDON
P. R. BLAKEY

Institute of Technology,
Bradford.

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A Phase Diagram for the Calcium Oxide–Carbon Dioxide System in the Eutectic Region

UNDER a pressure of carbon dioxide of 39·5 atmospheres, thermal dissociation of calcium carbonate takes place at 1,240°–1,242° C.[1,2] with the formation of a eutectic melt of lime and calcium carbonate. From determinations of the pressure–temperature relations below the eutectic point, and analyses of the melts formed by dissociation at pressures above the eutectic point, we have constructed a three-dimensional phase diagram for the system in the pressure-range 1–300 atmospheres (Fig. 1).

The surface *ABCD* represents the normal equilibrium between solid calcium carbonate and oxide below the eutectic point. Above the eutectic point, the system can best be considered in terms of two unidirectionally parallel surfaces *BHGC* and *BKMC*. The space behind the surfaces *ABCD* and *BHGC* can be regarded as the pressure–temperature region where solid calcium carbonate is stable, while the space above the surfaces *ABCD* and *BKMC* can be regarded as the pressure–temperature region where solid lime is stable. The region represented by the space between the surfaces *BHGC* and *BKMC* can only be bridged if melt formation takes place. Since the composition of any melt is determined by pressure and temperature, from the phase rule point of view, the all-liquid region will be represented by a surface (*ELF*). The lines *EF* and *EL*, formed by the intersections of the surfaces, consequently give the compositions of melts in equilibrium with the respective solid phases. In the diagram the composition scale refers solely to the compositions of the melts.

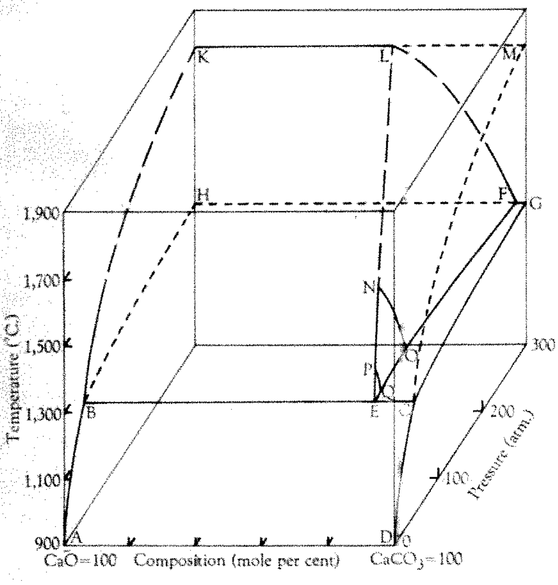

Fig. 1. Phase diagram for the eutectic region of the calcium oxide–carbon dioxide system

At the eutectic point, *E*, the composition is $CaCO_3$ 88·3 mole per cent, CaO 11·7 mole per cent. The melt in equilibrium with solid calcium carbonate at 300 atmospheres pressure and 1,308° C. (point *F*) contains 97 mole per cent of carbonate, and must therefore lie near the melting point of pure calcium carbonate. The latter is estimated to melt at about 1,325° C. at a pressure of 1,000 atmospheres or more; a conclusion supported to some extent by the work of Smyth and Adams[1].

The melt in equilibrium with solid lime at 100 atmospheres pressure of carbon dioxide and 1,457° C. (point *N*) contains 80 mole per cent of calcium carbonate. Experimental difficulties prevented determinations being made beyond this point, consequently the portion *NL* of the solid–liquid equilibrium line has been extrapolated in the direction of the melting point of lime.

The curved nature of the all-liquid surface was elucidated from analyses of melts with compositions lying on the isobars at 56 and 100 atmospheres in the all-liquid region (lines *PQ* and *NO* respectively).

It is to be expected that oxide–carbon dioxide systems in which melt formation occurs will in general be represented by phase diagrams similar to the above. The diagram for the strontium oxide–carbon dioxide system, for example, is very similar and differs only in the actual values of the parameters. Details of this system are to be published shortly.

We thank the Department of Scientific and Industrial Research for financing this work.

E. H. BAKER
J. BUTLER

Nuffield Research Group in Extraction Metallurgy,
Imperial College of Science and Technology,
London, S.W.7.

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Methionine, the *N*-Terminal Amino-Acid of Phycoerythrins

THE phycoerythrins are photosynthetically active chromoproteins of algae[1]. We have performed *N*-terminal amino-acid analyses on spectrally different phycoerythrins from *Ceramium rubrum* (*R*-phycoerythrin, absorption maxima at 497, 542 and 568 mμ); from *Rhodochorton floridulum* (*B*-phycoerythrin, λ_{max} 544 and 566 mμ), and from *Phormidium persicinum* (*C*-phycoerythrin, λ_{max} 562 mμ). All three plants are marine algae, *Ceramium* and *Rhodochorton* being Rhodophyta, and *Phormidium* a member of the Cyanophyta.

Phycoerythrins were extracted from the algae into distilled water and were purified by precipitation with ammonium sulphate, dialysis against running water, chromatography (twice) on tricalcium phosphate-'Supercel' columns[2] and finally, except in the case of C-phycoerythrin, by crystallization from ammonium sulphate solution. Dinitrophenylation of the phycoerythrins was performed by Sanger's method[3].

Only one DNP-amino-acid was visible on paper chromatography of ether extracts of diluted hydrolysates of the DNP-phycoerythrins. The chromatograms were run on Whatman No. 1 paper and in a variety of solvent systems, including those described for DNP-amino-acids by Fraenkel-Conrat *et al.*[4]. Freshly prepared DNP-derivatives of the amino-acids which have been reported present in phycoerythrins were also chromatographed in these systems. Of these, only DNP-methionine behaved identically with the DNP-amino-acid from *R*-phycoerythrin in our six solvent systems. The identity of the two was confirmed by co-chromatography, and by alkaline hydrolysis of the DNP-derivatives[5]. The free amino-acid formed on hydrolysis was identified as methionine by paper chromatography in butanol/acetic acid/water (4 : 1 : 5), followed by development with ninhydrin. A small quantity of methionine sulphone, which was also present on the paper, was assumed to result from oxidation of methionine during hydrolysis. *R*-phycoerythrin, which had been oxidized with performic acid, yielded DNP-methionine sulphone after dinitrophenylation and hydrolysis; identification was by paper chromatography in four solvent systems with an authentic sample of DNP-methionine sulphone.

These results, which were not altered by further initial purification of the chromoprotein, show that methionine is the *N*-terminal amino-acid of *R*-phycoerythrin[6]. We have found by the same methods that methionine is also the *N*-terminal residue of *B*- and of *C*-phycoerythrins.

We thank L. Provasoli for the *Phormidium* culture and Miss Dani Curley for growing the alga and purifying its phycoerythrin. This investigation was supported by the U.S. Air Force through its European Office (contract No. *AF* 61 (052)–409).

PÁDRAIG Ó CARRA
COLM Ó hEOCHA

Department of Chemistry,
University College, Galway, Ireland.

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Chromatography of Some Phenolic Amines

LITTLE work has, as yet, been published dealing with the paper chromatography of urinary phenolic amines. Solvent systems used have been isopropanol–ammonia[1] and *n*-butanol–ammonia or –methylamine[2,3] for the first direction, followed by *n*-butanol–acetic acid[1–3]. Such systems, however, give poor resolutions, particularly in the group of amines derived from phenylethylamine, and have little potential use except for revealing grossly abnormal excretions of the few compounds that can be unambiguously identified: probably the demonstration of increased excretion of metanephrine and normetanephrine in phæochromocytoma[3] or stress represents the limit of their usefulness.

Further investigations in these laboratories indicate that the general separation of phenolic amines thus far identified in human urine (Table 1) from each other and from other diazo-reacting amines requires the use of *tert.*-amyl alcohol/17 per cent aqueous methylamine (4 : 1) (solvent *A*) in the first direction; on Whatman paper No. 1 a 10-in. overnight ascending run must be carried out at about 32° C. Two solvent systems have been found to be particularly suitable for development in the second direction at about 21°. The first of these, solvent *B*, is an example of the type *sec.*-butanol/aqueous pyridine buffer (4 : 1); the buffer solution, *p*H 4, has the composition water 100 : pyridine 10, acetic acid 41 by volume. The second, solvent *C*, has the composition nitroethane/70 per cent aqueous acetic acid (9 : 4). For 10-in. ascending runs about 16 hr. (*B*) and 3 hr. (*C*) are required. Approximate distances travelled by authentic amines under the conditions outlined above are indicated in Table 1.

Table 1. MOBILITIES OF AMINES IN SOLVENTS *A*, *B* AND *C*

Amine	*A*	*B*	*C*
Tyramine (ref. 1)	166	100	121
m-Tyramine (ref. 1)	150	103	131
o-Tyramine (ref. 1)	191	125	153
3-Methoxytyramine (refs. 2 and 3)	130	73	152
p-Octopamine (refs. 2 and 4)	69	70	70
p-Sympatol (refs. 2 and 4)	116	83	124
Normetanephrine (refs. 1, 2 and 3)	51	41	89
Metanephrine (refs. 1, 2 and 3)	96	64	145
N-Methylmetanephrine (ref. 3)	142	61	188

Figures indicate typical distances run (mm.) measured to centre of spot. For composition of solvents and approximate conditions, see text. Amines applied to paper as hydrochlorides, except *p*-sympatol (tartrate).

Both the combinations *A*–*B* and *A*–*C* have been applied to the two-dimensional chromatography of urine extracts. Some anomalies have been noted: thus *m*-tyramine may give rise to multiple spots; absolute and relative movements, particularly in *C*, are somewhat different from the values recorded in Table 1; extensive decomposition of the 4-hydroxy-3-methoxyphenyl amines in *C* has been observed on occasion. Any disadvantages appear, however, to be small by comparison with the enormously improved resolutions effected. For example, one of a group of very similar compounds, previously provisionally identified as *p*-sympatol[2], proved almost impossible to identify with certainty in any given urine. It now seems probable that it may be isolated as a single spot using the *A*–*C* combination and that other systems used fail to reveal the presence of a contaminating compound which is rather commonly present in urine extracts and which may sometimes be the major constituent of a single mixed spot. This substance shows no evidence of being an artefact from *p*-sympatol and has an R_F value slightly less than has that amine in *A* and distinctly less so in *C*. Other spots which could have been confused with *p*-sympatol have been observed less frequently and are completely separated from it in either of the systems *A*–*B* or *A*–*C*.

P. SMITH

Royal Air Force Institute of Aviation Medicine,
Farnborough, Hants.

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BIOPHYSICS

Mechanical Damage of Keratin Proteins observed by Electron Spin Resonance

It has been shown by electron spin resonance that mechanical damage to biological material causes the formation of free radicals and changes of chemical valence. The change of hæmoglobin to methæmoglobin and the appearance of an unidentified free radical have been observed, and were due to mechanical damage of whole blood by freeze drying[1]. Mechanically excited electron spin resonance spectra of synthetic organic polymers were studied by Bresler *et al.*[2].

In the experiments with mechanically damaged keratin carried out in this laboratory, intense electron spin resonance spectra were obtained. Most experiments were performed with hoof keratin, but other keratinous substances, such as finger nails, feather, etc., have similar spectra. The material was filed with a rough file, in atmospheres of both air and nitrogen with 7 per cent oxygen.

The powder was inserted into the cavity of a standard spectrometer operating at 9 kMc./s. and with 100 kc./s. magnetic modulation, in open or closed sample tubes, at room temperature. Fig. 1 shows the spectra obtained by filing the hoof in air and their change with time; Fig. 2 is a recording of the spectrum from the material treated in nitrogen.

The spectra are very similar to those of X-irradiated keratin proteins[3-5], but show greater intensity and are better resolved. Just as with X-irradiated keratin, they can be assigned to the presence of amino-acids containing sulphur.

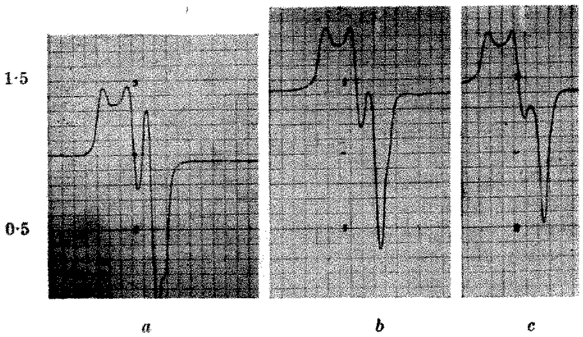

Fig. 1. Electron spin resonance spectra of hoof proteins excited by filing in air. (*a*) Fresh powder; (*b*) after 4 min.; (*c*) after 10 min.

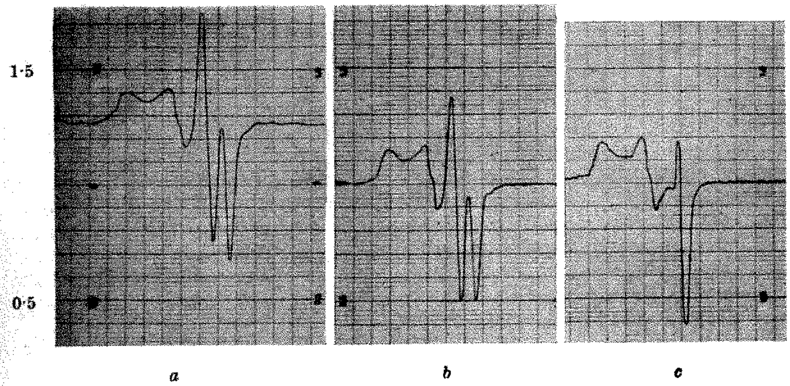

Fig. 2. Electron spin resonance spectra of hoof proteins excited by filing and measuring the samples in nitrogen. (*a*) Fresh powder; (*b*) after 80 min.; (*c*) after 3 days

The spectra obtained in air (Fig. 1) closely resemble those of cysteine and cystine[3-5], and the spectra observed in nitrogen are similar to those of glutathione, observed by Henriksen and Pihl[6]. It may be noted that McCormick and Gordy[4] found no distinct difference between the spectra of cystine, cysteine and reduced glutathione, while oxidized glutathione was not clearly resolved.

Due to the ease with which mechanical excitation of the electron spin resonance spectra is brought about, it is probable that many effects in the spectra of biological objects, which have been thought previously to be of metabolic origin, are artefacts caused by mechanical damage during the treatment of the samples. This is true especially in the case of substances containing electron sinks[7].

The effects observed may be of importance in connexion with the processes of wounding and healing, for the initiation of cancer by mechanical stimulation etc.

K. Ulbert

Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Science, Prague.

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Generation Time of Epithelial Cells in the Human Colon

The duration of the mitotic cycle in intestinal epithelial cells of rodents has been measured by radioautography after tritiated thymidine[1,2]. Proliferating jejunal and colonic epithelial cells in rodents have total generation times of about 18 hr., DNA synthesis (*S* phase) times of 5–8 hr., and pre-mitosis (G_2) and mitosis times of 1·5–2 hr. In man, investigations of mitotic indices and autoradiographic studies with tritiated thymidine have suggested turnover times of intestinal epithelial cells of several days[3].

This investigation gives measurements of the component parts of the mitotic cycle of human intestinal epithelial cells after tritiated thymidine and the effect of 5-fluorouracil on the proliferative cycle. Two patients having a colostomy opening with normal mucosa of the transverse colon attached to the anterior abdominal wall after resection of carcinoma of the rectum were examined; both had liver metastases. The first was given intravenously 0·057 μc./gm. of tritiated thymidine, the second 0·26 μc./gm. (specific activity 6·59 curies/mM dissolved in 10 ml. of water). Three months later the second patient was treated with 5-fluorouracil, total dose 105 mgm./kgm., for 16 days. At the onset of toxicity, the drug was discontinued and a second tritiated thymidine injection given. After each injection, serial biopsies of colonic mucosa were taken from the colostomy opening from the surface to a distance of 2 in. below. Tissue specimens were fixed in neutral formol-saline, embedded in paraffin, and sectioned at 5μ. For radioautography, slides were dipped in *NTB* (Kodak) liquid emulsion, exposed for 2–3 months, developed and stained with hæmatoxylin and eosin. The fraction of labelled mitoses in colonic crypts, as well as the rate of duplication of labelled cells, were determined.

Fig. 1 shows the fraction of labelled mitoses at various times after tritiated thymidine in the first patient. Each circle represents results from 2–4 biopsy specimens taken at the same time. The number of mitoses at each point range from 7 to 58 with a mean of 27·4. The vertical bars delineate the 95 per cent confidence limits. Although many labelled cells are seen at 15 min., almost no labelled mitoses are present. This represents the minimum for the premitotic (G_2) phase of the generation cycle.

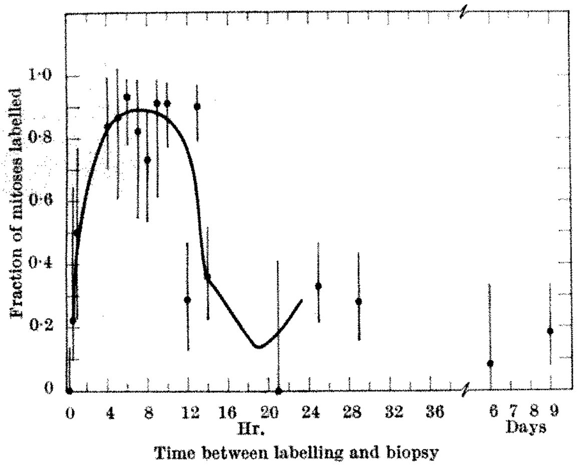

Fig. 1. Fraction of mitoses labelled at various intervals after injection of tritiated thymidine in subject 1. Each symbol represents pooled data from 2–4 biopsy specimens. The vertical lines define 95 per cent confidence intervals

The earliest part of the ascending wave of labelled mitoses records those mitoses derived from cells leaving DNA synthesis (*S* phase) at the time of injection. The descending part of the wave represents labelled mitoses derived from cells entering *S* phase at the time of injection. After 1 hr., 50 per cent of mitoses were labelled (Fig. 1). This is approximately the mean duration of G_2 plus about half the mitosis time[1]. The fraction of mitoses labelled rose rapidly to about 0·92. The midpoint of the subsequent decline of the wave of labelled mitoses was about 12–15 hr. The mean duration of the *S* phase is measured by the distance between the midpoints of the rise and fall of the wave of labelled mitoses; this is about 11–14 hr. Labelled mitoses are lowest at 14–24 hr. This trough represents cells in post-mitosis (G_1), mitosis, and pre-mitosis (G_2) phases respectively at the time of injection; these were therefore not labelled. Since G_2 is known to be short, the distal part of the trough can be used to provide a minimum estimate of the total generation time—about one day. Subtraction of 15 hr. for the *S*, G_2 and mitosis phases gives an estimated 10 hours or longer for the G_1 phase. Counts of labelled cells at 30 min. and 24 hr. show a corresponding rise in numbers of labelled cells from a mean of 14·5 ± 1·4 (*S.E.*) to 31·0 ± 1·6 (*S.E.*), and an obvious reduction in the number of grains per cell at 12 hr. and later.

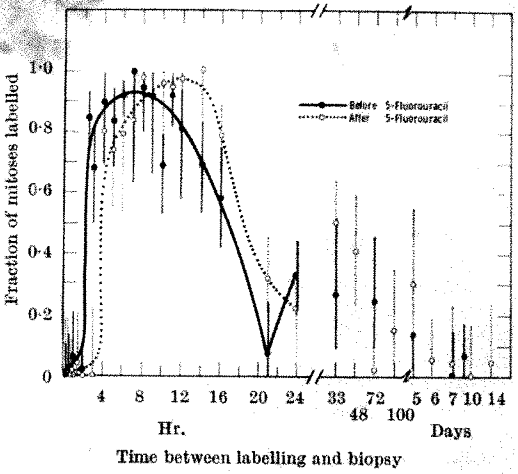

Fig. 2. Fraction of mitoses labelled at various intervals after injection of tritiated thymidine in subject 2. Solid line drawn through values obtained before and dotted line after administration of 5-fluorouracil

Fig. 2 shows the fraction of mitoses labelled at various times after tritiated thymidine in the second patient. The solid line records values before, and the dotted line after, 5-fluorouracil. The number of mitoses at each point was 8–93 with a mean of 37·2. In the control measurements, practically all mitoses were unlabelled until 2 hr. after thymidine. This is the minimum time of G_2 in Subject 2. The wave of labelled mitoses reached a peak at about 6–8 hr. The wave of labelled mitoses then declined; the distance between the midpoints of rise and fall, *S* phase, is close to 15 hr. The duration of the entire generation cycle from these measurements is also about one day.

After 5-fluorouracil, there were abnormalities in the mucosal epithelial cells; nuclei were large, stained lightly, and there were irregularities in chromatin pattern. One day after stopping 5-fluorouracil these abnormalities disappeared. There was a delay in the onset of labelled mitoses, the G_2 duration being increased to about 4 hr. The wave of labelled mitoses was also shifted to the right after 5-fluorouracil. The *S* phase—about 13–17 hr.—was unchanged. The *S* phase might have been prolonged if the drug had not been stopped at the time of injection of thymidine.

Human specimens were also examined two weeks after thymidine. At this time one would expect most labelled cells to have been removed from the mucosa. In previous investigations with animals, proliferating crypt cells divided rapidly and diluted their label to background-level within several days. Other heavily labelled cells migrate to the surface of the crypts and are extruded into the lumen. Labelled cells therefore rapidly disappear from the mucosa. However, in the colonic mucosa of several species, a few epithelial cells do not follow the pattern of proliferation and migration described for most cells; a few heavily labelled cells are retained in the mucosa for prolonged periods[2,4]. Prolonged retention of heavily labelled epithelial cells is more marked in distal colon than in duodenum or jejunum of other species[5]. In man, although there was rapid dilution of label and removal of most heavily labelled cells two weeks after injection of tritiated thymidine, a few heavily labelled epithelial cells persisted in colonic crypts, indicating prolonged retention.

We thank Drs. H. Quastler and L. D. Hamilton for their review of this work, and Dr. B. Clarkson for assistance in selection of patients. This work was aided by U.S. Public Health Service grants *A*-3165

and *C*–3697 and the John A. Hartford Foundation, Inc.

MARTIN LIPKIN
PAUL SHERLOCK
BERTRAND M. BELL

Department of Medicine,
Cornell University Medical College,
Second (Cornell) Medical Division,
Bellevue Hospital and Department of Medicine,
Memorial Center for Cancer and Allied Diseases,
New York.

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BIOCHEMISTRY

Electron Microscope Examination of some Globular Proteins

A MODEL of a protein molecule should be compatible with all experimental evidence available, it should explain changes of these properties corresponding to various experimental conditions, and give a feasible concept of its synthesis in the cell. From these criteria, Segal, Dornberger and Kalaidjiev have proposed a model for globular proteins: the "Faltentrommel"[1].

According to this hypothesis the protein should contain a cylindrical hole, coaxial with the outer limiting cylinder. This hole should be large enough to be seen in the electron microscope. Proteins have been examined by various authors in the electron microscope, but to our knowledge no holes have so far been found (for example, refs. 2–5). For our experiments we have used albumins (ovalbumin and bovine serum albumin (our own preparations) as well as bovine serum albumin from Sera, Heidelberg, and ovalbumin from Kebo, Stockholm) dissolved in distilled water (1 mgm./l.). A small drop of the solution was deposited on the specimen grid and allowed to dry. Some of the grids were examined directly, some were covered by a very thin layer of carbon, some were shadowed (platinum at an angle of 20°), and some were stained with osmium tetroxide. Pictures were usually taken at an instrumental magnification of 20,000, and at a voltage of 48 kV. Selected parts of the electronmicrographs were enlarged photographically (× 30, Figs. 1–4).

The electron microscope does not permit very accurate measurement of the particle-size of proteins[2], but our values are in agreement with results published by other authors. At very high magnifications some particles are represented as rings in the electronmicrographs (Figs. 1–4). They are sometimes particularly noticeable in agglomerations (Fig. 4). Osmium-stained particles show more contrast than unstained ones, if photographs have been taken under the same conditions. The results of a qualitative evaluation of osmium staining will be reported later.

An inspection of blank collodion films shows that these rings are not structures of the supporting film and that they have not been introduced by the solvent. By careful focusing also artefacts have been avoided which may be introduced by the formation of Fresnel rings. According to its position on the supporting film a hollow cylinder will give a picture of either roughly uniform or roughly annular density distribution. Thus from the electron microscopic evidence we may draw the conclusion that the proteins which we have examined have roughly the shape of hollow cylinders, as postulated by the hypothesis of Segal, Dornberger and Kalaidjiev. They have apparently a tendency to 'tumble over', as annular structures are not too frequently found. Sometimes, if they are more closely packed they seem to support each other, and a greater number of annular structures is observed (Fig. 4).

We think there are a few reasons why these annular structures have not been observed before. They are not too frequent, and they can only be detected at very high magnifications. Shadowing makes their detection more difficult, though they are often indicated by a blob of metal. Furthermore, the contrast in unshadowed specimens is very poor, and it may have been enhanced by working at a very low voltage, as we did. Finally, we would like to mention that one of us[6] found annular structures in the protein-containing sub-units of the chromatoid body of *Entamoeba invadens*, and that also X-ray findings

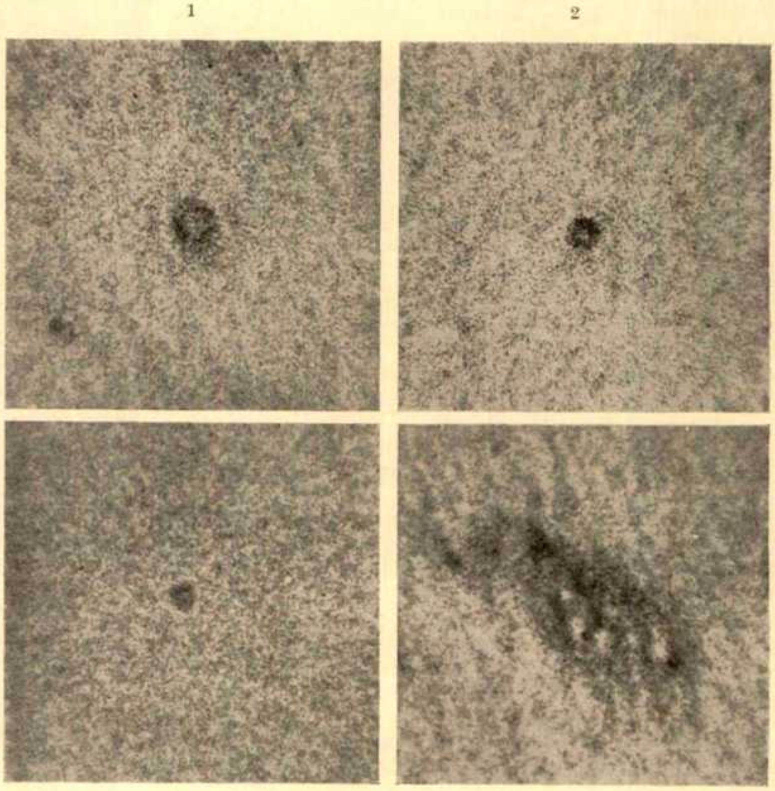

Fig. 1. 1 mol. of serum albumin, osmium stained (30,000); Fig. 2, 1 mol. of serum albumin, untreated (20,000); Fig. 3, 1 mol. of ovalbumin, untreated (20,000); Fig. 4, agglomeration of molecules of serum albumin, osmium stained (30,000). (All photographs enlarged × 30)

indicate the existence of a hole in the molecule of a globular protein[7].

K. Deutsch

Department of Electronmicroscopy,
University, Greifswald.

J. Segal
A. Kalaidjiev

Department of General Biology,
University, Berlin.

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Separation of Catecholamines by Paper Chromatography

One of the difficulties encountered in investigating the metabolism and distribution of catecholamines has been the inadequate separation and isolation of these substances from tissue extracts[1]. Various solvent systems have been used for developing chromatograms of the catecholamines[2-4]; but none has effected a complete separation. The method described here involves a novel technique of 180° solvent-reversal paper chromatography utilizing two different solvent systems, which makes possible separation of 3,4-dihydroxyphenylalanine (dopa), 3-hydroxytyramine (dopamine), norepinephrine, epinephrine, and 3,4-dihydroxyphenylacetic acid, on a single paper strip.

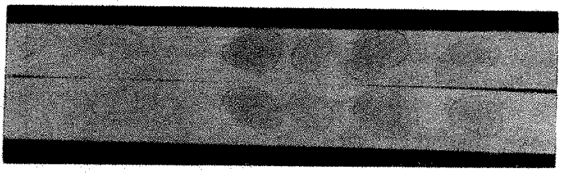

Fig. 1. Separation of catecholamines. From left to right: 3,4-dihydroxyphenylacetic acid; 3,4-dihydroxyphenylalanine; norepinephrine; 3-hydroxytyramine; epinephrine

Approximately 25 μgm. of each of five catecholamines, dissolved in 1 per cent hydrochloric acid in 95 per cent ethanol[5], were spotted 7 cm. from the end of a strip or sheet of Whatman No. 1 filter paper, 57 cm. in length. The papers were developed by the descending technique for 28 hr. with an 88 per cent aqueous solution of phenol in an atmosphere of 1 *N* hydrochloric acid. The chromatograms were washed with benzene to remove the phenol and allowed to dry completely. The papers were then redeveloped with *n*-butanol saturated with 1 *N* hydrochloric acid[3], again by the descending technique for 25 hr. However, the second solvent was run in a direction opposite (180°) to the phenol. After drying completely, the spots were developed by spraying with 0·44 per cent potassium ferricyanide solution in phosphate buffer *p*H 7·8 (ref. 2). When the colours did not appear immediately, the papers were exposed to ammonia vapour to enhance their colour development. For further identification of the spots, the papers were viewed under ultra-violet light (Table 1).

The R_F values for the individual catecholamines developed with each solvent system separately are indicated in Table 1. To separate the catecholamines, the two solvent systems were run in an opposite direction, therefore a net *R* value (*Re*; based on epinephrine) was calculated for each compound. Epinephrine was considered arbitrarily to represent the solvent front and was given a value of 1·0 based on the starting line of the first solvent system. Generally, two compounds will be separated if the difference between their net *Re* values is greater than 0·1. Chromatographic separation of radioactive catecholamines in tissue extracts was improved by addition of 20–40 μgm. of each of the corresponding carriers to the extract before application to the paper.

Table 1. Staining Characteristics and R_F Values of Catecholamines

Compound	Colour with potassium ferricyanide	Colour under ultra-violet light	R_F in phenol	R_F in butanol	Net *Re** value phenol and butanol
3,4-Dihydroxyphenylacetic acid	faint purple	purple	0·61	0·85	0·17
3,4-Dihydroxyphenylalanine (dopa)	purple	dark purple	0·26	0·30	0·50
Norepinephrine	pink-purple	yellow	0·30	0·22	0·63
3-Hydroxytyramine (dopamine)	purple	dark purple	0·48	0·31	0·78
Epinephrine	pink-mauve	yellow	0·54	0·25	1·00

* *Re*, net *R* values based on epinephrine which was considered arbitrarily to represent the solvent front and was given a value of 1·0.

Benjamin Weiss
G. Victor Rossi

Department of Pharmacology,
Philadelphia College of Pharmacy and Science,
Philadelphia 4.

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Oxygen Consumption, Lactic Acid Production and Protein Synthesis of Peritoneal Macrophages

It has been shown that macrophages, obtained by injection of paraffin oil into the peritoneum of the rabbit, are facultative anaerobic cells[1]. In order to evaluate the ability of respiration and glycolysis to supply energy for biosyntheses in these cells, the incorporation-rate of a labelled amino-acid into protein, as a model of a synthetic process, was investigated in aerobic and anaerobic conditions[2,3]. At the same time, consumption of oxygen and production of lactic acid were also measured.

Table 1. Lactic Acid Production by Peritoneal Macrophages

	No. of experiments	μmoles/mgm./N/hr. (1)	μmoles/10^6 cells/hr. (2)
Aerobiosis	7	4·9 ± 1·17	0·09 ± 0·021
Anaerobiosis	10	8·2 ± 1·09	0·15 ± 0·019

Incubation mixture (final concentration): NaCl, 108 m.moles; KCl, 4·7 m.moles; $CaCl_2$, 1·3 m.moles; $MgSO_4$, 1·2 m.moles; sodium phosphate buffer, 5·0 m.moles; sodium bicarbonate, 25·0 m.moles; glucose, 15 m.moles. Incubation lasted 60 min. at 38° C. in a metabolic shaking incubator. The reaction was stopped adding 10 per cent trichloroacetic acid and lactic acid was estimated by the method of Barker and Summerson (ref. 8). Gas phase: 95 per cent oxygen + 5 per cent carbon dioxide; 95 per cent nitrogen + 5 per cent carbon dioxide. Figures are means ± *S.E.*

(1) Nitrogen estimated by the Nessler method (ref. 9).

(2) Cell counts made in a Thoma–Zeiss hæmocytometer.

Table 2. OXYGEN CONSUMPTION BY PERITONEAL MACROPHAGES

	No. of experiments	μmoles/mgm. N/hr. (1)	μmoles/10⁶ cells/hr. (2)	QO_2	No. of experiments
Endogenous	24	2·96 ± 0·118	0·053 ± 0·0021	6·4	7
Succinate	17	2·78 ± 0·124	0·050 ± 0·0022	6·5	8
Glucose	17	2·64 ± 0·169	0·047 ± 0·0030	5·1	4

Incubation mixture (final concentration): NaCl, 127 m.moles; KCl, 5·1 m.moles; $CaCl_2$, 2·7 m.moles; $MgSO_4$, 1·3 m.moles; sodium phosphate buffer (*p*H 7·4), 10·3 m.moles. When added, succinate and glucose were, respectively, 10 m.moles and 15 m.moles. The incubation was carried out in a Warburg apparatus at 38° C. for 60 min. Readings were taken every 10 min. Gas phase: air. 30 per cent potassium hydroxide in the central well. Figures are means ± *S.E.*

(1) and (2), same legends as in Table 1.

10 ml. of sterile saline containing 0·1 mgm. glycogen[4] were injected intraperitoneally in each rat used throughout these experiments. Seven days later, the rats were killed (10–15 rats for each experiment) and the peritoneal cavity rinsed with Ringer-phosphate in order to collect the cells; the suspensions were pooled and then centrifuged at 150*g*. The precipitate was resuspended in the incubation mixtures as shown in Tables 1 and 2. Mononuclear cells (macrophages) accounted for 85 per cent of the whole picture as microscopically checked; other cells were small lymphocytes, eosinophils and mast-cells. Glycogen was used because of its strong chemotactic property on migrating cells[5] and because it eliminates a possible metabolic impairment caused by paraffin oil.

Aerobic and anaerobic lactic acid production, oxygen consumption, either endogenous or with sodium succinate and with glucose as substrates, and leucine-1-C^{14} incorporation-rate into protein were measured.

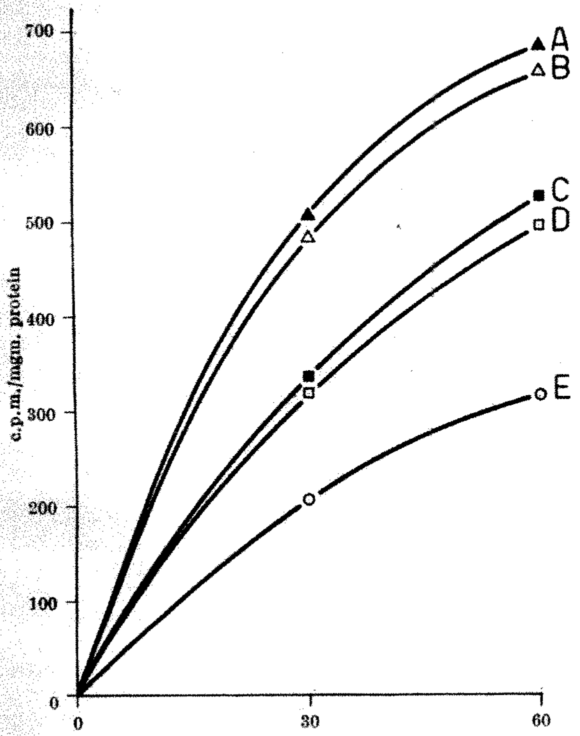

Fig. 1. Incorporation of DL-leucine-1-^{14}C into protein by peritoneal macrophages. Incubation mixtures were the same as in Tables 1 and 2: 0·526 μmole (4 μc.) leucine added. Incubation lasted 30 and 60 min. at 38° C. Cell proteins were purified (ref. 10), weighed, suspended in absolute ethanol and plated as infinitely thin samples. Radioactivity was measured with a mica-window Geiger–Müller counter. Counting error was less than 3 per cent. *A*, Incorporation in phosphate medium, glucose added, air phase; *B*, incorporation in bicarbonate medium, glucose added, 95 per cent oxygen + 5 per cent carbon dioxide phase; *C*, incorporation in phosphate medium, no substrates, air phase; *D*, incorporation in phosphate medium, succinate added, air phase; *E*, incorporation in bicarbonate medium, glucose added, 95 per cent nitrogen + 5 per cent carbon dioxide phase

Results are summarized in Tables 1 and 2 and in Fig. 1. It is evident that macrophages produce lactic acid under aerobic conditions. The values are nearly a half of those produced anaerobically. In both cases the lactic acid produced is less than that observed by Harris and Barclay[1]. Addition of glucose to the incubation medium depresses oxygen consumption (in some experiments inhibition was more than 30 per cent), suggesting the presence of a Crabtree effect in these cells[6]. Succinate, on the other hand, does not stimulate the respiratory activity. In spite of the Crabtree effect, top values of incorporation of amino-acid were attained in aerobic conditions with glucose as substrate; the incorporation-rate with succinate as substrate was lower than before and was not statistically different from that observed in absence of any substrate. Least incorporation-rates were reached in anaerobic conditions.

These results show that glucose in aerobic conditions stimulates protein synthesis despite its inhibitory effect on consumption of oxygen; on the other hand, succinate fosters neither endogenous respiration nor incorporation of amino-acid into protein. This may suggest that glucose stimulates protein synthesis in conditions in which the supply of oxidative energy is reduced.

A similar behaviour was reported by Ciaranfi and Fonnesu for Yoshida hepatoma-ascites cells[7]. Experiments with specific inhibitors of oxidative and glycolytic pathways are in progress to clarify this problem.

ROBERTO COMOLLI

Istituto di Patologia Generale
dell' Università,
Milano.

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Immunoelectrophoresis of Bovine Growth Hormone

As part of my investigations on the production of growth hormone in the embryo, I have performed immunoelectrophoresis on bovine growth hormone.

Recently, Ferguson and Wallace[1] reported that starch-gel electrophoresis revealed two components in bovine growth hormone preparations with approximately equal biological activity. My findings seem to support their results.

Rabbits were injected subcutaneously with 5 mgm. highly purified bovine growth hormone (Organon) in 1 ml. of physiological saline, mixed with an equal volume of Freund's adjuvant complete (Difco). This was followed by weekly injections of 2 mgm. suspended in the same mixture. The animals were bled at intervals and antibody titres were determined with a micro precipitin interfacial ring test[2]. Injections were continued until a titre of at least 1 : 12,800 (antigen dilution) was reached, usually after 6–8 weeks.

In most cases a total dose of about 20 mgm. bovine growth hormone was quite sufficient to obtain this titre, contrary to the findings of Hayashida and Li[3], who needed 80 mgm., using a different immunization method.

Electrophoresis was performed after the method of Wieme[4], which gives a separation in very sharp bands. It was carried out for 45 min. with a 0·5 or 1 per cent solution of bovine growth hormone on microscopic slides (76 mm. × 26 mm.) covered with 2 ml. of gel (1·5 per cent Difco 'Special Agar Noble' in barbiturate buffer, *p*H 8·6, 0·05μ). With 130 V. applied across the electrodes, the amperage was 3–4 m.amp.

Antiserum was applied as usual, and the reactions were read after 24 and 48 hr.

In all cases one single precipitation line was observed, either a long asymmetrical line (Fig. 1*A*) or a two-peaked line (Fig. 1*B*). This indicates the presence of substances with different electrophoretic mobilities, but with the same antigenic properties.

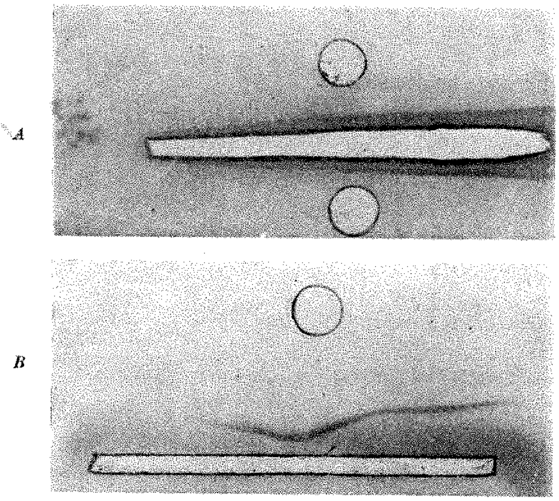

Fig. 1. Immunoelectrophoretic pattern after staining with amido-black. *A*, Antiserum 1 against bovine growth hormone, 0·5 per cent (upper basin) and 1 per cent (lower basin); *B*, antiserum 2 against bovine growth hormone, 1 per cent

Some of the antisera also reacted with bovine serum proteins: precipitation lines, different from those with bovine growth hormone, were seen with albumin and with γ-globulin.

As these lines were not found with the bovine growth hormone itself, the amount of serum proteins contaminating the hormone samples must have been very small, although sufficient to evoke antibody formation.

The aspecific antibodies could be removed by absorption, and the reaction with bovine growth hormone remained the same after this procedure.

My findings can be explained most easily by the existence of electrophoretically different forms of growth hormone. As the same line was observed with saline extracts of single bovine pituitaries, put up against the absorbed antiserum, the possibilities of degradation of the hormone during the isolation process or of polymorphism seem to be excluded.

It is interesting to note that Hirschfeld, Gemzell and Wide[5] found the same pattern of lines with immunoelectrophoresis of human growth hormone as I obtained with the bovine preparation, while Ferguson and Wallace[1,6] reported a very different behaviour of human and bovine growth hormone in starch-gel electrophoresis.

I thank Dr. J. D. H. Homan (Organon, Oss, Holland) for gifts of bovine growth hormone. Part of this investigation was made possible by a grant from the Dutch Ministry of Education, Arts and Sciences (1959–80.004).

J. Zwaan

Laboratory for Anatomy and Embryology,
University of Amsterdam.

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Dialysable Neuraminic Acid in Human Cerebrospinal Fluid

Because of the presence of interfering sugars in great excess, it has not been possible heretofore to determine with any certainty in other laboratories as well as our own[1] whether neuraminic acid occurs in dialysable form in cerebrospinal fluid. Recent methods developed in this laboratory for the quantitative fractionation of all the carbohydrate constituents of specimens of human cerebrospinal fluid have been described in the first paper of this series on the cerebrospinal fluid[2]. By using concentration by lyophilization and exhaustive dialysis, all the solids of cerebral spinal fluid have been separated quantitatively into non-dialysable and dialysable fractions. An accurate estimate of the neuraminic acid bound to protein has thereby been obtained without interference from dialysable sugars[2,3]. Evidence is now presented that neuraminic acid does indeed occur in dialysable form as well as in protein-bound form in specimens of human cerebrospinal fluid. The dialysable neuraminic acid does not, however, appear to be free, but rather to be bound to hexose and nitrogenous residues in glycopeptide-like low molecular weight compounds.

The methods used for the fractionation of whole cerebrospinal fluid are as outlined in the previous communication[2]. Neuraminic acid was determined by means of the thiobarbituric acid method[4]. Neuraminic acid was separated from the other constituents in the dialysable fraction of cerebrospinal fluid by means of a 3-column system in series consisting of 'Amberlite *IR*–120', 'Dowex 3–*OH*', and 'Dowex 1-formate'. 'Amberlite *IR*–120' was used to remove sodium ions. 'Dowex 3–*OH*', used to remove chloride ions, was shown to allow free neuraminic acid to pass through unadsorbed. Free neuraminic acid was then adsorbed to the 'Dowex 1-formate', allowing all the free hexoses to pass through unadsorbed, and eluted therefrom with 0·3 *N* formic acid. Recovery with neuraminic acid standards prepared by hydrolysis of a preparation of bovine brain ganglioside[5] were found to be quantitative in this 3-column system. Paper chromatography was performed with Whatman No. 2 paper, using downward irrigation with a solvent system, butanol/acetic acid/water (4 : 1 : 5). Strips were developed utilizing the thiobarbituric acid spray[6] and ninhydrin reagent. Nitrogen was determined colorimetrically after hydrolysis and hexoses by the method of Sorensen-Haugaard[5].

One hundred random consecutive samples of cerebrospinal fluid were first tested for the presence of 'free' neuraminic acid by means of the thiobarbituric acid reaction. The concentration was shown to vary from 2–12 μgm./c.c. (mean = 5.60 ± 1.90 (*S.E.*)). Only eleven of the specimens showed values greater than 6 μgm./c.c. and nine of these were from patients in the process of being treated with electroshock therapy. On the other hand, seven other specimens which were from patients on this therapy did not show elevated 'free' neuraminic acid. Since the isolated glycoprotein fraction was shown to be negative for neuraminic acid with this reaction (without hydrolysis), the presence of 'free' neuraminic acid was indicated.

In the dialysable fraction (fraction II[2]) neuraminic acid was shown to be present by the thiobarbituric acid method before any further fractionation in amounts equivalent to those observed in whole non-dialysed cerebrospinal fluid. Further, individual dialysate fractions chromatographed on the 3-column system described above yielded an amount of neuraminic acid which had been adsorbed to the 'Dowex 1-formate' in quantities equivalent to the amount observed in the dialysate material before fractionation with a yield of 70–85 per cent.

Starting with 258.9 gm. of whole dialysate from 248 individual specimens (approximately 20 c.c. each) the material eluted with 0.3 *N* formic acid from the 'Dowex 1-formate' column was lyophilized, then further partitioned. By washing the lyophilized material above with cold methanol, two fractions, consisting of methanol-soluble and methanol-insoluble material were prepared. By successive precipitation of the methanol-soluble material with ether in the cold, six precipitates were obtained with generally increasing amounts of neuraminic acid (precipitates I–VI). The methanol-insoluble material was dried in air, taken up with water and separated by centrifugation into a water-soluble 'supernate *A*' and water-insoluble precipitate. The material in supernate *A* was not precipitable with ether. Determination of the content of neuraminic acid, nitrogen and hexose in these subfractions showed the presence of varying amounts of these substances in each of the fractions, the bulk of the neuraminic acid being found in the three fractions in Table 1. The absorption spectrum of the coloured product obtained with the thiobarbituric reaction in each of these three fractions was identical with that produced by our standard neuraminic acid. Each of the three fractions shown in Table 1 demonstrated one spot, respectively, on paper chromatography which was not identical to free neuraminic acid. With an R_F of the neuraminic acid standard of 0.13–0.15, precipitates I and VI and supernate *A* ran with slower R_F's as shown in Table 1, but one of these (precipitate VI) gave a strong 'free' neuraminic acid reaction with the thiobarbituric acid spray, and one (supernate *A*) gave a weak reaction with fluorescence. Hydrolysis of each of these fractions with 0.1 *N* sulphuric acid at 80° C. for 1 hr. produced a second more rapidly moving thiobarbituric acid-positive product with R_F 0.30–0.35 corresponding to the R_F of the product obtained by hydrolysis of the neuraminic acid standard under the same conditions.

Table 1. MOLAR RATIOS NEURAMINIC ACID, NITROGEN (AS AMINO-ACIDS), AND HEXOSE, AND R_F VALUES OF THREE FRACTIONS ISOLATED FROM DIALYSABLE CONSTITUENTS OF HUMAN CEREBROSPINAL FLUID*

	Molar ratios			
	Neuraminic acid	Amino acid	Hexose (glucose)	R_F
Supernate *A*	1.0	3.0	7.6	0.10
Precipitate I	1.0	2.6	4.1	0.10
Precipitate VI	1.0	4.0	5.6	0.07

* Hexosamines are totally absent from the dialysable fraction of human cerebrospinal fluid (ref. 2).

Since (1) the unhydrolysed fractions have slower mobility than the truly free neuraminic acid standard used, yet all showed the presence of 'free' neuraminic acid with the thiobarbituric acid reaction in test tube, (2) there was only one definite spot for each of these fractions in terms of ninhydrin-positive material and thiobarbituric-positive material, (3) hydrolysis produced an identical split product in the case of the neuraminic acid standard and of the three fractions, it is concluded that a sialic acid bound with hexose in ninhydrin-positive glycopeptide-like substances are present in dialysable form in human cerebrospinal fluid. These substances, not previously described in cerebrospinal fluid, are being further characterized and structurally studied. Since 'free' neuraminic acid is present in whole cerebrospinal fluid prior to fractionation, in isolated whole dialysate, and in isolated subfractions of the dialysate after column separation, all in equivalent amounts, these substances could not be derived by splitting from the larger molecular weight glycoproteins of cerebrospinal fluid during the isolation procedure. On the other hand, their biosynthetic origin, which *in situ* may be from the glycoproteins of cerebrospinal fluid, and their function, are the subjects of further study.

The 'whole dialysate' fractions were prepared by Mr. Peter C. Belval. This work was done with the support of grants from the U.S. Public Health Service (*B*-3612) and from the Scottish Rite Committee for Research on Schizophrenia.

SAMUEL BOGOCH
PETER EVANS

Foundation for Research on the Nervous System,
36 The Fenway, Boston,
and Department of Psychiatry,
Boston University School of Medicine.

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Diminution of β-Glucuronidase Activity in Post-Heparin Human Sera

HAHN[1] discovered that after intravenous administration of heparin an enzyme appears in the blood, known as the clearing factor or lipoprotein-lipase. Later, Levy and Swank[2] observed in animal experiments an increase of tributyrinase. Skorepa and Todorovicova[3] found a 50 per cent rise of aliesterase activity and Pav and Wenkeova[4] indicated that, in addition to aliesterase, at least two other lipases are present in the post-heparin plasma. In this study it has been found that β-glucuronidase activity is significantly lowered in human post-heparin sera.

Blood serum β-glucuronidase activity was determined in normal individuals from 5 to 10 years of age, males and females, 1 hr. following an ordinary meal. Blood was drawn before and 15 min. after intravenous administration of 30 mgm. (30,000 units) of heparin. Dohrmann's modification[5] of Fishman's method[6] was used with an incubation period of 18 hr. A 43–64 per cent decrease of β-glucuronidase activity was found in the post-heparin sera. Cajani[7] noted that β-glucuronidase showed a 2,000-times greater affinity for heparin than for phenolphthalein β-glucuronide. Klesper and Dohrmann[8] demonstrated an anti-coagulant effect of β-glucuronidase *in vitro*. Kayahan[9] found a similar effect *in vivo* and also showed a decrease of lipæmia after administration of β-glucuronidase.

From these observations the following hypothesis, among others, appears reasonable: β-glucuronidase and heparin form a complex having not only an anti-coagulant power but also a lipoprotein–lipase activating or enhancing effect.

MIHÁLY BARTALOS
FERENC GYORKEY

Departments of Pathology,
The Johns Hopkins University
and Baltimore City Hospitals,
Baltimore, Maryland

ZSOLT KOPPÁNYI

Department of Pediatrics,
Baltimore City Hospitals,
Baltimore, Maryland

---

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Excretion of 5-Hydroxy Indolyl-Acetic Acid after Surgery

ALTHOUGH the healing of a wound is an essentially local event, it must inevitably also involve wider metabolic processes in the whole organism. One metabolic excretory product of dietary tryptophan is 5-hydroxy indolyl-acetic acid (5-HIAA) derived via the intermediate metabolites 5-hydroxytryptophan (5-HTP) and 5-hydroxytryptamine (5-HT). Special interest has been devoted to this pathway because of its significance in carcinoid tumours. Certain of the metabolic and clinical changes produced by 5-HT may, however, also be important in tissue regeneration and the healing of wounds. As a method of investigating this, it was decided to study the excretion of 5-HIAA following wounding.

5-Hydroxytryptamine metabolism is notoriously difficult to study as it varies from animal to animal, and a number of confusions and contradictions observed in the past stemmed from this difficulty. The investigation reported here was carried out on human subjects. A 'standard experimental' wound was made by using patients with the same clinical condition and operation.

One of the most uniform operations is the repair of a moderate-sized inguinal hernia in the adult male. We therefore used 14 patients on whom this operation was to be carried out, by the same surgeon (G. T. W.). The patients were of a similar age-group (25–35 years) and all were admitted to the same ward. By this means it was hoped to standardize the form of mental and physical stress to which the patients were subjected. The diet was the regular hospital one, modified by exclusion of any items known to be high in tryptophan metabolites (for example, bananas, spinach, pineapple juice). The men were admitted to hospital 2 days before operation and no specimens were collected in the first 12 hr. The urine was then collected over each 24 hr. That passed during the day of operation was discarded. All specimens were collected in bottles containing 10 ml. glacial acetic acid and analyses made as soon as possible. Experiments showed, however, that if longer storage was unavoidable no significant change in 5-hydroxy indolyl-acetic acid content occurred for at least one week if the acidified sample was deep-frozen to − 20° C. Assays were made using a modification of the method of Udenfriend, Titus and Weissbach[1]. The results were expressed in mgm. of 5-hydroxy indolyl-acetic acid excreted every 24 hr. With this method the normal range for laboratory staff fell between the values 2–9 mgm./24 hr.

Table 1. URINARY 24-HR. 5-HIAA OUTPUT EXPRESSED IN MGM./24 HR.

Case No.	Day before operation	Days after operation First	Second	Third	Fourth
1	less than 1	10.5	—	—	—
2	1.2	9.9	—	—	—
3	4.5	8.5	—	—	—
4	2.6	6.4	—	—	—
5	6.0	6.1	—	—	—
6	3.8	10.6	13.2	7.4	6.8
7	4.0	9.4	5.9	7.0	—
8	6.3	11.3	8.7	—	—
9	8.7	10.2	4.5	10.2	—
10	10.3	15.8	8.1	6.7	—
11	1.5	10.6	2.9	4.8	5.8
12	4.9	24.2	11.6	10.6	—
13	5.8	17.2	—	10.2	12.1
14	4.9	10.1	5.1	3.5	6.1

Table 1 shows the pre- and post-operative levels of urinary 5-HIAA in the 14 patients examined. These figures indicate that with but one exception (case 5) the urinary 5-HIAA output was significantly increased on the first day following surgery. That such changes were not a function of a generalized increase in non-protein nitrogen metabolism was excluded in parallel urea nitrogen and creatinine measurements on the same samples. These showed no such changes after surgery. Parallel daily platelet counts were made in 6 cases; no significant correlation was evident. This shows that the alterations in 5-HIAA output are not related to an enhanced 5-HT metabolism consequent on an increase in post-operative circulating platelets. This does not of course exclude the possibility that an abnormal release of 5-HT from platelets may occur without change in the number circulating.

The urine samples from 6 cases were examined chromatographically. The results for 5-HIAA when assessed by a semiquantitative method were in general agreement with the colorimetric assays. It was also noted that in some instances certain other indole metabolites, notably indolyl acetic acid (IAA) and potassium indoxyl sulphate (indican), were also increased post-operatively and to approximately the same degree as 5-HIAA.

We thank Prof. F. A. R. Stammers, and Dr. G. B. Leyton of the Hollymoor Hospital for assistance with the chromatographic studies. This work was supported by the Medical Research Council and U.S. National Heart Institute (Grant No. *H*5772).

NEVILLE CRAWFORD
GEORGE T. WATTS

Division of Wound Healing Research,
Department of Surgery,
University of Birmingham

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N-Acetyl-dopamine as Sclerotizing Agent of the Insect Cuticle

SCLEROTIZATION, that is, the hardening and darkening of the insect cuticle, is generally believed[1-3] to be due to the incorporation of *o*-quinones, the latter arising from the corresponding *o*-diphenols through the action of phenoloxidases. Tracer investigations[4] confirmed the view that tyrosine metabolites are incorporated into the cuticle. However, the nature of the metabolites has not been identified, though several *o*-diphenolic compounds have been isolated[5,6] from insect sources and tentatively regarded as precursors of sclerotin.

The diphenols discussed so far are all acidic in nature. We have recently detected an amine derivative, *N*-acetyl-dopamine, as a metabolite of tyrosine in blowfly larvæ (*Calliphora erythrocephala*) and have identified this compound as the tanning agent in puparium formation. The evidence is as follows:

(1) *N*-acetyl-dopamine (a compound not yet described in the literature) is a normal constituent of the larvæ. It can be demonstrated by paper chromatography of extracts from *Calliphora* larvæ ($R_F = 0{\cdot}80$ in butanol/acetic acid/water, 4 : 1 : 5; colour reactions: purple with diazosulphanilic acid, blue with Folin's reagent), and has been isolated and identified[7]. Quantitative determinations show that *N*-acetyl-dopamine is accumulated in late larvæ and disappears abruptly at the time of pupa formation.

(2) Radioactive *N*-acetyl-dopamine labelled at the β-C-atom is readily incorporated into the cuticle (Table 1). The incorporation is as high as that of tyrosine, within the experimental error. Also, if only the acetyl group of *N*-acetyl-dopamine is labelled, the radioactivity is found mainly in the cuticle[8]. From this we conclude that *N*-acetyl-dopamine is incorporated as a whole molecule.

(3) The enzyme phenoloxidase is presumably involved in the sclerotization process. This enzyme has recently been purified and obtained in the crystalline form in our laboratory[9], and its substrate specificity has been studied. Monophenols are not attacked, at least not with measurable velocity; the enzyme is a true *o*-diphenoloxidase. Also, *o*-diphenols with acidic side-chains, like protocatechuic acid, 3,4-dihydroxyphenylacetic and 3,4-dihydroxyphenylpyruvic acid, are not oxidized by the enzyme isolated from *Calliphora*. The best substrates are *N*-acetyl-dopamine ($K_m = 3 \times 10^{-3}$), dopamine ($K_m = 2 \times 10^{-4}$), and dopa ($K_m = 8 \times 10^{-3}$). Thus, the substrate specificity strongly suggests that not the dihydroxyphenolic acids isolated earlier but *N*-acetyl-dopamine is the sclerotizing agent in *Calliphora*.

(4) A survey of the tyrosine metabolism revealed remarkable differences between younger and older larvæ. In the early III instar (3–5 days old) tyrosine is mainly catabolized to *p*-hydroxyphenylpyruvic and *p*-hydroxyphenylpropionic acid. These metabolites have been identified by paper chromatography in combination with the tracer technique. In older larvæ (7–8 days old, about one day before pupation) these metabolites are only minor ones; the main product of injected tyrosine is *N*-acetyl-dopamine. Eventually, its 4-*O*-β-glucoside is also formed; this substance has also been detected in *Drosophila* pupæ[10].

Table 1

Substance injected	Percentage of radioactivity incorporated into the cuticle*
N-Acetyl-dopamine-β-^{14}C	63
N-(1-^{14}C-acetyl)-dopamine (ref. 8)	50
Dopamine-β-^{14}C	66
Tyrosine, generally labelled (ref. 8)	74

* Percentage of the recovered activity

(5) The shift in tyrosine metabolism from degradation (through transamination and oxidative decarboxylation) to the production of *N*-acetyl-dopamine is brought about by the hormone of the ring gland, ecdysone. It is inhibited by ligation or destruction of the ring gland. Thus, the hormone which causes pupa formation is also responsible for the metabolic changes which give rise to *N*-acetyl-dopamine.

Dennell[11] has postulated a metabolic pathway of tyrosine which, through unspecific hydroxylation and elimination of the side-chain, would give rise to *p*-dihydroxy compounds and *p*-quinones which are believed to be involved in sclerotization. This hypothesis, based on only a few model experiments, has been criticized by several authors[3,4]. The results described here do not support the hypothesis of Dennell; instead, they suggest the pathway of tyrosine metabolism in *Calliphora* as shown in Fig. 1.

It is likely that *N*-acetyl-dopamine is also the sclerotizing agent in other insects as well. By paper chromatography it has been identified in extracts from the mealworm (*Tenebrio molitor*, fully grown larvæ and adult insects) and from the desert locust, *Schistocerca gregaria* Forsk. On the other hand, protocatechuic acid has been identified as tanning agent in the hardening of the oothecæ of the cockroach, and the accumulation of its precursor, the 4-*O*-β-gluco-

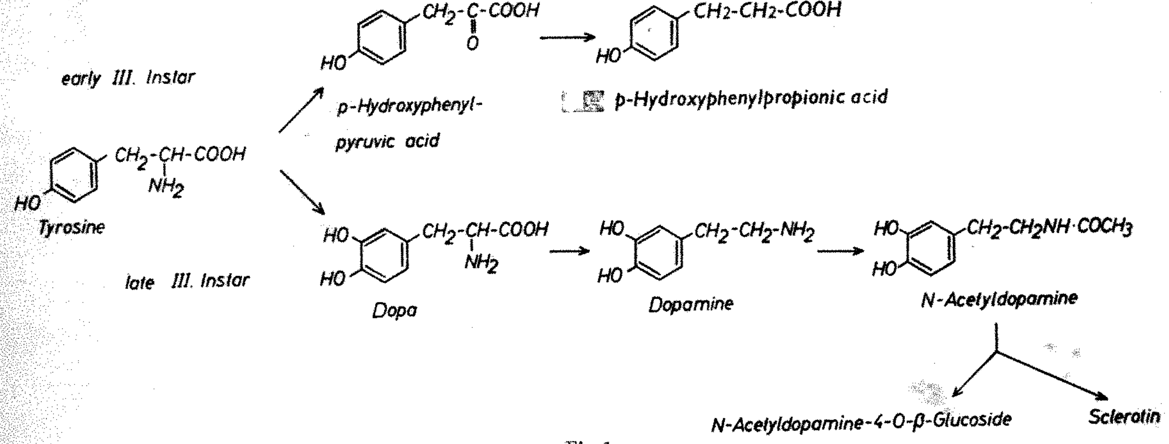

Fig. 1

side, has been demonstrated[12]. Production of ootheca, however, is a very specialized process, and its biochemistry may be different from the general picture of cuticle sclerotization outlined here.

This work was financially supported by the Deutsche Forschungsgemeinschaft.

PETER KARLSON
CONSTANTIN E. SEKERIS
Physiolog. Chem. Institut der Universität München,
Germany.

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Investigation of Phenolic Components of Brewing Materials by Gel Filtration

POLYPHENOLS, especially anthocyanogens, are of interest in brewing because of their tendency to form insoluble complexes with protein. These phenols are somewhat labile and difficult to isolate completely from colouring matter and low-molecular-weight contaminants. Separation can be achieved efficiently on 'Sephadex' and no reagents damaging to the phenols need be used.

A 2·5 × 30 cm. column of medium grade *G*.50 'Sephadex' was used. Before packing, the gel was allowed to swell in the eluting medium for several hours. The column was loaded with a 1-ml. sample and elution was carried out with water or 0·001 *M* phosphate buffer, *p*H 6·7. The eluate from the column was fed into an auto-analyser assembly where it was mixed continuously with diazotized *p*-amino benzoic acid. Any phenolic material gave coloured compounds, the absorption of which was measured at 420 mμ and continuously recorded.

Fig. 1 shows a typical elution curve obtained using a 1 ml. sample of unhopped brewery wort. The first peak ($K_D = 0$) contains about 30 per cent of the total nitrogen and consists of the high-molecular-weight material (> 8,000), which is mainly protein and melanin pigments. Low-molecular-weight simple phenols (for example, tyrosine) together with sugars and amino-acids are then eluted and, finally, a second peak of yellow material which is actually weakly adsorbed on the 'Sephadex' ($K_D > 1$). This fraction contains all the materials which yield red pigments on heating with hydrochloric acid (anthocyanogens). About 30 per cent of the total nitrogen is also present in this fraction, possibly as preformed soluble protein–polyphenol complex.

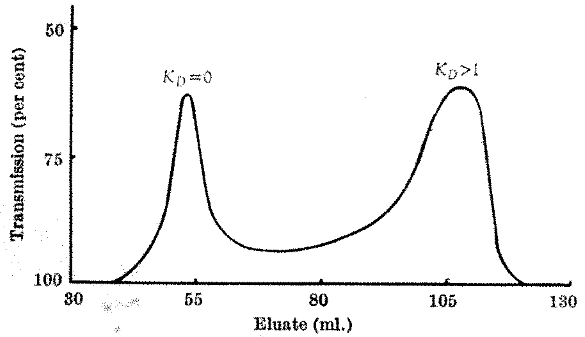

Fig. 1. Chromatography of unhopped brewery wort on a *G*.50 'Sephadex' column. Elution with 0·001 *M* phosphate buffer, *p*H 6·7. Material with $K_D = 0$ is excluded from the gel grain (mol. wt. > 8,000). $K_D > 1$ indicates adsorption on the 'Sephadex'

Nylon and polyvinyl pyrrolidone are known to improve shelf life of beer by adsorbing polyphenolic materials. 'Sephadex' columns are being used to investigate the nature of the polyphenols and their reaction with proteins, and to study similar compounds which occur in barley and hops.

J. B. WOOF
Research Laboratory,
Arthur Guinness Son and Co. (Park Royal), Ltd.,
Park Royal Brewery, London, N.W.10.

PHYSIOLOGY

Climatic Variations of Plasma Potassium in Men

PLASMA potassium has been determined in South Indian and European subjects residing permanently in Madras, and in Europeans in Liège—all male, adult and healthy. The samples of blood were taken from the vein in the morning before breakfast and immediately centrifuged. The potassium was determined in the plasma by flame photometry. The meteorological data were obtained from the Regional Meteorological Center, Madras, and the Institut Royal Météorologique, Uccle, Belgium.

The plasma potassium of one European subject (J. G. H.) was repeatedly determined during several trips in India and Europe during June 1959–April 1961. The 24-hr. average temperature of each experimental day is plotted in the upper graph (Fig. 1).

The other subjects were tested only once, either during summer 1959 in India (9 White Europeans or Americans and 22 Indians), during winter 1960 in Europe (23 Europeans), during July–August 1960 in India (7 Europeans, 27 Indians), in September (24 Indians), during the monsoon (29 Indians), during December, January or May in India (respectively 17, 26 and 30 Indian subjects). The average potassium values and the standard errors (*m.* ± *s.e.*) are indicated for each group. The lower graph shows the mean temperature per month or half-month (Fig. 1).

No racial differences between Western and Indian men can be observed. On the contrary, climatic

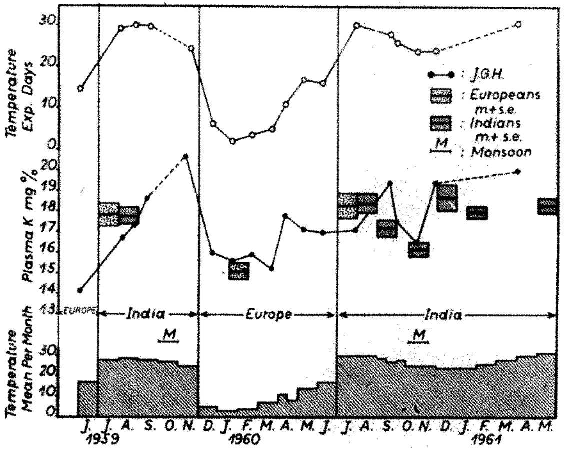

Fig. 1

variations are important and significant ($P < 0{\cdot}001$); potassium values are obviously correlated with dry bulb temperatures (Fig. 1). However, other potassium fluctuations seem to be superimposed over the main heat–cold effect. During the monsoon, the time–potassium curve shows a minimum corresponding to a sudden but slight decrease of temperature which in itself appears to be too small to affect the basic potassium value of the population later on, during December–January. There is no correlation between potassium values and relative humidity.

The potassium changes could be related to modifications in the activity of the suprarenal glands in hot and cold weather as suggested by previous investigations[1,2]. Influence of other meteorological factors and of sudden changes of temperature will be further investigated.

J. G. HENROTTE
P. S. KRISHNARAJ

Laboratoire de Pathologie et Thérapeutique
Générales,
University of Liège,
and Laboratory of Biochemistry,
Madras Medical College, Madras.

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Effect of Glutamine on Intoxication caused by Ethyl Alcohol

IN their work on the effect of alcohol on the amino-acids of the brain of the rat, Kulonen and Häkkinen[1] observed that animals receiving glutamine seemed to be far less intoxicated than those receiving none. Ravel *et al.*[2] isolated a factor from *Streptococcus* bacteria which made this organism resistant to alcohol. The factor proved to be glutamine. This prompted me to examine the effect of glutamine on alcohol intoxication. For such purposes, these laboratories have devised a tilting-plane technique[3–5], which is described in the first-mentioned work as follows: The rat is placed on a plane with a rough surface; the plane is tilted and at a certain angle the animal slides down. An intoxicated rat slides down at a lower angle than a sober rat. The performance in intoxication is expressed as a percentage of the sober value.

Seven groups of rats were used, all of them originating from two litters, aged 2–5 months and weighing 200–300 gm. (females) or 250–450 gm. (males). The number of rats in the groups was 6, 8, 7, 10, 10, 8 and 8 (total 57). The animals were fasted overnight before the experiments. Alcohol was given by stomach tube as a 30 per cent v/v solution; glutamine was administered in the same solution as alcohol. Three replications were used in all groups except two, in which there was only one (4 and 5) from which alcohol in the blood was determined.

The results are given in Table 1. There is great intergroup variation in the effect of glutamine. There is no effect in the females with the smaller dosage of alcohol, but this might be due to the fact that the females tolerate alcohol well[6] and do not become markedly intoxicated by it even if no glutamine is given; with the larger dosage of alcohol the glutamine effect appears even among them. The groups of males show great variation in the glutamine effect; the only one in which there was no effect was a group with only one replication.

Glutamine did not affect the alcohol-level in the blood, as is seen from Table 1. Obviously the reason for the effect of glutamine lies in the part played by this substance in brain metabolism during alcohol intoxication, as shown by Kulonen and Häkkinen[1]. The degree of the effect varies greatly according to environmental conditions, and most probably also according to genetic constitution.

LASSE SAMMALISTO

Research Laboratories of the
State Alcohol Monopoly (Alko),
Helsinki, Finland.

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Table 1. FIGURES GIVE THE MEAN PERFORMANCE OF EACH GROUP, EXPRESSED AS A PERCENTAGE OF THE SOBER VALUE. MEAN BLOOD ALCOHOL IS GIVEN IN BRACKETS IF MEASURED

Alcohol dosage (mgm./kgm. body-wt.)	Group		Hr. after administration			Group	Hr. after administration
			½	1	3		1
3·0	1	a		77·9 ± 3·80	84·4 ± 3·55	6	95·6 ± 1·14
		$a+g$		86·7 ± 3·16	95·3 ± 2·11		94·6 ± 1·25
		$P<$		0·01	0·01		
	2	a	83·8 ± 1·77	84·5 ± 1·73	93·5 ± 1·45	7	91·2 ± 1·12
		$a+g$	95·1 ± 0·86	96·7 ± 0·65	98·7 ± 0·47		92·2 ± 1·05
		$P<$	0·001	0·001	0·001		
	3	a	84·7 ± 2·72	83·9 ± 2·20	90·8 ± 1·32		
		$a+g$	90·1 ± 2·64	90·5 ± 2·32	94·0 ± 2·07		
		$P<$		0·05			
	4	a		78·1 ± 5·20 (207)			
		$a+g$		74·9 ± 5·45 (208)			
		$P<$		**Not significant**			
	5	a		62·1 ± 6·05 (203)			
		$a+g$		81·6 ± 4·18 (185)			
		$P<$		0·001			
4·5	1	a		63·9 ± 3·90		6	74·0 ± 1·19
		$a+g$		57·3 ± 2·82			78·6 ± 2·34
		$P<$					0·05

Effects on Sensory Nerves of injecting 5-Hydroxytryptamine into the Skin

CUTANEOUS mast cells in the rat differ from those of other species in that they contain, besides histamine, 5-hydroxytryptamine (5-HT)[1]. It is known that in the cat 5-HT excites certain cutaneous sensory nerve endings[2] and, since 5-HT is normally present in the rat's skin, there may be a possibility

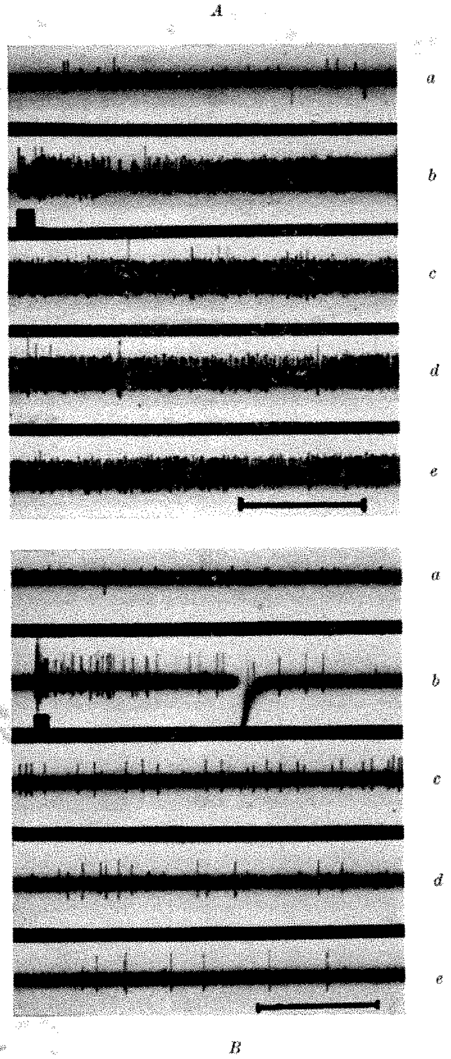

Fig. 1. Stimulation of sensory nerves produced by intra-arterial injection of 0·01 μmole 5-HT into the rat's skin (*A*) and the action of LSD (10 μgm./kgm.), administered intravenously 2 hr. previously, in antagonizing this effect (*B*). *a*, Spontaneous activity while artery is open; *b*, at signal, artery was closed and 0·1 ml. 5-HT injected; *c*, *d* and *e* show, respectively, the action of the drug 30 sec., 60 sec. and 105 sec. after time of injection. Time trace represents 5 sec.

that in this animal it has in certain circumstances a physiological action on cutaneous excitability. Results presented here show that the presence of 5-HT in small concentrations in the skin leads to a pronounced discharge of impulses in cutaneous sensory nerves.

Drugs were injected 'close-arterially' into a region of the skin innervated by a small branch of the saphenous nerve, from which impulses were recorded. Recordings were continued for 2 min. following each injection, after which time the blood supply to the skin was restored. All substances were dissolved in mammalian Ringer–Locke at *p*H 7·2 and the volume injected was 0·1 ml.

Injection of 0·01 μmole (1·8 μgm.) 5-HT produced a pronounced and long-lasting sensory discharge which began 1–2 sec. after completion of the injection (Fig. 1*A*). The latency of the response to 5-HT was always longer than to an injection of acetylcholine. The latter is known to excite sensory endings[3], but in these experiments the effective dose of acetylcholine was 50 times (0·5 μmole) larger than that of 5-HT.

The discharge evoked by 5-HT always occurred in two distinct phases. The first phase, consisting of a burst of impulses, lasted approximately 5 sec., after which time the activity diminished for 3–10 sec. Following this period of diminished activity, a renewed discharge occurred which then persisted for at least 2 min. When 5-HT injections were made after the rat had received 10 μgm./kgm. (3 μgm./300 gm.) lysergic acid diethylamine (LSD) intravenously, the second phase of the response gradually diminished, and after 2–3 hr. was abolished completely (Fig. 1*B*). The first phase of the response was little affected. LSD at no time affected the response evoked by mechanical stimulation of the skin or by an injection of acetylcholine.

The present work did not indicate whether 5-HT acted directly on certain sensory endings or whether the sensory stimulation was produced indirectly, for example, by vasoconstriction. However, the action of 5-HT could not be attributed to an indirect release of acetylcholine since hexamethonium (5 mgm./kgm. intravenously) did not affect the 5-HT response but did abolish the effects of injected acetylcholine. Histamine in amounts up to 1 μmole had little effect in these preparations, which also makes it unlikely that the 5-HT effect was due to a release of histamine.

According to Benditt[4], the concentration of 5-HT in the skin of the rat is approximately 2 μgm./gm. of tissue, which is roughly equivalent to 0·7 μgm./cm.^{2} of skin. Evans's blue injections indicated that, in the present experiments, at least 10 cm.^{2} (and probably more) of skin were perfused by the injected fluid. Since the total amount of 5-HT injected was 2 μgm., the maximum concentration in the skin would be about 0·2 μgm./cm.^{2}. It may be concluded, therefore, that disruption of cutaneous mast cells in the rat would result in strong stimulation of sensory nerves in the region affected. In view of the differences in mast cell content, it would be of interest to determine whether in other species histamine is more effective than 5-HT.

I thank Dr. J. H. Gaddum for his interest in this work, which was carried out during the tenure of a postdoctoral fellowship from the Life Insurance Medical Research Fund.

N. M. van Gelder*
Agricultural Research Council,
Institute of Animal Physiology, Cambridge.

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* Present address: Department of Pharmacology, Tufts University School of Medicine, Boston 11, Mass.

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Influence of the Growth of the Walker 256 Carcinoma on Hepatic Transaminases

In the course of a study of enzymes associated with protein metabolism in the livers of tumour-bearing rats we have observed certain significant changes in the pattern of transaminase activities.

Female albino rats weighing 140–160 gm. were injected intramuscularly into the right thigh with 1 ml. portions of a suspension of Walker 256 carcinoma[1]. A control group received an intramuscular injection of 1 ml. 0·9 per cent saline. Throughout the experimental period both groups of rats were fed on synthetic diets described previously[2]. Groups of rats were killed at 3, 5, 7 and 9 days after inoculation and hepatic L-alanine-α-oxoglutarate and L-aspartate-α-oxoglutarate transaminases measured by colorimetric methods[3]. Under the experimental conditions used the mean weights of the tumours at 3, 5, 7 and 9 days after implantation were found to be 0·1 ± 0·08, 2·9 ± 0·5, 9·7 ± 0·9 and 14·6 ± 1·0 gm. respectively.

The concentration of hepatic alanine transaminase activity fell to about 50 per cent of the control value by the fifth day of tumour growth and this fall persisted over the remainder of the period studied. The total hepatic activity was also significantly lowered at the same stage of tumour growth (Table 1). There was a concomitant increase in the total activity of the closely related aspartate transaminase although the concentration of this enzyme was unaltered (Table 2).

Table 1. EFFECT OF TUMOUR GROWTH ON HEPATIC L-ALANINE-α-OXOGLUTARATE TRANSAMINASE ACTIVITY

Alanine transaminase activity*	Group	Time after injection 3 days	5 days	7 days	9 days
Per gm. liver	Control	0·76 ± 0·07 (5)	0·95 ± 0·06 (6)	1·02 ± 0·06 (6)	1·02 ± 0·06 (6)
	Tumour-bearing	0·61 ± 0·10 (6)	0·52 ± 0·03 (6)	0·49 ± 0·03 (6)	0·52 ± 0·09 (6)
	P	> 0·10	< 0·001	< 0·001	< 0·001
Per liver/100 gm. initial body-wt.	Control	2·22 ± 0·25 (5)	2·93 ± 0·16 (6)	3·06 ± 0·18 (6)	3·26 ± 0·15 (6)
	Tumour-bearing	2·18 ± 0·28 (6)	2·18 ± 0·22 (6)	2·23 ± 0·27 (6)	2·11 ± 0·31 (6)
	P	> 0·10	< 0·02	< 0·05	< 0·01

Results are expressed as the means ± *S.E.* Figures in parentheses refer to the number of animals in each group.

* The enzyme activities are expressed as m.moles pyruvic acid formed per 10 min. at 37° C.

Table 2. EFFECT OF TUMOUR GROWTH ON HEPATIC L-ASPARTATE-α-OXOGLUTARATE TRANSAMINASE ACTIVITY

Aspartate transaminase activity*	Group	Time after injection 3 days	5 days	7 days	9 days
Per gm. liver	Control	1·94 ± 0·12 (5)	1·97 ± 0·06 (6)	2·11 ± 0·08 (6)	2·04 ± 0·22 (6)
	Tumour-bearing	2·05 ± 0·21 (6)	2·00 ± 0·15 (6)	2·01 ± 0·09 (6)	2·54 ± 0·41 (6)
	P	> 0·10	> 0·10	> 0·10	> 0·10
Per liver/100 gm. initial body-wt.	Control	5·65 ± 0·33 (5)	6·05 ± 0·13 (6)	6·36 ± 0·31 (6)	6·39 ± 0·41 (6)
	Tumour-bearing	5·61 ± 0·27 (6)	8·30 ± 0·55 (6)	8·95 ± 0·49 (6)	10·20 ± 0·81 (6)
	P	> 0·10	< 0·01	< 0·002	< 0·002

Results are expressed as the means ± *S.E.* Figures in parentheses refer to the number of animals in each group.

* The enzyme activities are expressed as m.moles oxaloacetic acid formed per 10 min. at 37° C.

It has been shown previously[2] that these animals exhibited a considerable hepatomegaly throughout the experimental period. Thus it would appear that while the level of aspartate transaminase is keeping pace with the increase in liver size there is a definite depression of the alanine transaminase.

The activity of hepatic alanine transaminase has been shown to be related to the level of circulating glucocorticoids. Thus administration of corticotrophin or corticosteroids with gluconeogenic activity was found to cause a marked increase in the activity of this enzyme[4] whereas adrenalectomy resulted in reduced hepatic alanine transaminase activity[5]. It might be inferred, therefore, that the fall in hepatic alanine transaminase activity observed after 5 days of tumour growth might reflect a reduction in the level of circulating glucocorticoids. This view would be compatible with a previous finding that livers of rats bearing the Walker 256 carcinoma have an increased capacity to inactivate corticosterone as early as 30 hr. after tumour implantation[2].

This investigation was supported by a grant from the Medical Research Council.

G. A. J. GOODLAD
C. M. CLARK

Department of Physiology and Biochemistry,
St. Salvator's College,
University of St. Andrews,
Fife.

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HISTOCHEMISTRY

A Quantitative Histochemical Method for estimating Phospholipids

THE affinity of the 'Luxol' solvent dyes for phospholipids has been investigated. One of these dyes, 'Luxol' fast blue *MBS*, was introduced by Kluver and Barrera[1] as a myelin stain. It is a diarylguanidine salt of a sulphonated copper phthalocyanine dye. The dyes used in this investigation are diarylguanidine salts of sulphonated azo dyes. 'Luxol' fast blue *G*, 'Luxol' fast blue *ARN*, and 'Luxol' fast black *L*, have been found to form complexes with phosphotidyl choline, phosphotidyl ethanolamine, and phosphotidyl serine. Pearse[2] has already noted that 'Luxol' fast blue *MBS* forms precipitates with certain phospholipids in *in vitro* tests. He believed it was possibly an acid base reaction and that the base of the phospholipid replaced the base of the dye. He found his precipitates confined to the choline containing phospholipids. I have found that the dyes 'Luxol' fast blue *G* and *ARN*, and the phospholipids, phosphotidyl choline and phosphotidyl serine, form complexes stoichiometrically. Furthermore, there is a reversal of their common solubility in absolute ethanol (Table 1).

Table 1

Dye or dye complex	Solubility at 25° C. Water *p*H 7	Ethanol absolute	50 per cent ethanol
'Luxol' fast blue *G* 'Luxol' fast blue *ARN*	Insoluble	Readily	Slightly soluble
Dye-phosphotidyl choline complex	Soluble	Insoluble	Fairly
Dye-phosphotidyl ethanolamine complex	Soluble	Slowly soluble	Fairly
Dye-phosphotidyl serine complex	Soluble	Insoluble	Fairly

The phospholipid-dye complexes are soluble in neutral or alkaline water. They are not soluble in acidified water, however. These findings have been adapted as a staining method for phospholipids which I believe is quantitative or at least comparative. Riemersma[3] recently has stated that lecithin binds the anionic dye Ponceau 4*RS* in molecular proportions. He believes the quaternary ammonium group of the lecithin molecule to be involved, and that Ponceau 4*RS* can be regarded as a reagent for the positively charged choline group. However, this theory would not seem to account for the formation of a phosphotidyl serine-dye complex. In textile dyeing, direct dyes have been 'fixed' *in situ* with quaternary phosphonium compounds containing pentavalent phosphorus as well as with quaternary ammonium compounds.

The dye solution used was 0·1 per cent solution of either 'Luxol' fast blue *G* or *ARN* in absolute ethanol.

Measured quantities of lecithin and phosphotidyl serine in pyridine solution were placed on filter paper strips, and the pyridine allowed to evaporate completely. The strips were then placed in the dye solution for 1 hr. at room temperature. They were then rinsed in several changes of fresh absolute ethanol until all the uncomplexed dye was removed. Even leaving the strips for 24 hr. in absolute ethanol has not been found to remove the dye-phospholipid complex. The complex can be removed from the strips by eluting with slightly alkaline water. A 1 per cent solution of concentrated ammonia solution in water was used. The dye-phospholipid complex in this aqueous solution was then examined spectrophotometrically and found to be in direct proportion to the amount of phospholipid deposited originally on the strips.

Tissues of rat and dog brain were examined. The material was fixed in 10 per cent formalin, calcium formol or the following mixture[4]: water 90 ml., formaldehyde solution 10 ml., calcium chloride 1 gm., and cetyltrimethylammonium bromide 0·5 gm. Paraffin sections were cut of this material at 10μ, and the sections mounted on slides. When dried the paraffin was removed with xylene, and the sections rinsed in three changes of absolute ethanol and then placed in the above dye solution for 1 hr. at room temperature. They were then rinsed in several changes of absolute ethanol, cleared in xylene and mounted. In some cases 2 per cent pyridine was added to the dye solution, as this and small amounts of other bases appear to prevent any tendency for other tissue elements to stain. In the case of brain sections only phospholipid-containing tissue elements were stained. These findings were compared with the staining obtained with Baker's hæmatein method for phospholipids, and found to be very similar.

The affinity of these dyes for phospholipids is being further examined.

The samples of dye were supplied by the Organic Chemicals Department, E.I. du Pont de Nemours Co., Wilmington 98, Delaware.

T. N. SALTHOUSE *

Haskell Laboratory,
E.I. du Pont de Nemours Co.,
Newark,
Delaware.

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* Present address: Pathology Department, The Squibb Institute for Medical Research, New Brunswick, N.J.

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An Improved Intravital Dye Iron-Prussian Blue Method for the *in vitro* Examination of Living Lymphatic Vessels

IN the nineteenth century Fodéra[1] used ferrous sulphate, converted to prussian blue, to demonstrate lymphatic vascular absorption in the experimental animal. With the introduction of pharmaceutical iron colloids, Turner-Warwick[2] found that 'Inferon' Benger (iron-oxide dextran complex) could similarly be used to demonstrate the afferent lymphatic vessels of the living female breast. For cleared specimens the staining method had certain disadvantages chiefly due to the solubility of prussian blue in alcohol during dehydration[3].

In the method described here, pharmaceutical 'Ferrivenin' Benger (colloidal saccharated iron oxide) is prepared as an isotonic solution (300 m.osmols/gm. water) by dilution in distilled water. When this solution (rendered visible by addition of patent blue violet) is injected intradermally by the method of Hudack and McMaster[4], it enters the fine lymphatic capillaries where it can be readily demonstrated by routine histology. For cleared whole specimens the disadvantages noted here[3] are overcome by treatment of the freshly stained material with alcoholic ammonia. This converts prussian blue to its alcohol-insoluble ammonium complex (stable in the presence of a very small excess of ammonia) and at the same time removes extraneous dye by alkali decomposition. The fixation method depends on the fact that 'Ferrivenin', though soluble in water, is insoluble in alcohols.

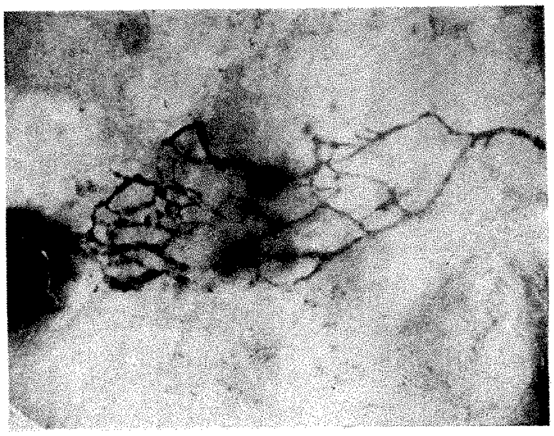

Fig. 1. Normal mouse ear. 10 min. before injection a light stroke with a blunt probe was made across the ear. (× *c*. 12·5. Cleared in oil of wintergreen)

Foci of abnormal lymphatic permeability, calibre and distribution are then clearly demarcated. (In practice iron diffusion from normal lymphatics is never seen (Fig. 1).) The illustration shows how this can be demonstrated by McMaster's experiment[5], in which a normal mouse ear is stroked transversely with a blunt probe 10 min. before injection. The cleared specimen shows a zone of increased lymphatic permeability at the line of the stroke. Distal to it the lymphatics are dilated, while the proximal vessels show a noticeable narrowing.

The method is as follows (sterile materials required for work on human beings):

(1) Dilute 5 ml. sterile 'Ferrivenin' Benger in 18 ml. sterile distilled water (about 21·7 per cent v/v).

(2) Add 2 ml. sterile isotonic patent blue violet solution (11 gm. per cent in distilled water) as a marker.

(3) Store in a rubber-capped vaccine bottle. This prevents ingress of moisture which lowers the osmotic pressure. (Solutions of less than 240 m.osmols/gm. water are of little value.)

(4) Inject this mixture by any technique based on that of Hudack and McMaster[4]. For work with small animals the microneedle of Florey *et al.*[6] is recommended for controlled studies. An 8 × red filter gives good contrast photography with patent blue violet.

(5) Resect the specimen with the minimum of trauma, in the mouse ear clamping should be avoided A delay of about 5 min (for example, for photography) does not harm the result in animal work In human skin special methods are necessary if delay is unavoidable

(6) Fix in a mixture of 10 parts 40 per cent aqueous formaldehyde and 90 parts absolute alcohol (for example, 74° proof industrial spirit) or 'Cellosolve' (2 ethoxy-ethanol B D H.) This latter substance does not have the hardening effects associated with alcohols (Leach, personal communication)

(7) After fixation for 4–12 hr , depending on size, the specimen is ready for routine histology A celloidin method (for example, that of Chesterman and Leach, personal communication) gives good results, since the embedded iron is not labile to acid distilled water and forms a well-defined blue 'mass' by Perl's method Paraffin and 'Estax' methods do not hold the iron so well and give a fine granular precipitate of prussian blue

(8) For cleared whole specimens, transfer the fixed material to 'Cellosolve' for 24 hr to remove traces of blue dye

(9) Trim and blot dry (Mouse ears should be trimmed off at the root)

(10) Stain 5–10 min in a warm acid ferro-cyanide mixture (for example, 1 part 25 per cent hydrochloric acid and 9 parts potassium ferrocyanide under a 60-W lamp)

(11) Observe in a clear solution (for example, distilled water) The injection site and (in thin specimens) the adjacent lymphatics should now be clearly visible Specimens more than 1 0 cm from injection site to cut edge may require a further 5–10 min staining to differentiate the remoter vessels

(12) Wash in tap-water and transfer to 'Cellosolve' containing 5 per cent v/v of strong (specific gravity 0 880) ammonia Shake The alkali decomposes extraneous dye, bleaching the specimen, the injected material is also changed to a deep navy blue colour

(13) Transfer to 'Cellosolve' containing 0 25 per cent v/v ammonia The number of changes depends on specimen size

(14) Clear in oil of wintergreen for 24–48 hr The specimen is then ready for photography Selected portions may be embedded directly in nitro-cellulose for histology or returned to 0 25 per cent ammonia 'Cellosolve' for storage

A method of delaying lymphatic flow for work on normal skin is now being investigated Abnormal skin may give good results without special measures if the flow-rate is pathologically reduced The technique of Hudack and McMaster[7] is used for the injection In practice a 'first' needle is not required if the injecting needle is first inserted point downward and then rotated

F R WELLS

Department of Plastic Surgery,
Churchill Hospital,
Headington,
Oxford

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HÆMATOLOGY

Penicillin Sensitization of Paroxysmal Nocturnal Hæmoglobinuria Erythrocytes

THE ability of penicillin to function as a hapten can be detected by a hæmagglutination technique involving the use of penicillin-coated erythrocytes[1,2] It appears that all normal human erythrocytes as well as those from rabbits and sheep can be successfully sensitized with the antibiotic As part of an investigation to characterize the antibody concerned, it was found that the majority of sera giving positive direct hæmagglutination of sensitized cells suspended in saline failed to cause hæmolysis with added complement, although hæmolysis occurred if enzyme-treated penicillin-sensitized cells were used instead Since sera from patients with certain forms of auto-immune hæmolytic anæmia will also cause hæmolysis of paroxysmal nocturnal hæmoglobinuria erythrocytes, experiments were carried out to determine whether paroxysmal nocturnal hæmoglobinuria penicillin-sensitized cells would undergo hæmolysis in the presence of antibody and complement/properdin complex

Erythrocytes from a patient with paroxysmal nocturnal hæmoglobinuria and normal, group O, Rh-negative cells were sensitized with crystalline penicillin *G* as described previously[2] Hæmagglutination was tested for by adding two volumes of a 5 0 per cent suspension of washed cells in 0 85 per cent saline to six volumes of serial dilutions of known positive serum (titre 1/512) After incubation at 37 0° C for 1 5 hr , the tubes were centrifuged at 1,000 r p m for 1 min and hæmagglutination read by light resuspension of the cell deposits For the hæmolysis experiments dilutions of positive serum were made in fresh normal group AB serum Results are shown in Table 1

Table 1

	Hæmagglutinin titre	Hæmolysin titre
Penicillin-coated paroxysmal nocturnal hæmoglobinuria cells	<1/2	nil
Penicillin-coated normal cells	1/512	nil

Not only did paroxysmal nocturnal hæmoglobinuria cells fail to hæmolyse but also they failed to agglutinate at serum titres as low as 1/2, and repeated attempts to sensitize them with varying concentrations of penicillin were unsuccessful Antiglobulin tests performed on the washed cells were also negative Similarly, enzyme-treated penicillin-sensitized paroxysmal nocturnal hæmoglobinuria cells failed to react. When washed 'ghost' membranes, prepared by lysis of normal penicillin-sensitized cells and paroxysmal nocturnal hæmoglobinuria penicillin-treated 'cells in distilled water, were plated on to an agar plate seeded with a highly sensitive strain of *Sarcina lutea*, wide zones of inhibition surrounded the membranes from normal cells whereas no inhibition zones were noted around paroxysmal nocturnal hæmoglobinuria membranes This suggests that the phenomenon is due to a failure of adsorption of penicillin to paroxysmal nocturnal hæmoglobinuria cell stromal tissue

Further investigation showed that the adsorption defect was a selective one since paroxysmal nocturnal hæmoglobinuria cells were fully capable of adsorbing *Vi* antigen from a saline extract of acetone dried *Vi*-containing organisms, giving identical titres with *Vi* antiserum as did normal cells sensitized with *Vi* extract Penicillin sensitization of erythrocytes from a patient with congenital spherocytosis indicated that they adsorbed the antibiotic in the same way as normal cells

The nature of the stromal abnormality of paroxysmal nocturnal hæmoglobinuria cells has not been determined Electron microscopy has revealed a patchy pitted surface with many clefts[3] Chemical studies have indicated a decrease in phosphorylcholine and an increase in phosphorylserine[4], although these findings have since been refuted[5] Similarly, abnormalities in stromal lipoprotein described by Munn and Crosby[6] may be dependent on variations in the dietary consumption of fat[7] A marked reduction in the uptake of radioactive phosphorus by paroxysmal nocturnal hæmoglobinuria cells as compared with normal cells has been described[8] and also a deficiency of acetylcholinesterase[9], but the significance of these observations is obscure

K C Watson

Department of Pathology,
University of Natal,
Durban

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Effect of Anæmic Anoxia on Erythropoiesis of Nephrectomized Dog

Experiments performed on the dog suggest that the kidney is a source of production of an erythropoietic factor. Erythropoiesis is suppressed by bilateral nephrectomy while this function is maintained after ureteral ligation[1,2] Furthermore, erythropoietin plasma-level decreases after nephrectomy in an anæmic dog and is unaffected by ureteral ligation, despite the same accumulation of metabolic waste products[3] The present investigation was designed to determine whether it is possible to stimulate erythropoiesis in dogs made anæmic by severe bleedings performed after nephrectomy

Control measurements were made in 22 normal dogs, eight dogs were subjected to bilateral nephrectomy only (details and results concerning this group have been reported elsewhere[2]), eight dogs were severely bled immediately after nephrectomy three daily withdrawals of blood were made on four animals (each bleeding was equal to 2 5 per cent of the body-weight), four other animals were bled twice only and the quantity of blood withdrawn each time was equal to 2 per cent of the body-weight The first bleeding was performed 30 min after the surgical procedures The volume of blood was maintained by infusion of dextran

Erythropoiesis was measured by numeration of marrow erythroblasts, reticulocytes and plasma iron turn-over carried out 3 days after nephrectomy Results are summarized in Table 1

Normal values for the percentage of erythroblasts and reticulocytes and for turn-over of plasma iron were respectively 23 3 per cent, 3 1 parts per thousand and 0 78 mgm /kgm /day Three days after nephrectomy, these values averaged 0 6 per cent, 0 9 part per thousand and 0 24 mgm /kgm /day respectively When bleedings were performed, no stimulation of erythropoiesis could be disclosed on the third day, despite severe anæmia (hæmatocrit between 18 and 29 per cent) The same percentage disappearance of erythroblasts (1 8 per cent) and percentage reduction of reticulocytes (0 8 per cent) occurred as in non-anæmic nephrectomized dogs. the turn-over of plasma iron was of the same magnitude (0 26 mgm /kgm /day) in both groups

Table 1 Effect of Bleeding on the Erythropoiesis of Nephrectomized Dogs

Group	No	Hæmatocrit (per cent)	Reticulocytes (parts per thousand)	Erythroblasts (per cent)	Iron turn-over mgm / kgm /day
I Normal	22	49 ± 0 96	3 1 ± 0 68*	23 3 ± 2 23	0 78 ± 0 06
II Nephrectomized	8	44 ± 1 57	0 9 ± 0 41	0 6 ± 0 18	0 24 ± 0 05
III Bled after nephrectomy	8	24 ± 1 43	0 8 ± 0 18	1 8 ± 0 50	0 26 ± 0 04
P of differences					
I–II		< 0 01	*N S*	< 0 001	< 0 001
II–III		< 0 001	*N S*	< 0 05	*N S*

* Values are means ± standard error of the mean

According to these observations, it can be concluded that in the absence of the kidney there is no more regulation of erythropoiesis probably because the source of erythropoietin has been removed This does not support the observation published by Erslev[4], who has demonstrated an increased erythropoietic activity in nephrectomized bled rabbits

Nevertheless, our results lead to the same conclusion as that drawn from the disappearance of erythropoietin from plasma of anæmic dogs following nephrectomy These observations suggest that, in the dog, there is no other site of production of an erythropoietic factor outside the kidney

As has been shown by previous experiments[2], retention of toxic products does not seem to be responsible for the non-regulation of erythropoiesis, after nephrectomy, but this possibility is now under investigation

J P Naets
A F Heuse

Clinique Médicale de l'Hôpital Brugmann,
Université Libre de Bruxelles,
et Laboratoire de Biologie clinique,
Fondation Médicale Reine Elisabeth

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PATHOLOGY

Cariogenicity of Common Sugars as evaluated in the Artificial Mouth

The artificial mouth is a device for simulating *in vitro* the principal oral conditions presumably involved in dental caries[1] Qualitatively, the *in vitro* lesions produced in human teeth are similar to those produced naturally[2-4] The method has been used for the quantitative evaluation of anti-caries agents and dentifrices[5-7]

The procedure consists of exposing extracted human teeth to a mixed culture of oral micro-organisms[5,6] A bacteriological medium is dripped continuously on the tooth, which is covered with a mixed bacterial plaque derived from oral micro-organisms The rate of softening of the external enamel is followed by measurements of the change of hardness The sugar used in the medium has generally been D-glucose, the concentration of which has been shown to be very important in determining the type of attack[8] When the sugar was present in low concentrations (< 0 10 gm /100 ml), the teeth were not attacked unless decalcified dentin was present For concentrations of 0 5 gm /100 ml , the attack was mainly a decalcification, and the dentinal matrix was not attacked For concentrations of about 0 2–0 3 gm /100 ml , both decalcification and dentinal matrix destruction occurred

The present work was done in order to determine whether other common sugars can be used instead of D-glucose and to compare their cariogenicity under these conditions

The effect of the following sugars in the bacteriological medium used in the artificial mouth was studied D-glucose, D-fructose, D-mannose, D-galactose, lactose, maltose and sucrose Soluble starch was also included in the investigation The original plaques were grown in the presence of these sugars, and they were also present during the tooth-softening period Some difference in amount of bacterial plaque formation was noted, but the amount was always sufficient to cover the experimental tooth surfaces The amount of sugar (0 3 gm /100 ml of hexose equivalent) was the same as the optimal amount found previously for D-glucose to produce maximal decalcification and dentinal matrix destruction

The average softening-rates are given in Table 1 The rate for D-glucose was similar to that previously reported[6] and was used as the standard for comparisons For D-fructose, D-mannose and sucrose, the softening rates were similar to those for D-glucose Those for D-galactose, maltose and lactose seemed to be less than for the other sugars However, analysis by the Student *t* test indicated that only maltose was significantly different from D-glucose at the 0 01-level of confidence

When soluble starch was present in the medium, the teeth showed no change during the experimental period

Table 1 COMPARISON OF DIFFERENT SUGARS* ON THE RATE OF SOFTENING OF ENAMEL SURFACES IN THE ARTIFICIAL MOUTH FOR 8 HR

Sugar	No of teeth	Softening-rate† (percentage per hr)	*S D*
D-Glucose	23	6 1	2 4
D-Fructose	12	6 2	3 3
D-Mannose	12	7 3	3 1
D-Galactose	10	4 8	2 2
Lactose	12	4 9	2 3
Maltose	12	3 8	1 8
Sucrose	16	5 2	3 8
Soluble starch	12	0 0‡	—

* Present as hexose equivalent of 0 3 gm /100 ml of culture medium

† $\dfrac{\text{Decrease in Knoop hardness No.} \times 100}{\text{Time (hr)} \times \text{initial hardness}}$

‡ The teeth of this group all showed a slightly greater hardness than the initial value

The *p*H and titratable acidity of the effluent media were measured after the 8 hr operation of the artificial mouth The absolute values have no significance, since they are dependent on the rate of flow For all the effluent media which contained sugars, the *p*H was in the range 5 0–6 0, whereas that of the original media was 6 4–6 7 The production of acids was also shown by an increase of the titratable acidity (to *p*H 8 0) of the medium, but no consistent pattern was evident for the sugars For the soluble starch solutions, the *p*H was 6 3–6 4, and some titratable acid was formed

The two most common dietary sugars, D-glucose and sucrose, thus produced similar rates of softening of teeth in the artificial mouth when incorporated in the bacteriological medium This result is not surprising since many bacteria can use either interchangeably Slower rates produced by galactose and lactose, if significant, might be explained by their utilization by fewer micro-organisms, actually the oral micro-organisms did utilize them and produced enamel softening

The lack of any softening by soluble starch is probably related to the slight ability of the oral micro-organisms to break down the starch to fermentable sugars This particular result has no direct relation to oral conditions, however, since saliva contains amylases which hydrolyse starches at least partially to fermentable oligosaccharides The low rate produced by maltose is interesting in this connexion since its rate was the lowest of the sugars, and it is a major product of amylase action

As shown earlier[9], the softening produced by the medium containing D-glucose was completely prevented in the presence of calcium phosphate solutions which will reharden softened surfaces Presumably, the effects of other sugars would be similarly inhibited

This work was supported by the Office of Naval Research, Dental Branch (*NONR* 3139(00)), and by the American Chicle Co

WARD PIGMAN
JAMES BRASHER
THEODORE KOULOURIDES

Biochemistry Departments,
University of Alabama Medical Center,
Birmingham, Alabama, and
New York Medical College,
New York

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IMMUNOLOGY

Reduced Antibody Response in Thymectomized Rabbits

DESPITE considerable experimental work, the function of the thymus has not yet been clearly delineated Although it is an organ rich in lymphoid cells, it differs from other lymphoid organs in its failure to produce significant amounts of antibody after antigenic stimulation[1–3] and in its embryonic origin from the endoderm of the third pharyngeal pouch The thymus is largest relative to body-weight during the period of immunological deficiency early in life and it becomes progressively smaller after the immune responses are fully developed[4], a growth pattern which suggests its relation to the development of immunological mechanisms The occurrence in the same patient of the two rare conditions, namely, benign thymoma and acquired agammaglobulinaemia[5–7], suggested a relationship of the thymus to immunological processes Thymectomy in adult or even in young rabbits[6,8,9] had no effect on their antibody response, but subtotal thymectomy performed on young guinea pigs caused reduced antibody formation in some of the experimental groups[10]

Other work which suggested neonatal thymectomy in rabbits was that of Mueller *et al* [11] Removal of the bursa of Fabricius, a lymphoid organ of endodermal origin, also reduced antibody production Because of these observations thymectomy in the experiments reported here was performed in neonatal rabbits the antibody response of which was assessed at a later time. A preliminary report has been presented[12]

New Zealand albino rabbits were anæsthetized with ether and their thymuses were removed through a median incision of the chest wall either by surgical dissection or with a glass pipette connected to an aspirator The incision was closed with silk suture Litter mates were either sham operated or left unoperated as controls Three separate experiments were performed In the first, thymectomy was carried out at five days and the rabbits were challenged with bovine serum albumin 50 mgm /kgm of body-weight intravenously at 7–8 weeks of age The anti-bovine serum albumin content of the serum was measured by the capillary precipitin method (Table 1) and by the ammonium sulphate precipitation technique of Farr[13] (Table 2) These tables show that the thymectomized rabbits had a diminished antibody response to bovine serum albumin as compared with litter-mate controls

In the second experiment, the rabbits were thymectomized at 5–7 days of age and challenged with bovine serum albumin at 5–7 weeks The results of similar determinations in this series are given in Tables 3 and 4 Although the thymectomized rabbits responded less frequently and, as a group, formed less antibody than the controls, the difference was not significant

Table 1 ANTIBODY RESPONSE TO BOVINE SERUM ALBUMIN AT 14 DAYS BY CAPILLARY PRECIPITIN METHOD

	No of animals	Percentage of positive antibody response
Thymectomized	7	0
Unthymectomized (sham- and un-operated)	10	80

Table 2. ANTIBODY RESPONSE TO BOVINE SERUM ALBUMIN IN EXP 1 (AMMONIUM SULPHATE PRECIPITATION TECHNIQUE OF FARR)

	14 days			21 days		
	No. of animals	Mean	Range	No of animals	Mean	Range
Thymectomized	7	6 6*	0–19 5	7	9 8	0–42 5
Unthymectomized (sham- and un-operated)	10	52 4	0–129 0	10	62 2	14 5–207 9
		$P < 0\ 01$			$P < 0\ 01$	

* Results are expressed as the gamma of bovine serum albumin labelled with iodine-131 bound per 100 ml of undiluted serum at that dilution of antiserum (which bound 33 per cent of 0 01 gamma of bovine serum albumin nitrogen added)

Table 3 ANTIBODY RESPONSE TO BOVINE SERUM ALBUMIN AT 14 DAYS BY CAPILLARY PRECIPITIN METHOD

	No of animals	Percentage of positive antibody response
Thymectomized	9	44
Unthymectomized	27	60

Table 4 ANTIBODY RESPONSE TO BOVINE SERUM ALBUMIN IN EXP 2 (AMMONIUM SULPHATE PRECIPITATION TECHNIQUE OF FARR)

	14 days			21 days		
	No of animals	Mean	Range	No of animals	Mean	Range
Thymectomized	9	23 3*	0–57 0	8	25 1	7 8–58 4
Unthymectomized (sham- and un-operated)	26	31 0	0–95 7	21	41 8	0–178 0
		P value is not significant			P value is not significant	

* Results are expressed as the gamma of bovine serum albumin labelled with iodine-131 bound per 100 ml of undiluted serum at that dilution of antiserum (which bound 33 per cent of 0 01 gamma of bovine serum albumin nitrogen added).

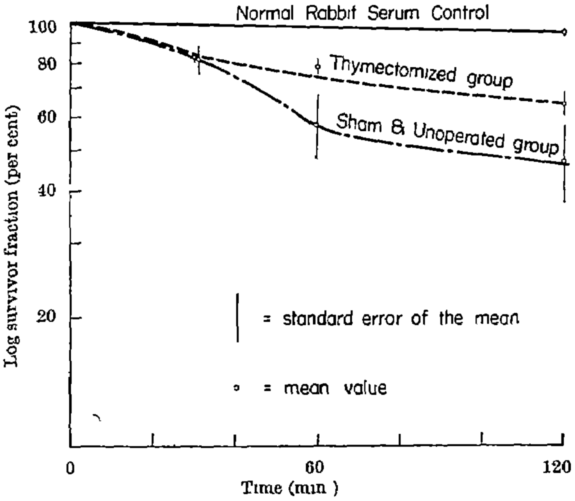

Fig 1

In the third experiment the rabbits were thymectomized at 0–3 days and were immunized with 8 × 10^9 T_2 coliphage particles/kgm at 14–18 weeks of age Phage neutralization-rates[14] on 7-day serum samples are given in Fig 1 The thymectomized rabbits formed significantly less neutralizing antibody against the bacteriophage than did the control animals

These experiments indicate that thymectomy in the new-born rabbit depresses its antibody response at maturity In the first and third experiments the differences between the thymectomized and control groups were highly significant The second experiment showed the same trend although the results fell below the level of significance, reflecting in part a poor antibody response of control rabbits Also, it is probable that the time of thymectomy in the new-born rabbit is very critical because significant results were recorded only when the operation was performed at 5 days of age or less Very early thymectomy may be especially important in animals such as the rabbit, which appear immunologically more mature at birth than animals such as the mouse in transplantation and tolerance studies[15]

These experiments on rabbits, and recent work showing that transplantation barriers can be bridged in mature mice thymectomized in the neonatal period[16,17], point to the importance of the thymus in the development of the immune response Whether this effect is consequent to humoral or cellular mechanisms remains to be defined The experiments of Comsa[18] and Metcalfe[19] suggesting that extracts of thymus can cause lymphocytosis point to a humoral mechanism Whether neonatal thymectomy quantitatively reduces the total number of cells available in the lympho-reticular system or whether it removes elements which are qualitatively distinct is likewise undefined Our present hypothesis is that the thymus in new-born mammals and the bursa of Fabricius in newly hatched fowl represent important centres for centrifugal distribution of immunologically competent cells to other lymphatic organs A similar concept has been discussed elsewhere[20-22]

This work was aided by grants from the U S Public Health Service American Heart Association, Minnesota Heart Association and National Foundation

Olga K Archer*
James C Pierce
Ben W Papermaster
Robert A Good†
Pediatric and Surgical Research Laboratories,
Variety Club Heart Hospital,
University of Minnesota

* Fellow of Pediatric Research HTS 5462

† American Legion Memorial Heart research professor of pediatrics

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ANATOMY

Prediction of Muscle Content of Steer Carcasses

With the emphasis in beef production shifting rapidly to the development of cattle with a high lean content, it is becoming increasingly important that methods should be available whereby accurate prediction of carcass muscle can be made The accepted practice of using measurements of a cross-section of the longissimus dorsi muscle has been shown to have a low degree of accuracy[1,2]

It was found by Orme *et al* [3] that the weights of some individual muscles and muscle groups of a line of mature Hereford cows were closely correlated to their total carcass muscle, and these authors suggest that the relationships might apply over a wide range of carcasses

This communication, based on the complete individual muscle dissection of thirty-five steers of various breeds and ages, records the similarity of results obtained irrespective of breed and age, and indicates the greater degree of accuracy attained by proper anatomical dissection of all muscles than by either dissection of lean from commercial cuts[1], or the gross separation of lean from other tissues[3] The steers dissected were drawn from the following breeds Poll Hereford, 19, Hereford, 4, Brahman/Hereford Crossbred, 8, and $\frac{3}{4}$ Bred Brahman, 4 The ages of the cattle ranged from six months to four years The dissection technique, to be published elsewhere, was carried out by one individual and was less time-consuming than the procedure reported by Walker[4]

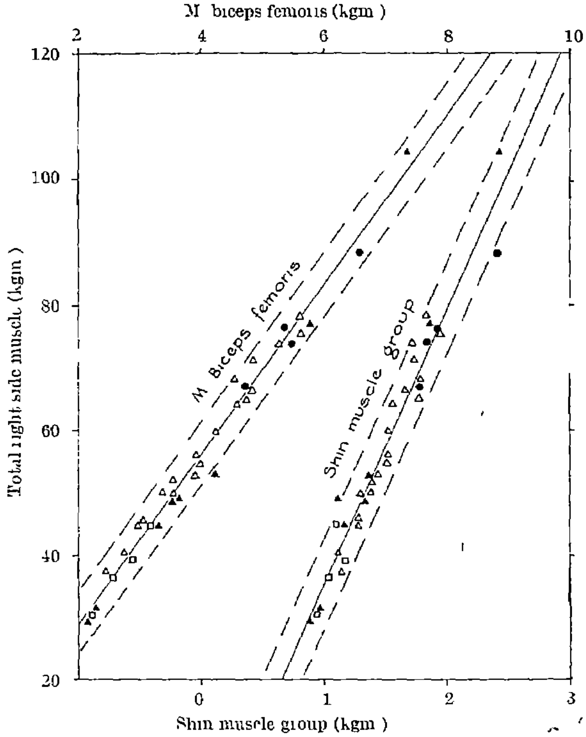

Fig 1 Regression of total right side muscle weight on the weight of biceps femoris muscle and shin muscle group M biceps femoris, $y = 1,344 + 13\ 6578\ x$(gm), $r = 0\ 9908$ Shin muscle group, $y = -7,905 + 43\ 6127x$(gm), $r = 0\ 9760$ □, Hereford, ⊿, Polled Hereford, ▲, Brahman × Hereford, ●, $\frac{3}{4}$ Bred Brahman

All muscles were weighed and data from the analysis of the weights of selected individual muscles versus total carcass muscle weight of this heterogeneous group of cattle are given in Table 1 The data presented are for right sides of carcasses only, and justification for the assumption of bilateral symmetry will be published later

Coefficients of correlation obtained were significantly better at the 5 per cent level than those published by Orme *et al* [3] This indicates that data

Table 1 Regression of Total Right Side Muscle Weight on Weight of Certain Muscles or Muscle Groups for Thirty-Five Steers

Muscle	Regression equation	Error Syx*	Error $Sbyx$†	Correlation coefficient r	Significances of differences of correlation coefficients‡ t
Biceps femoris	$y=1,344+13\ 6578x$(gm)	2,330	0 3234	0 9908	2 258§ (ref 3)
Semimembranosis plus adductor femoris	$y=-926+14\ 2480x$(gm.)	2,759	0 4015	0 9866	3 800¶ (ref 3)
Infraspinatus	$y=3,295+44\ 2487x$(gm.)	3,763	1 7073	0 9760	4 696¶ (ref 3)
Longissimus dorsi	$y=2,317+14\ 7452x$(gm.)	2,923	0 4404	0 9855	1 9552¶ (ref 3)
Semitendinosis	$y=9,871+33\ 0438x$(gm.)	5,106	1 7771	0 9550	2 9816¶ (ref 3)
Shin muscle group	$y=-7,905+43\ 6127x$(gm.)	3,742	1 6859	0 9760	5 2412¶ (ref 1)

* $t_{0\ 05} \times Syx$ gives 95 per cent limits of error in estimation of total muscle weight (ref 7)

† $Sbyx$ is standard error of regression coefficient

‡ Test between differences in correlation coefficients of present data and those of references 1 and 3 using Z' transformation (refs 6, 7)

§ Significant at $P=0\ 05$

¶ Significant at $P=0\ 01$

obtained from my series of dissections are more consistent than those of Orme *et al* , and this probably reflects the differences in the accuracy of the various dissection techniques Table 1 shows that the total weight of the dissected individual muscles of the shin group is a more valuable prediction index than the muscle content of the commercial foreshank[1]

The relationship between the total carcass muscle weight and the weights of the biceps femoris muscle and the muscles of the shin group is shown in Fig 1, and indicates the value of a large individual muscle or a group of small muscles, for predicting total carcass muscle weight over a wide range of steer carcasses

Statistical analysis revealed no significant differences between the breed groups within the series, when compared for the weight of biceps femoris muscle, longissimus dorsi muscle, and the muscles of the shin group This result is interesting when the striking difference in the external shape of the extreme types of cattle in this series is considered

When the animals were grouped into the following age-groups 6–12 months, 3, 13–18 months, 7, 19–24 months, 7, 25–30 months, 10, and greater than 30 months, 7, with the same muscles and muscle group as above, statistical analysis could not detect any effect of age on the regression of the total muscle weight on individual muscle weights It is apparent that the major age differences expected in the light of the work of Hammond[5] were no longer apparent by the age of six months Further information on the age effect on muscle distribution in cattle is urgently needed

Statistical comparison of the regression coefficients of my data with those of Orme *et al* [3] gave significant differences with three of five muscles No explanation of these differences is attempted in this preliminary communication, which, it is hoped, will stimulate further investigation of the use of individual muscle dissection as a valuable tool for prediction of total muscle in various species, wherever these data are needed in research or commerce

This work was financed partly by the Commonwealth Scientific and Industrial Research Organization which also provided laboratory accommodation I thank Prof John Francis and Mr N D S May for their support and advice during the course of the work, Mr A Howard for statistical advice and Mr L E Brownlie for assistance with the calculations

R M BUTTERFIELD
Sub-Department of Veterinary Anatomy,
School of Veterinary Science,
University of Queensland, Brisbane

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Growth of Human Striated Muscle

IT has often been stated that hyperplasia in human striated muscle is completed early in fœtal life This belief is based on the single report of total fibre counts in the sartorius muscle by MacCallum[1] and on the negative findings of Cuajunco[2] that in the biceps brachialis muscle division of fibres could not be detected after mid-gestation Tello[3], on the other hand, deduced from the morphological appearances that new fibres might appear in human muscle up to the time of birth

MacCallum's conclusion was based on fibre counts made on five fœtal specimens and one specimen at term Two figures were given for the latter, there is no indication that the other figures represented more than one estimate per specimen

During a study of muscle changes in human malnutrition it was found that in controls the number of fibres and of sub-sarcolemmal nuclei continued to increase during the late fœtal period The number of nuclei increased by a further factor of 10 or more between birth and adult life

Sartorius muscle was examined at autopsy from 3 still-births (2 premature 1 at full term) 2 well-nourished infants and 2 adults One whole muscle was removed in each case, cleaned of visible fat and fixed in formol-saline Complete transverse blocks were taken from the midpoint and from the proximal and distal quarters of each muscle Slides of the whole muscle in cross-section were cut of uniform thickness and stained with hæmatoxylin and eosin

Low-power projections were made through a photographic enlarger and the area of primary bundles was outlined on paper and measured by planimetry A millimetre scale was similarly projected High-power projections were then made through a standard microscope using a strong light source Counts of individual fibres and sub-sarcolemmal nuclei were made as they were traced on paper, from fields which were as representative as possible of the total area included in the low-power projection The high-power scale was obtained from a hæmocytometer slide 200–500 fibres were included in each count (at least 100 in the case of adult specimens), and at least 5 areas were counted in each slide

Table 1 TOTAL COUNTS OF FIBRES AND SUB-SARCOLEMMAL NUCLEI IN TRANSVERSE SECTION OF HUMAN SARTORIUS MUSCLE

Age	Body-length (cm)	No of fibres ($\times 10^{-3}$) ($\pm$ *S E*)	No of nuclei ($\times 10^{-3}$) ($\pm$ *S E*)	No of nuclei ($\times$ body-length $\times 10^{-5}$)
31/52 fœtus	38	64 ± 3 15	34 ± 2 10	13
33/52 fœtus	44	76 ± 2 09	42 ± 2 29	18
Full-term	50	101 ± 4 75	56 ± 1 27	28
4 months	58	134 ± 7 36	64 ± 2 58	37
13 months	76	128 ± 5 35	85 ± 2 59	65
64 years	162	103 ± 3 59	233 ± 9 84	376
74 years	156	114 ± 2 70	201 ± 10 5	313

No significant differences in morphology or in the counts were found in transverse sections of the proximal and distal quarters of the muscle as compared to the midpoint The mean figures of total fibres and sub-sarcolemmal nuclei in sections from the midpoint are given in Table 1 Figures in the final column are an approximate index of the total number of sub-sarcolemmal nuclei in the whole muscle, there is an error due to the differential growth of the lower limbs which tends to underestimate the numbers in the adult relative to those of the infant

The fibre number is approximately doubled between the 32nd week of fœtal life and 4 months of age It is not certain whether the increase is complete at full-term A steady increase in sub-sarcolemmal nuclear number appears to accompany all stages of muscle growth

No evidence of splitting of fibres was found which could not be attributed to artefact, on the other hand, in the fœtal specimens the appearance of some fibres in longitudinal section strongly suggested a fine tapering to a termination, accounting for some of the marked variation in fibre diameter which was observed on transverse section. No process either of mitotic or amitotic division of sub-sarcolemmal nuclei could be found, and no centrally placed nuclei were seen.

The picture suggests that at least during this stage of fœtal development the increase in fibre number in transverse section may be due to lengthening of pre-existing fibres rather than to splitting[4]. The recent *in vitro* studies of Cooper and Konigsberg[5] offer a possible explanation of the increase in numbers of sub-sarcolemmal nuclei, in that mononuclear interstitial 'myoblasts' may be progressively incorporated into the developing fibre.

Although the problem may be different in the case of the mononuclear cardiac cell, it is of interest that growth of the piglet's heart appears also to involve a steady nuclear proliferation[6].

R. D. Montgomery

Medical Research Council,
Department of Experimental Medicine,
University of Cambridge.

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RADIOBIOLOGY

Kinetosomes of Tetrahymena

It has been reported that the kinetosomes of the ciliate *Tetrahymena* contain deoxyribonucleic acid (DNA)[1,2]. This finding is of considerable interest in view of its possible bearing on the problem of the function of the kinetosomes. Attempts have been made to use high-resolution autoradiography as a sensitive cytochemical tool in the detection of kinetosomal DNA in *Tetrahymena*.

The organisms, *Tetrahymena pyriformis* strain *S*, were grown in axenic culture on a proteose-peptone, yeast extract medium at 28·5° C; fresh cultures were used for each experiment, which was performed during the logarithmic phase of growth. The DNA of the organisms was labelled by incorporating precursors into the culture medium; tritiated thymidine of 200 mc./m*M* specific activity, tritiated cytidine 99 mc./m*M*, and tritiated uracil 123 mc./m*M* were used at concentrations of 0·5 μc./ml. to 5 μc./ml. After 24 hr. the organisms were fixed in an acetic acid–alcohol mixture the osmotic pressure of which had been adjusted to that of the culture medium. Whole mounts of the specimens were made by drying down on to clean slides. Autoradiographs were prepared with Kodak *AR* 10 stripping film, and were exposed in light-tight boxes at 5° C. and developed in Kodak *D* 19*b*. Controls were run consisting of specimens which had not received any radioactive material and of specimens which had been incubated with deoxyribonuclease and with ribonuclease.

No evidence for the existence of kinetosomal DNA in *Tetrahymena* was obtained from these experiments. Autoradiographs of tritiated thymidine showed strong nuclear labelling after an exposure of 40 days, there was no nuclear labelling after treatment with DNase, and the intensity of labelling was unaffected by the use of RNase. In some experiments a very low level of random cytoplasmic labelling was observed, and it was apparently removed by DNase. However, even after 61 days exposure this was only just at the level that could be regarded as sufficient under the most favourable conditions to indicate positive labelling[3].

Tritiated cytidine and tritiated-uracil are incorporated into both the nucleic acids of *Tetrahymena*, so the interpretation of the results depends on the enzyme digestions used. When RNA had been removed by enzyme digestion, there was no detectable cytoplasmic labelling above background, after 40 days exposure. There was strong labelling in the nucleus from tritiated-cytidine but only rather weak labelling from tritiated-uracil; the nuclear labelling was removed by DNase.

An examination of the autoradiographs under phase-contrast conditions showed that the kinetosomes were still identifiable after preparation of the specimen and, with the resolution available in tritium autoradiographs using Kodak *AR* 10—about 0·75μ—it would have been possible to see ordered rows of silver grains in the emulsion if the kinetosomes had become labelled. A calculation of the amount of tritium needed to produce a single silver grain in the autoradiograph after 40 days exposure shows that there is probably less than 10^{-15} gm. DNA per kinetosome in *Tetrahymena*.

The sensitivity of these experiments is not high enough to detect the DNA found by Seaman[2] in the isolated kinetosomes of *Tetrahymena*.

This work was carried out in the Wheatstone Laboratory, King's College, London, with the help and advice of Prof. J. T. Randall and advice on autoradiography from Dr. S. R. Pelc.

V. W. Rampton

Department of Physics,
University of Nottingham.

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Specific Radioprotective Effect of the Mitochondrial Matrix

The ability of the cellular contents to protect enzyme molecules from the destructive action of ionizing radiation is now clearly recognized[1]; but there is little quantitative information concerning the specific radio protection offered by the natural intracellular milieu. Irradiation of whole animals suggests that this natural environment affords a high degree of protection from the indirect effects of aqueous radicals and perhaps from direct effects as well[2]. The specificity of this protection can be demonstrated by the irradiation and assay of enzymes uniquely associated with organelles within and without their natural cellular environment. For example, diphosphopyridine nucleotide-pyrophosphorylase, an enzyme contained exclusively in the cell nucleus, is many times more resistant to X-rays in isolated nuclei than is an equally active solution free of nuclei[3]. In the experiments to be reported, glutamic dehydrogenase, an enzyme believed to be confined to the mitochondrial matrix[4,5], has been irradiated without and within the intact mitochondrion.

Mitochondria were prepared from albino rat liver homogenates in 0·25 *M* sucrose – 1 m*M* ethylenediamine tetraacetate *p*H 6·7 (ref. 5). After centrifugation of the nuclei, erythrocytes and cell debris at 7,000 *g* × min. the mitochondria were sedimented at 35,000 *g* × min. The mitochondrial pellet was resuspended in fresh sucrose and sedimented again at

Table 1. Irradiation of osmotically disrupted mitochondria (0·025 *M* sucrose) in which nearly half the glutamic dehydrogenase activity was extramitochondrial. Activities are expressed as *ΔD* 340 mμ per 5 min.; the numbers in parenthesis are the percentages of the control unirradiated suspensions

	Unirradiated	90,000 r.	150,000 r.
Total activity ('Triton *X*-100')	0·088 (100 per cent)	0·074 (84 per cent)	0·045 (51 per cent)
Activity of free enzyme (supernatant)	0·038 (100 per cent)	0·017 (45 per cent)	0·004 (9 per cent)
Intramitochondrial activity (difference)	0·050 (100 per cent)	0·057 (114 per cent)	0·041 (82 per cent)

Table 2. Irradiation of intact liver mitochondria to which pure beef liver glutamic dehydrogenase has been added. Activities are expressed as *ΔD* 340 mμ per 5 min.; the numbers in parentheses are the percentages of the control unirradiated suspension

	Unirradiated	76,000 r.	133,000 r.
Total activity ('Triton *X*-100')	0·240 (100 per cent)	0·112 (47 per cent)	0·092 (38 per cent)
Activity of free added enzyme (supernatant)	0·149 (100 per cent)	0·017 (11 per cent)	0·006 (4 per cent)
Intramitochondrial activity (difference)	0·091 (100 per cent)	0·095 (104 per cent)	0·086 (95 per cent)

7,000 $g \times$ min. to remove residual nuclei and again centrifuged for 35,000 $g \times$ min. This last centrifugation was repeated until the supernatant was clear. Suspensions of mitochondria were irradiated with 250 kV. unfiltered X-rays in aerated, ice-cooled glass beakers and a ferrous ammonium sulphate dosimeter was used. Glutamic dehydrogenase activity was assayed by following the oxidation of DPNH in the presence of 30 m*M* nicotinamide, 0·4 m*M* potassium cyanide, 1 m*M* ethylenediamine tetraacetate, 50 m*M* ammonium chloride, 0·5 m*M* α-oxoglutarate, 0·01 per cent 'Triton *X*-100' and 20 m*M* potassium phosphate *p*H 7·5.

When the intact mitochondrial suspensions were irradiated, no change in the total glutamic dehydrogenase activity could be found with doses up to 133,000 r. and no significant activity could be found in the supernatants from centrifuged irradiated and non-irradiated mitochondria.

Mitochondria were osmotically disrupted in 0·025 *M* sucrose[6]; in this instance approximately half the activity was to be found in the supernatant following centrifugation at 500,000 $g \times$ min. As shown in Table 1, irradiation of the mitochondrial suspension reduced the supernatant activity considerably while the activity of intramitochondrial enzyme was relatively undiminished.

A purified, crystalline preparation of beef liver glutamic dehydrogenase (2·5 μgm./ml.) was added to a suspension of intact mitochondria and the mixture irradiated. As shown in Table 2, the activity of the free added enzyme was markedly diminished while that of the mitochondrial enzyme was unchanged. However, the presence of the mitochondria did afford some protection to the added enzyme since irradiation of the pure enzyme in sucrose alone reduced the activity to 11 per cent with 32,000 r. instead of 76,000 r. as shown in Table 2.

The results demonstrate that glutamic dehydrogenase contained within the mitochondrial matrix is remarkably well protected from the destructive effects of ionizing radiation. Equally active extramitochondrial enzyme is not nearly as well protected and is 90 per cent inactivated by a dose 2·5–5 times that required to inactivate a pure sucrose solution of the enzyme to the same degree. The specific radioprotective property of the matrix presumably derives from the presence of bound and unbound scavenger molecules, the high local concentration of enzyme and, perhaps, from the contiguous membranous structure. In addition, there is no evidence that X-irradiation of the mitochondrion leads to leakage of glutamic dehydrogenase from the matrix, even at high doses. This failure of irradiation to alter the permeability of mitochondria to this enzyme has been reported recently at lower dosage (9,000 r.) (ref. 7) and is here confirmed.

This work was supported by a grant (*A*-4219) from the U.S. Public Health Service and the Medical Foundation, Inc.

S. J. ADELSTEIN

Departments of Anatomy and Radiology,
Harvard Medical School,
Boston 15.

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BIOLOGY

Occurrence of Glossopteridae Fructifications at Baralaba, Queensland, and their Evolution

A NUMBER of fructifications of the Glossopteridae occurred in shale in the roof of the Dawson Seam, Baralaba Colliery, Baralaba, near Rockhampton, Queensland. The specimens examined form part of

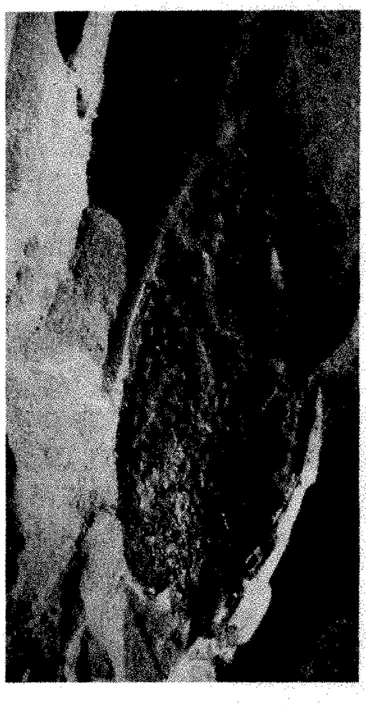

Fig. 1. *Lanceolatus* sp. attached to a leaf of *Glossopteris*. The adnate organ (upper third only is visible) does not appear to be sac-bearing, whereas the pedicellate bract-like organ appears to bear sacs. This latter organ is attached by a short, thick pedicel visible at the bottom of the photo. The leaf has taeniopteroid venation, and appears to be either *G. indica* or a very narrow *G. communis*. (× *c*. 2·7)

a collection belonging to the Geology Department, University of Queensland. The collection was made first of all by Miss B. Houston (now of the Mines Department, Queensland) and later supplemented by specimens collected by the colliery manager.

The fructifications were all unwinged, that is, of the *Cistella* and *Lanceolatus* types. They occurred as carbonaceous compressions. Unfortunately the carbonaceous material was dissected by a large number of fine parallel fractures simulating a microcleat, but it has been possible to prepare balsam transfers.

From the transfers one can see that the sacs must have represented quite a thickness of tissue as they are up to 0·4 mm. thick, but neither spores, nor pollen, nor seeds have been detected.

The nature of the probable ♀ fructification agreed with those illustrated elsewhere[1-4]. They consisted of a body bearing swollen sacs. These sacs sometimes had a rounded, convex top and at other times the top had a central circular depression with a raised central boss[1-3,5]. The latter appeared to be more mature sacs than the former. There appeared to be more 'exploded' sacs towards the base.

The only attached specimen is illustrated in Fig. 1 below. In some detached fructifications, the sacs appeared elongated, as much as three times as long as their diameter and free from adjacent sacs. The row of sacs around the margin (referred to as 'marginal sacs') appeared larger than those away from the margin (the 'body sacs'). Both marginal and body sacs showed rounding, then formation of the central depression. Plumstead's illustrations[3] of various *Cistella* species show these two types of sac clearly.

A transfer of a sac-bearing organ showed the sacs on both surfaces. Those on the reverse surface may have represented only the reflexion of the additional tissue being compressed from the obverse surface. Alternatively, it may have been derived from compression of a circular body similar to that illustrated by Sen (ref. 4, Fig. 5). There is no evidence to suggest a preference for either. Plumstead's interpretation of the nature of the fructification[2,3,6] is logical, even though largely unsubstantiated by lack of suitable material. Sen[4] and myself have both had carbonaceous material, but so far have been unable to find any definite clue to the identity of these organs.

The evidence from the Baralaba specimens supports the conclusions of Plumstead; but it is hoped with further work to be able to elucidate the true nature of these fructifications.

Based on fructifications seen by me of *Cistella* and *Lanceolatus* species from Baralaba, *Scutum thomasii* from Mt. Kembla, New South Wales[7], and *Hirsutum* sp. from the same district, along with published illustrations of fructifications[1-6], a possible line of evolutionary descent is suggested in Fig. 2 based on sac-bearing glossopterid fructifications.

The probable fructification, *Arberia*[8,9], appears ovuliferous and could have been an intermediate stage between the primeval fructification and *Ottokaria*. Particularly *A. minasica* White resembles *Ottokaria*.

Derbyella from Brazil[10] appeared to have borne spores in a manner similar to the scale leaves of *Glossopteris* first reported by Arber[11] and later reported by many others. No suggestion can be made along this line of descent.

Melville[12] has advanced an interesting theory regarding the development of the angiosperm flower. In this, the fructification of *Glossopteris* was a logical intermediary between a primitive fructification having single epiphyllous sporangia borne alternately and an inflorescence such as *Gnetum*. *Arberia* appeared a logical immediate progenitor of the *Glossopteris* fructifications if viewed in this light, although possibly removed from the main line as proposed here.

I thank Prof. Dorothy Hill, University of Queensland, for allowing me to examine the Baralaba collection; Dr. H. K. Worner, director of research, Broken Hill Pty., Central Research Laboratories, Shortland, New South Wales, for assistance; Mr. S. Thompson, of the same organization, for the photograph; and Prof. T. M. Harris, Univer-

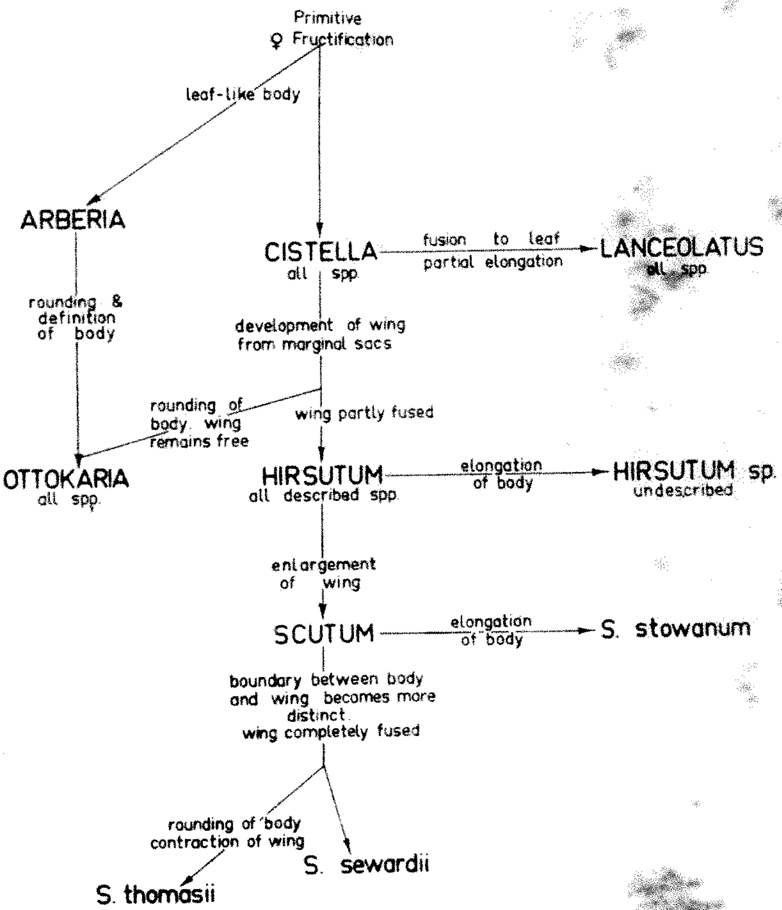

Fig. 2. Evolution of the sac-bearing organ in the Glossopteridae. Alternative lines of descent include: (*a*) primitive organ → *Arberia* → *Ottokaria* → *Cistella*; and (*b*) primitive organ → *Arberia* → *Ottokaria* and *Cistella*

sity of Reading, for suggestions concerning technique.

J. F. Rigby

Broken Hill Proprietary Co., Ltd.,
Central Research Laboratories,
Shortland, 2*N*,
New South Wales.

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A Membrane Peculiar to the Egg in the Gametophyte of *Pteridium aquilinum*

Investigations of the ultrastructure of the cells taking part in oogenesis in *Pteridium aquilinum* have shown that the mature egg possesses a membrane lying between the plasmalemma and the cellulose wall, and not present in adjacent cells (Fig. 1). After fixation in potassium permanganate and staining with uranyl acetate this additional membrane is electron dense, and it probably therefore consists of lipid or lipo-protein. The membrane is absent from the young egg and the cells preceding it, and there is evidence, which will be presented in detail elsewhere, that the material of which it is composed is derived from organelles which have degenerated during the maturation of the egg.

Although this membrane cannot be seen in sectioned material with the light microscope, DeMaggio[1] recently reports that the egg of *Todea barbara*, which unlike the egg of *Pteridium* can be viewed as a solid object lying in the venter of the archegonium, is enclosed in a thin transparent membrane. It seems likely that this membrane is similar in nature to that now detected in *Pteridium*, and such a membrane may be general in the egg cells of ferns, and possibly of other plants.

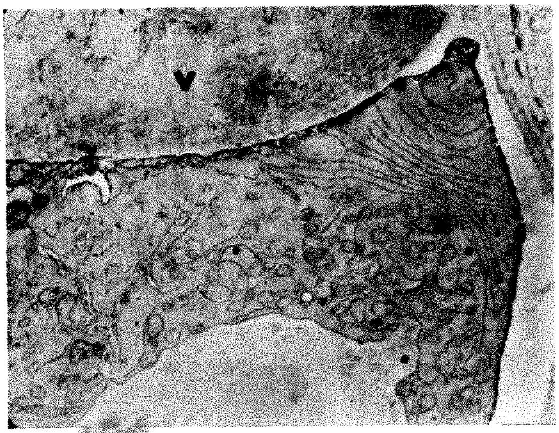

Fig. 1. *Pteridium aquilinum*. Section of mature egg stained with uranyl acetate. A densely staining membrane surrounds the egg. The egg is surmounted by the degenerating ventral canal cell (*v*). (× *c*. 5,630)

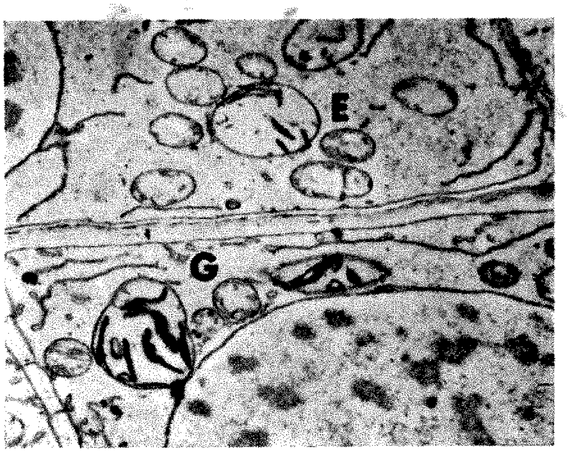

Fig. 2. *Pteridium aquilinum*. Section of portion of embryo about 15 days old and adjoining gametophyte, stained with uranyl acetate. Patches of the membrane formerly surrounding the egg can be seen lying between the plasmalemma of the embryo cell (*E*) and the cellulose wall forming the boundary between the embryo and the gametophyte (*G*). (× *c*. 10,670)

Sections of embryos of *Pteridium* show that this extra membrane persists during their early development as a layer on the inside of the outer walls of the peripheral cells. As the embryo expands and these cells divide the layer becomes attenuated and eventually discontinuous (Fig. 2).

A layer of this kind would be expected to influence the permeability of the boundary of the egg and young embryo. The failure of nucleotides to diffuse freely into the embryos of *Pteridium*, reported by Bell[2], is probably explained by its presence. Conversely the layer might also reduce the tendency of metabolites to diffuse out of the zygote and young embryo. This may explain the fact that according to DeMaggio and Wetmore[3] the isolated zygote of *Todea barbara* undergoes normal development in liquid culture up to the octant stage, but not afterwards. The irregular development which then ensures could be partly a consequence of the attenuation of the lipid layer in the outer cells of the embryo and a rapid loss by diffusion into the medium of metabolites essential for ordered growth.

P. R. Bell

Department of Botany,
University College,
London, W.C.1.

K. Mühlethaler

Laboratorium für Elektronenmikroskopie,
Institut für Allgemeine Botanik,
Technische Hochschule,
Zürich, 6.

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Source of Dichromatism in Two Maldanid Polychætes

The tubiculous Maldanid polychætes are among the most abundant infaunal components of intertidal and sub-tidal bottom communities of the North American Atlantic coast. Within their respective ranges the two species *Clymenella torquata* Leidy and *Axiothella mucosa* Andrews show a striking dichromatism: populations may be either green or orange.

Table 1. COLOUR OF GEOGRAPHICALLY SEPARATE POPULATIONS OF MALDANID POLYCHÆTES

Species	Source	Latitude	Colour
Clymenella torquata	Passamaquoddy Bay, New Brunswick	45° N.	Orange
	Western Bay, Maine	44° 30′ N.	Orange
	Vineyard Sound, Mass.	41° 30′ N.	Both
	Long Island Sound, Conn.	41° 15′ N.	Orange
	Isle of Wight Bay, Maryland	38° 20′ N.	Orange
	Newport River, North Carolina	34° 40′ N.	Green
	Summer River, Florida	29° 50′ N.	Green
Axiothella mucosa	Newport River, North Carolina	34° 40′ N.	Green
	Summer River, Florida	29° 50′ N.	Orange
	*Alligator Harbour, Florida	29° N.	Green
Euclymene collaris	Western Bay, Maine	44° 30′ N.	Orange
	Vineyard Sound, Mass.	41° 30′ N.	Orange
	Newport River, North Carolina	34° 40′ N.	Orange

*Specimens from the coast of the Gulf of Mexico obtained through the courtesy of Dr. Alan J. Kohn, Department of Biology, Florida State University, Tallahassee.

A population inhabiting a particular microgeographic area is always consistent, although the distance over which a colour change occurs may be only a few miles. Continuous intergraduations do not occur. A third closely related species, *Euclymene collaris* Claparède, does not undergo the colour change; it exists only in the orange form even where it is sympatric with the green forms of the first two. Table 1 lists the colour of populations found at each of seven macrogeographical locations sampled by me.

The green coloration of the so-designated populations appears to be due to the accumulation of the bile pigment mesobiliverdin in the epidermal tissues of the body-wall. In the absence of mesobiliverdin the tissues derive their colour solely from yellow-orange pigments, probably carotenoids.

Individuals of all three species taken from the Newport River, North Carolina, were first placed in a 5 per cent mixture of acetic acid and ethyl alcohol. The yellow-orange extract failed to give the characteristic Gmelin reaction of bile pigments, and produced an indeterminate spectrum. The green pigment was then readily extracted from *C. torquata* and *A. mucosa* into a 5 per cent mixture of hydrochloric acid and methyl alcohol. The resulting blue-green solution elicited a distinct Gmelin reaction. Its absorption maxima at 358 and 670 mμ (Beckman *DK–2* spectrophotometer) lie extremely close to those of mesobiliverdin in hydrochloric acid: methyl alcohol (363 and 670 mμ)[1]. The solution was then diluted with an equal volume of water and extracted with chloroform. The yellow-brown hypophase was discarded, and the blue-green epiphase lyophilized and redissolved in dry methanol. The methanol solution showed fluorescence in long-wave ultra-violet light, and gave a positive zinc bilipurpurin test with an absorption maximum at 625 mμ. Following evaporation *in vacuo*, the pigment was resistant to destruction with concentrated sulphuric acid. The latter two tests confirm the presence of ethyl rather than vinyl side-chains, thus distinguishing mesobiliverdin from the related proto-compound. The initial procedure was repeated on *Eu. collaris* and orange individuals of *C. torquata* from Long Island Sound, but all attempts to extract a bile pigment of any kind were unsuccessful.

The occurrence of mesobiliverdin in animals was thought to be restricted to a few of the phytophagous insects[2] until Dales[3] recently obtained it from the terebellid polychæte *Eupolymnia heterobranchia* Johnson of the North American Pacific coast. The possibility of an analogous dichromatism within this species was mentioned, although the orange form was evidently not available for direct examination.

The most probable explanation of the observed dichromatism would attribute such sporadic accumulation of the pigment to properties of the available food in sediments inhabited by different populations. Maldanids of the sub-family Euclymeninae obtain their food by ingesting sand grains at approximately 20 cm. depth below the water-substratum interface, the lower end of their vertical tubes. Organic matter clinging to the sand particle surfaces is digested, and the particles defæcated into the water at the upper end of the tube. Since mesobiliverdin is related to a chromophoric group of phycocyanin[4], it is suggested that the colour of the polychæte reflects the density of suitable algal food organisms in the sediment. It is not known that the diet of green populations includes living algæ taken at that depth; it may consist of detrital pigment.

The complete absence of mesobiliverdin in *Eu. collaris*, which obtains its food in the same manner, indicates a subtle diversification either in the feeding processes or the digestive metabolism of these closely related sympatric species. It would be most enlightening to know whether the pigment undergoes further breakdown in the metabolism of *Eu. collaris*, or whether the animal simply returns the pigment intact to the sediment.

This investigation was aided by a research grant from the National Science Foundation (*G*.17862), L. M. Passano, principal investigator.

CHARLOTTE P. MANGUM

Department of Zoology,
Yale University,
New Haven, Conn.,
and
Duke University Marine Laboratory,
Beaufort, North Carolina.

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Promotion of Germination of *Striga asiatica* Seed by Coumarin Derivatives and Effects on Seedling Development

OUR efforts to isolate and characterize the *Striga* germination stimulant in root exudates of maize were accompanied by testing more than 270 compounds for their capacity to stimulate germination. A previous paper[1] reported the stimulation of *Striga asiatica* (witchweed) seed germination and described morphogenic effects on the seedlings induced by kinetin and certain other 6-(substituted) purines. This communication reports the results obtained with 41 coumarin derivatives, two of which stimulated germination of witchweed seeds.

Williams[2] confirmed the morphogenic effects of kinetin on young seedlings and further reported a resemblance of the effects of kinetin and red light. He suggested that since the production of shoots and the branching of roots were stimulated by kinetin and gibberellin, the development of the parasite might depend on a supply of growth factors of this type from the host. Williams also stated that the concentration of the compound required to stimulate germination appeared to be considerably less than that required to cause morphogenic changes.

Coumarin and certain of its derivatives have been reported to inhibit and stimulate plant growth processes, but coumarin has usually been regarded as an inhibitor. Neumann[3], however, has shown an auxin-like action of coumarin in promoting elongation of *Helianthus* hypocotyls, oat coleoptiles, and bean hypocotyls. He suggested that coumarin should be regarded as a naturally occurring growth regulator. Ishikawa[4] reported a stimulation of germination of *Setaria italica* seed by a 0·1 p.p.m. coumarin solution. Scopoletin (6-methoxy-7-hydroxycoumarin) has been shown to inhibit growth of oat roots at a concentration of 10^{-5} M (ref. 5), and to promote growth of pea roots at a 1 p.p.m. concentration[6].

Preparation of witchweed seeds for testing and techniques of the assay used in the present work were the same as those given in the previous paper[1]. Most of the compounds reported were dissolved in distilled water. If a compound appeared not to be water-soluble, the initial stock mixture was shaken under hot tap water and the resulting solution used in making the dilutions.

Results obtained with the two active compounds, scopoletin and 4-hydroxycoumarin, are given in Table 1. The concentrations shown include those most effective in stimulating germination.

Table 1. STIMULATION OF *Striga asiatica* SEED GERMINATION BY TWO COUMARIN DERIVATIVES

	Mean germination* (per cent) at indicated concentrations (p.p.m.)							
Compound	80	40	20	10	8	5	2·5	1·25
6-Methoxy-7-hydroxy-coumarin (scopoletin)†	10	23	34	34	29	15	5	2
	100	10	1	0·1				
4-Hydroxycoumarin‡	0	36	10	1				

* Average of three replications.

† Purchased from Delta Chemical Works, Inc., 23 West 60th Street, New York 23.

‡ Supplied by Amchem Products, Inc., Ambler, Pa.

Of the coumarin derivatives considered in this study having substituent groups on the lactone portion of the molecule, only the 4-hydroxy substitution gave an active compound. 3-carbethoxy-, 3-carboxy-, 3-chloro-, 3-*p*-chlorophenyl-, 3-ethylcarboxy-, 3-methylcarboxy-, 3-methoxy-, and 4-methyl-coumarin were inactive. Substitution at the 3-position apparently nullified the stimulatory effect of the 4-hydroxy constituent since 3-acetamido-, 3-amino-, 3-carbethoxy-, 3-carboxy-, 3-ethylcarboxy-, 3-ethyl-, and 3-phenyl-4-hydroxycoumarin were inactive.

No conclusions were drawn concerning structural requirements for activity on the phenyl portion of the molecule, since only a limited number of these compounds were tested. Two naturally occurring inactive coumarin derivatives were umbelliferone (7-hydroxycoumarin) and β-methylumbelliferone (4-methyl-7-hydroxycoumarin). Other inactive compounds were: 4-amino-7-hydroxy-, 4-amino-7-hydroxy-3-phenyl-, 3-carboxy-7-hydroxy-, 3-carboxy-6-methoxy-, 3-carboxy-8-methoxy-, 3-carboxy-7-propoxy-, 3-cyano-6-methoxy-, 3-ethylcarboxy-7-hydroxy-, 3-ethylcarboxy-7-methoxy-, 6-β-glucoxy-7-hydroxy-, 6-methyl-, and 4-methyl-6-hydroxy-coumarin. Coumarin; 3,3′-*p*-chlorobenzylidine-bis-(4,7-dihydroxycoumarin); 2,7-dihydroxy-4-iminobenzopyran; 5,7-dihydroxy-4-methylcoumarin; 3-ethylcarboxy-6,7-dihydroxycoumarin; *N*-(2′-hydroxyethyl)-7-hydroxycoumarin-4-acetamide ethanol; 3,3′-methylene-bis-(4-hydroxycoumarin); methylene-bis-(4-propionoxy-3-coumarin); 8-piperidinomethyl-7-hydroxy-4-methylcoumarin hydrochloride; and 3,3′-thio-bis-(4-hydroxycoumarin) were also inactive.

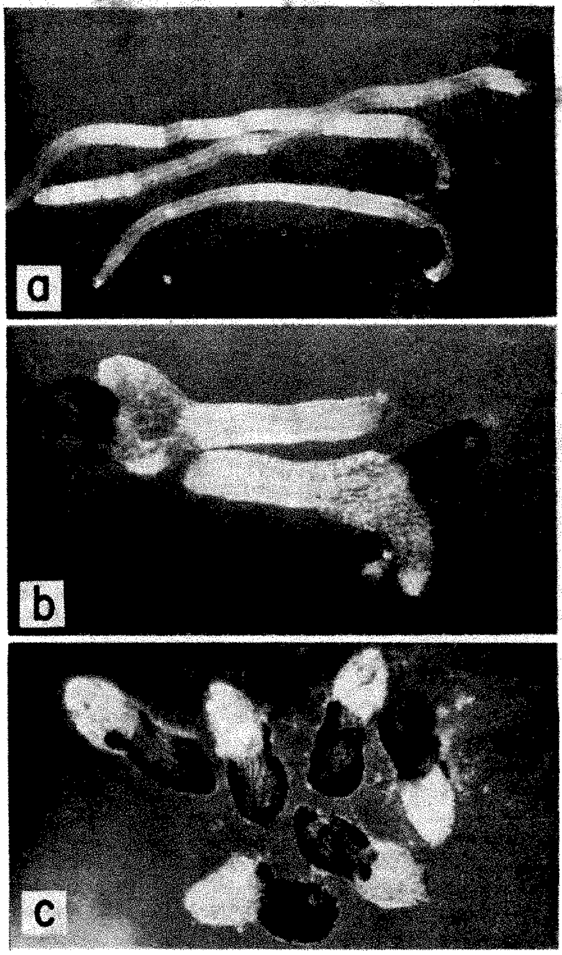

Fig. 1. *Striga asiatica* seedlings stimulated to germinate by, and left in, contact with: *a*, 2·5 p.p.m. scopoletin solution for three days. Radicles were similar in appearance to those developed in the presence of the natural stimulant (× *c*. 33); *b*, 10 p.p.m. scopoletin solution for four days. Radicle elongation has been somewhat inhibited and branching has occurred (× *c*. 67); *c*, 80 p.p.m. scopoletin solution for five days. Radicle elongation has been greatly inhibited (× *c*. 67)

The radicles of seeds stimulated to germinate by scopoletin solutions of 5 p.p.m. or lower and by 4-hydroxycoumarin at all concentrations were similar in appearance to radicles of seeds stimulated to germinate by the natural stimulant. The seedlings shown in Fig. 1*a* were floated on a 2·5 p.p.m. scopoletin solution for three days. This concentration of scopoletin appeared to promote greater elongation of the radicles than did the natural stimulant. All active concentrations of 4-hydroxycoumarin appeared to stimulate elongation.

Concentrations of scopoletin of 8, 10, and 20 p.p.m. inhibited elongation of the radicle and resulted in branching of the radicles. These morphogenic effects are shown in Fig. 1*b*. Almost no elongation of radicles occurred in scopoletin solutions above 20 p.p.m. Seeds germinating in these solutions had the short, thickened radicles shown in Fig. 1c.

No development of cotyledons, shoots, or root hairs was noted on seedlings stimulated to germinate by either of the two active coumarin derivatives.

In view of the results reported in this paper the morphogenic effects reported by Williams on parasite radicles growing in close proximity to a host root could also be attributed to a coumarin derivative instead of a kinetin-like compound. Stimulation of cotyledon enlargement and shoot growth, however, might still depend on a supply of kinetin-like growth factors from the host.

From the reports cited by Börner[7] of liberation of scopoletin and related compounds from plant roots and the finding of Brown *et al.*[8] that the *Striga* germination factor was a root-extension stimulator, the possibility that a coumarin derivative is the naturally occurring germination stimulant cannot be overlooked.

Additional indications obtained from work on the chemical nature of the natural stimulant from maize roots (to be published elsewhere) further suggest that it might be a coumarin derivative. Neither of the two active compounds reported here, however, is believed to be present in the natural stimulant. The natural stimulant appeared to be effective at much lower concentrations than were these two compounds, and Goodwin and Taves[9] reported that most naturally occurring coumarin derivatives isolated appeared not to have substitutions in the four-position. Sunderland[10], however, has recently indicated that the *Striga* stimulant from maize roots may be a complex of substances. Conceivably a coumarin compound could be one of the constituents, possibly the main constituent.

A. D. WORSHAM
G. C. KLINGMAN

Department of Field Crops,
North Carolina State College,
Raleigh.

D. E. MORELAND

Crops Research Division,
Agricultural Research Service,
U.S. Department of Agriculture.

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Production of Fusion Organs by the Species of *Phytophthora* which causes Foot Rot of *Piper nigrum* L. in Sarawak

A SERIOUS root disease of black pepper (*Piper nigrum* L.) was reported to be present in Sarawak by Robertson[1] in 1955. Holliday and Mowat[2] discovered that the causal organism was a species of *Phytophthora* and suggested the name 'foot rot'. In their communication it was stated that they had been unable to find the oospores of the fungus.

In July 1961, the majority of 31 cultures, remaining from a total of 81 isolates from black pepper, were found to be producing oospores after 2–4 months in pure culture on oatmeal agar. A description of the fusion organs (from isolate 78) of this *Phytophthora* species follows.

Oogonia are terminal or lateral, globose, thin-walled and hyaline when young but become thick-walled and amber to golden brown when mature; their diameter is 26–45μ (mean 35μ); the wall is smooth, or occasionally rough, 1·8–7·5μ thick (mean 3·8μ). Antheridia are terminal or lateral, thin-walled, hyaline and amphigynous; 11–15 × 11–23μ (mean 13 × 15μ). Oospores are spherical, thin-walled and hyaline when young but become thick-walled and amber to golden brown when mature; their diameter is 19–38μ (mean 26μ); the wall is smooth, 1·8–5·6μ thick (mean 3·2μ).

The identity of the species is still uncertain. Muller[3] named a species of *Phytophthora* causing foot rot of black pepper in Indonesia, *P. palmivora* var. *piperis*. Isolates from *Piper betle* L. have been referred to as *P. parasitica* Dast., by McRae[4], and *P. colocasiae* Rac., by Thompson[5], while Dastur[6] gave the name *P. parasitica* var. *piperina* to isolates from *Piper betle* and *Piper longum* L. It is thus obvious that further work is required before the Sarawak species can be finally identified.

I thank the Director of Agriculture for permission to publish this communication. The work on foot rot, initiated by the Sarawak Government, is continuing.

G. J. TURNER

Department of Agriculture,
Kuching.
Sarawak.

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MICROBIOLOGY

Production of Vitamin B_{12} and Vitamin B_{12} Analogues by Pure Cultures of Ruminal Bacteria

MICROBIAL fermentation in the rumen was early recognized as the primary source of vitamin B_{12} for the cow[1]. Rumen contents contain not only the vitamin itself but also certain analogues in which the nucleotide portion of the vitamin is missing or modified by the substitution of a different benzimidazole or a purine for the base (5 : 6-dimethylbenzimidazole). In general, the analogues containing nucleotides with benzimidazole derivatives tend to possess some vitamin activity for animals while those containing purines tend to have activity only for micro-organisms[2]. The relative activities vary with different micro-organisms and even with the particular assay technique used[3]. In rumen contents, vitamin B_{12} itself makes up about 10 per cent of the activity for *Escherichia coli*, factor *A* (containing 2-methyl adenine) composes about 60 per cent, while pseudovitamin B_{12} (containing adenine), factor *B* (containing no nucleotide) and factor *C* (containing guanine) account for most of the remainder[4]. Relatively little work has been reported in regard to the part played in the synthesis of these compounds by the individual species of micro-organisms obtained from the rumen and in general such studies have not included true or functional rumen organisms[5].

In the present work, the synthetic capability of 48 strains representing 21 different species of functional rumen micro-organisms have been tested for the production of vitamin B_{12} and vitamin B_{12} analogues. These included representatives of most of the important genera isolated from the rumen of the cow[6]. The growth medium for these organisms was that of Bryant and Robinson[7] except that 20 per cent (v/v) of rumen fluid clarified by centrifugation replaced vitamins, sodium monosulphide and salts of volatile fatty acids; glucose replaced cellobiose except for the ruminococci; and the casein hydrolysate was not treated with 'Norit'. A 10-ml. culture was incubated until maximum growth was obtained, its *p*H adjusted to 4·8–5·0 and a small amount of potassium cyanide added. The sample was heated in a steam oven for 40 min. and then refrigerated until tested. All samples were assayed with *E. coli* mutant 113–3 (Davis), using the cup-plate assay technique of Harrison *et al.*[8] with their medium or that of Burkholder[9] as modified by Ford and Holdsworth[10]. Active samples were further tested with *E. coli* by the tube test of Burkholder[9] and with *Ochromonas malhamensis* (A.T.C.C. 11532) by the assay of Ford[11] as modified by Lichtenstein *et al.*[12]. All assay values were corrected for the vitamin B_{12} activity of the control medium, which contained an average of 0·45 mμgm./ml. by the cup-plate technique, 0·16 mμgm./ml. by the tube test and 0·05 mμgm./ml. by the Ochromonas assay.

Table 1. COMPARATIVE PRODUCTION OF VITAMIN B_{12} AND ITS ANALOGUES BY SYNTHESIZING STRAINS OF RUMINAL BACTERIA

	Vitamin B_{12} activity		
Species and strain	*E. coli* plate assay (mμgm./ml.)	*E. coli* tube assay (mμgm./ml.)	*O. malhamensis* assay (mμgm./ml.)
Butyrivibrio fibrisolvens			
49	3·0 ± 0·3* (6)†	1·5 ± 0·1 (3)	0·14 ± 0·04 (3)
Peptostreptococcus elsdenii			
*B*159	8·8 ± 1·2 (5)	6·3 ± 0·4 (3)	0·24 ± 0·00 (3)
*T*81	4·7 ± 0·4 (5)	2·1 ± 0·2 (6)	0·26 ± 0·02 (3)
Selenomonas ruminantium			
*GA*192	35·8 ± 3·6 (6)	15·6 ± 2·3 (5)	0·56 ± 0·07 (3)
*HD*4	78·8 ± 7·5 (7)	62·4 ± 6·8 (6)	1·26 ± 0·08 (5)
*PC*18	47·8 ± 5·3 (7)	12·3 ± 0·6 (3)	0·31 ± 0·02 (2)
*GA*31	52·6 ± 9·3 (5)	18·9 ± 2·9 (6)	0·15 ± 0·01 (3)
Unnamed group			
*B*385-1	19·5 ± 2·4 (8)	10·1 ± 2·1 (4)	0·41 ± 0·01 (3)

* Standard error. † No. of assays.

The organisms tested by the cup-plate assay consisted of the following species and strains: *Bacteroides ruminicola* (23, $B_1$18, *GA*20, 118*B*, *GA*33, *GA*103), *Bacteroides succinogenes* (44, *S*85), *Borrelia* sp. ($B_2$5), *Butyrivibrio fibrisolvens* (49, 2–2, 3–4, *A*38, *D*1), *Eubacterium ruminantium* (*B*4, B_1C23, *GA*195), *Eubacterium* sp. (*B*17), *Lachnospira multiparus* (40, *D*32), anaerobic lactobacilli (*GA*1, *GA*19), *Peptostreptococcus elsdenii* (*B*159, *T*81), *Propionibacterium acnes* (*CL*1–2), *Ruminococcus albus* (7, 20, *B*199, $B_3$37, *D*89), *Ruminococcus flavefaciens* ($B_1$46, *C*94, *FD*1), *Selenomonas ruminantium* (*GA*192, *GA*31, *HD*4, *PC*18), *Succinivibrio dextrinosolven* (24), *Succinimonas amylolytica* ($B_2$4), *Streptococcus bovis* (*FD*10), an unnamed group, *B* 385-like (*B*385–1, 2–33, 3–18, 5–4), and 4 strains of unidentified anaerobic, motile, lancet-shaped, Gram-negative organisms. (For characteristics of these organisms, see review by Bryant[6].) Of the foregoing, only 8 strains representing 4 different species gave evidence of appreciable synthesis of the vitamin or its analogues. Results obtained with the active strains are shown in Table 1. Of these four species, *S. ruminantium* produced the largest amounts and all strains tested were active. Both strains of *P. elsdenii* tested yielded somewhat smaller amounts, while one strain each of *B. fibrisolvens* and of the unnamed *B*385-like group synthesized appreciable amounts. *S. ruminantium* and *P. elsdenii* resemble each other in that both ferment lactate and both produce propionic acid. Vitamin B_{12} is known to form part of a coenzyme system which functions in propionate metabolism[13]. Of the two organisms, *S. ruminantium* is the more important in the rumen of the mature cow, while the other predominates in calves and under special feeding conditions.

The assays with *O. malhamensis*, which reflect true animal vitamin activity, indicate that these organisms were not in general, under these pure culture conditions, synthesizing as large a proportion of vitamin B_{12} relative to the analogues as they do in the rumen (0·3–5·6 per cent of the *E. coli* plate assay value as compared to 10 per cent). While clarified rumen fluid was added to the culture medium to simulate as nearly as possible the conditions prevailing in the rumen, such conditions could obviously not be completely obtained, since, in the latter case, the various species of micro-organisms may interact.

Activity indicated by the *E. coli* tube test was only about one-fourth to four-fifths of that shown by the plate assay. This is further evidence that much of the activity shown by the synthesizing strains in the *E. coli* plate test was due to analogues of the vitamin. Preliminary ionophoretic and chromatographic studies of some of the *Selenomonas* cultures indicate that the activity present consisted primarily of two factors, one moving faster than vitamin B_{12} on chromatography and having an ionophoretic mobility corresponding to that of factor *B* and the other moving more slowly than vitamin B_{12}, possibly being factor *A*.

Modifications of the culture medium in respect to energy sources, precursor bases and the like might be expected to change the relative amounts and nature of the vitamin and its analogues present in the culture. However, it would appear from the present study that *S. ruminantium* and *P. elsdenii* are among the chief organisms responsible for the production of vitamin B_{12} and its analogues in the cow.

L. P. DRYDEN
A. M. HARTMAN
M. P. BRYANT
I. M. ROBINSON
L. A. MOORE

Dairy Cattle Research Branch,
Animal Husbandry Research Division,
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville,
Maryland.

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Taxonomy of *Haemophilus vaginalis*

THE numerous authors who have reported the isolation of '*Haemophilus vaginalis*' from cases of vaginitis, or of non-specific urethritis in men, have not succeeded in determining its *X* and *V* requirements; thus its taxonomic position is uncertain. We have therefore examined six strains of *H. vaginalis*—two from C. D. Dukes, who with Gardner first described this species[1], and four from P. N. Edmunds[2].

It was found that these strains, inoculated on fresh blood agar[2] and incubated microærophilically with increased carbon dioxide at 37° C., grow within three days as pinpoint hæmolytic colonies of Gram-negative rods. If, however, films are made after 18–24 hr. from hæmolytic zones in which no colonies are visible, the rods are found to be Gram-positive, with an arrangement suggestive of corynebacteria.

Moreover, all six strains grow comparatively copiously on nutrient media with a high serum content. On Roux inspissated serum, single colonies reach a diameter of 5 mm. after incubation for 7 days. All strains grown on Roux serum slopes are constantly Gram-positive, the majority of rods showing well-developed polar granules and some clubbing. They are catalase-negative and do not grow on a 0·04 per cent potassium tellurite medium suitable for the growth of *C. diphtheriae*[3].

Some of these characteristics are shown, according to Bergey's *Manual of Determinative Bacteriology*[4], by *C. parvum*. Two authentic strains of this species were obtained and compared with the six cultures of '*H. vaginalis*'.

The following constant differences were found on repeated examination. *C. parvum* stains Gram-positive uniformly at all stages of growth, and is catalase-positive. Unlike '*H. vaginalis*', it ferments sorbitol, adonitol and mannose; on the other hand, '*H. vaginalis*' strains ferment maltose, dextrin and starch, which are not attacked by *C. parvum*. Both species ferment glucose, lævulose and xylose. There is therefore little doubt that these '*H. vaginalis*' strains are not identical with *C. parvum* but are a species of *Corynebacterium* not previously described, for which the name *Corynebacterium vaginale* is suggested.

K. ZINNEMANN
G. C. TURNER

Department of Bacteriology,
School of Medicine,
Leeds, 2.

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GENETICS

Fertility and Chromosome Pairing in Autotetraploid Rye

IT is generally accepted that the fertility of autotetraploids depends in part on the types and distributions of chromosome associations at meiosis. On this basis selection for heritable change in the distributions of the associations should be effective in improving fertility. There is, however, little experimental evidence to confirm this[1–3]. On the contrary the evidence of Morrison[4] and of Morrison and Rajhathy[5,6] indicates that selection would be ineffective. They find no evidence either for heritable variation in the pattern of chromosome association at meiosis or for its effect on fertility.

These results are surprising. There are at least two good reasons for expecting heritable variation in the chromosome association pattern: (1) Theoretically, the types and frequencies of the associations: quadrivalent, trivalent plus univalent, bivalent pair, bivalent plus two univalents and four univalents, must depend partly on chiasma frequency[7]. (2) Chiasma frequencies are genotypically controlled[8].

That both factors are relevant and operate to control the fertility of autotetraploids is in fact established by our own investigations on rye. The results which follow here are from induced autotetraploids of two F_2 families derived from crossing three diploid inbred rye lines in pairs. Firstly, the range of chiasma frequencies found in these and other F_2 families indicates segregation of genes controlling chiasma frequencies. Secondly, when quadrivalent and univalent frequencies are plotted against the chiasma frequencies of the F_2 plants, as in Fig. 1 *A* and *B*, it will be seen that the distributions of the various chromosome configurations are dependent on the chiasma frequencies. In each case the regressions are significant. The frequencies of the other associations show exactly the same dependence on chiasma frequencies. Clearly the adjustment of chromosome association frequency is a consequence of change in

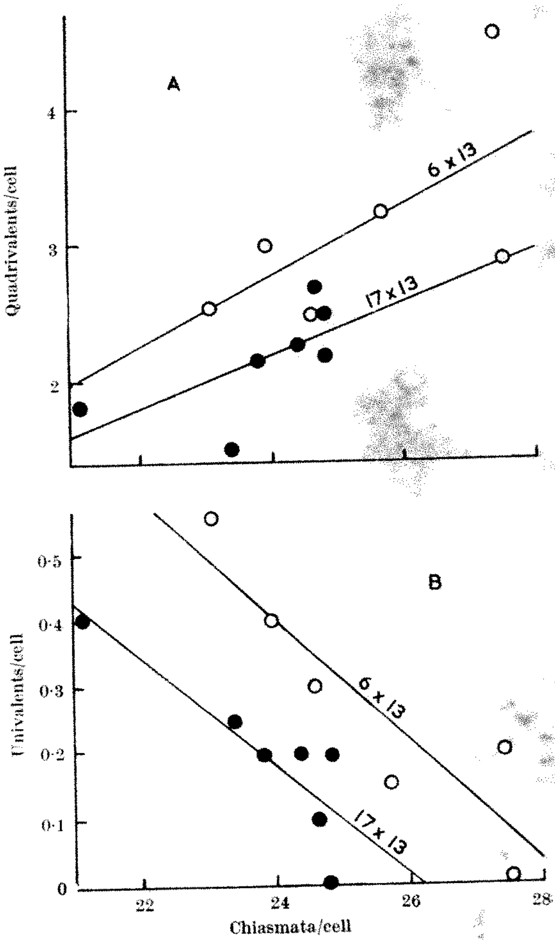

Fig. 1. Regression of association frequencies on chiasma frequencies in the two F_2 families. *A*, Quadrivalent distribution; *B*, univalent distribution calculated from the frequencies of the associations with univalents, III. I and II. 2I

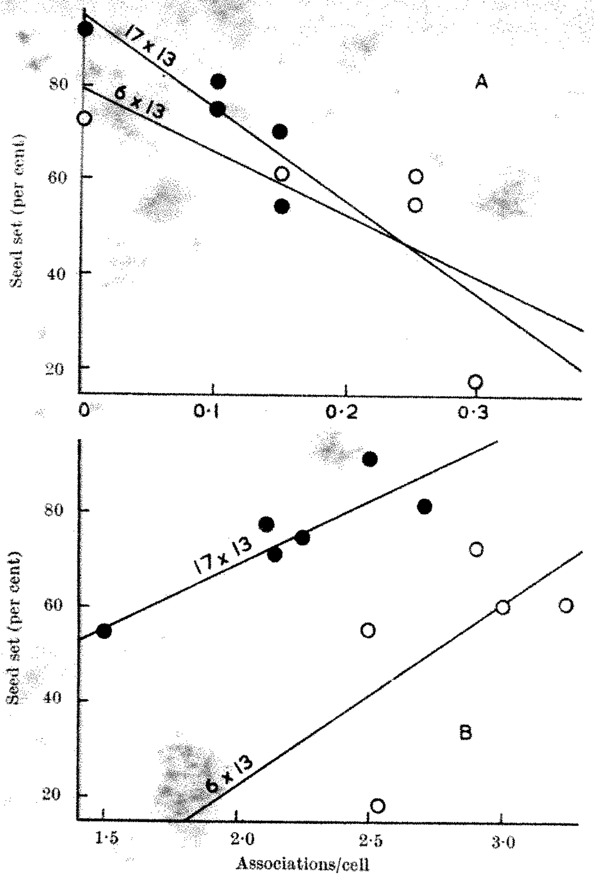

Fig. 2. Regression of fertility (percentage of seed set) on association frequencies in the two F_2 families. *A*, Trivalent frequencies; *B*, quadrivalent frequencies

chiasma frequency which, as we have inferred earlier, is genotypically controlled. It will also be observed in Fig. 1 that whereas in both F_2 families the association frequencies change with chiasma frequencies, 6 × 13, over the same range of chiasma frequencies, has more quadrivalents and more univalents than 17 × 13 ($P = 0·001$). There is, therefore, variation in the mean number of quadrivalents and univalents in the two families that is partly independent of their chiasma frequencies. This also must be assumed to be heritable, and provides an additional source of variation on which selection could act in altering the pattern of chromosome associations.

When we come to consider the relation between chromosome behaviour and fertility it is surely to be expected that variation in univalent frequency alone must influence the viability of the gametes produced. Also since many of the univalents are associated with trivalent formation a negative correlation between trivalent frequency and fertility should be expected. In Fig. 2 *A* fertility, measured as per cent seed set, is plotted against trivalent frequency. The regressions are significant and a negative correlation is confirmed. When, as in Fig. 2 *B*, the per cent seed set is plotted against quadrivalent frequency the regressions are positive.

From these results it is evident that the fertility of autotetraploid rye is dependent on the pattern of chromosome association at meiosis. Since the association pattern is under genotypic control it follows that selection for high fertility in rye must be effective and should be based on increasing the quadrivalent frequency and reducing the trivalent frequency. Both, as we have seen, would result from increasing the chiasma frequency. Finally, it is worth pointing out that the somewhat surprising relation between high quadrivalent frequency and high fertility found in rye may not hold for all other autotetraploids. In rye the chiasmata are distal and relatively few in number. This allows for efficient and regular separation of quadrivalent chromosomes at first anaphase of meiosis.

One of us (J. R.) acknowledges the award of an Agricultural Research Council postgraduate studentship.

J. ROSEWEIR
H. REES

Department of Agricultural Botany,
University College of Wales,
Institute of Rural Science,
Aberystwyth.

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Differential Characteristics in Uniform Strains of Mice

THE pharmacological sensitivities of uniform mouse strains determined in specified conditions show clearly defined differential characteristics. The comparison is made in Fig. 1 for the same mouse strains between their approximate ED_{50} convulsive responses to doses of insulin, and their approximate LD_{50} to histamine. The colour density of the histogram is relative to the colour of the mouse strain.

The order of magnitude of these two quantal responses in any one strain of mouse is unrelated, although the *C57Br/cd* strain is the most sensitive for both. Also in each response the three white strains of mouse used resemble one another, possibly due to their derivation. The response of the *C3H* strain to insulin

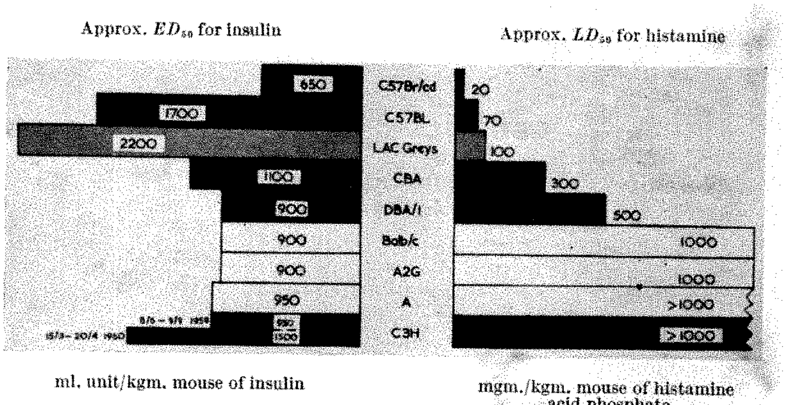

Fig. 1. Pharmacological sensitivities of some mouse strains determined in specified conditions

differed with samples of mouse, but the difference was not investigated for significance in these approximate determinations.

A further investigation of the quantal response to insulin was made with some of these strains and their F_1 crosses, and of their quantitative response to pentobarbitone sodium[1].

The results with the quantal response emphasized the genetic relation of the strains of mouse, as a species (*Mus musculus*), although specific differences were apparent between them. Of the two F_1 strains from *CBA* and *A2G* mice, the response by each more closely resembled that by its maternal parent, while the response of the hybrid from *A2G* females and *DBA*/1 males was well away from that of either parent and much less precise.

The study of the quantitative response to pentobarbitone sodium defined the necessity for using mice of the same age and sex when investigating strain differences. It also revealed that the precision of their response was distributed haphazardly between inbred and hybrid strains.

ANNIE M. BROWN

Laboratory Animals Centre,
Medical Research Council Laboratories,
Carshalton.

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Interactions of *S* Alleles in Diploid Pollen

SELF-INCOMPATIBILITY (*S*) alleles in the pollen of autotetraploids show either independent action or one or other of two types of interaction—'dominance' and 'competition'. While dominance interaction is common, competitive interaction is rare. When two *S* alleles compete for a presumably limited amount of substrate, a competitive interaction occurs which leads to self-compatibility owing to the lack of full expression of either of the *S* alleles[1]. A theory is proposed to explain the physiology of the different types of *S* allele behaviour in autotetraploids.

Studies in the diploid pollen of *Œnothera organensis*[1] and *Trifolium pratense*[2] have shown that the same pair of alleles could show both dominance and competitive interactions in the pollen. For example, a tetraploid plant of *T. pratense* having the constitution $S_{1\cdot1\cdot3\cdot3}$ may be self-compatible because of competitive interaction in the pollen $S_{1\cdot3}$. The same pollen when applied to a style of constitution $S_{1\cdot1\cdot2\cdot2}$ may be incompatible because S_1, which is also present in the style, is dominant over S_3 in the pollen; but it may be compatible in a style of constitution $S_{2\cdot2\cdot3\cdot3}$. This situation would suggest that the occurrence of the S_1 and S_3 alleles together in the pollen does not *per se* produce competitive interaction for, if it did, $S_{1\cdot3}$ pollen would be compatible on all styles. It is evident, therefore, that competitive and dominance interactions refer to two different aspects of the incompatibility reaction.

Competitive interaction occurs only when pollen grains germinate on styles that also possess the two *S* alleles present in the pollen. Thus competitive interaction in the pollen is governed not only by its own constitution but also by the constitution of the style. Dominance interaction, by contrast, is pre-determined in the pollen and its expression is not dependent on the constitution of the style.

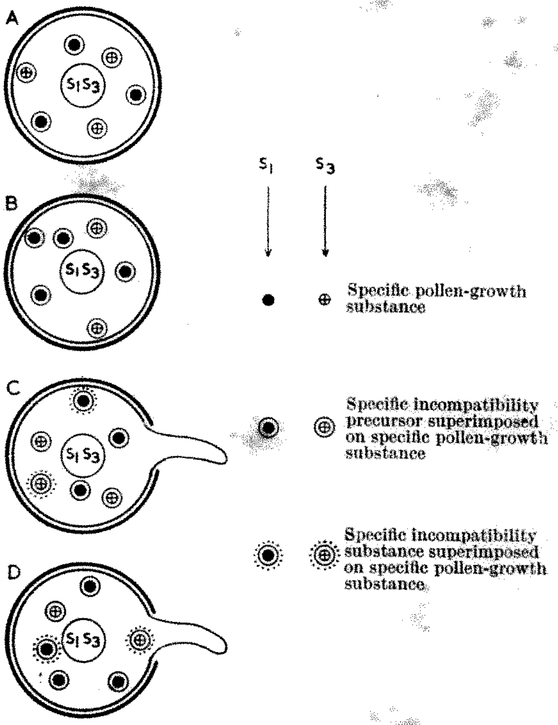
A
B
C
D
S_1S_3
S_1
S_3
Specific pollen-growth substance
Specific incompatibility precursor superimposed on specific pollen-growth substance
Specific incompatibility substance superimposed on specific pollen-growth substance

Fig. 1. Interactions of *S* alleles in diploid pollen. Three units are considered essential for the full expression of an *S* allele. *A*, independent action; *B*, dominance interaction, $S_1 > S_3$; *C*, competitive interaction; *D*, dominance and competitive interaction, $S_1 > S_3$

In *Œnothera organensis* there are two units of the *S* allele complex expressed in the pollen reaction : a unit controlling the pollen-growth substance and a unit controlling the specific pollen incompatibility precursors; and the latter is believed to be superimposed on the former[3,4]. It has been suggested that in the gametophytic species the action of both these units of *S* alleles occurs post-meiotically in the individual microspores[5]. However, the conversion of the incompatibility precursors into incompatibility substances seems to occur, after germination, during the growth of the pollen tube in the style.

Dominance may occur either at the level of specific pollen-growth substance only or at both levels of the specific incompatibility precursor as well as the specific pollen-growth substance. For example, in the hybrid *Œnothera trichocalyx* (self-compatible) ♀ × *Œ. pallida* (self-incompatible), the dominance interaction occurs only at the specific pollen growth substance level[3]. It occurs at both levels in the diploid pollen of an autotetraploid of gametophytic species as well as in the typically sporophytic species[3,5]. The competitive interaction, however, may be a secondary phenomenon and occurs later in the pollen tube when the specific incompatibility precursors are converted into specific incompatibility substances and probably involves competition for the specific enzymes presumably responsible for the conversion (Fig. 1).

S alleles, being neomorphs[6], are more likely to produce physiological interaction at some stage between gene action and final phenotypic expression than most mutant alleles of ordinary genes which are hypomorphs. The former kind of alleles produce qualitatively different effects while the latter produce similar effects but of different degree. This rare property of competitive interaction within a compound locus has also been observed for the scute alleles of *Drosophila melanogaster*[7,8].

In gametophytic species when there is an independent action of alleles in the pollen the phenotypic expression is always limited to the incompatible allele[1,2,9]. A similar situation is the rule in the diploid style in which, while both alleles are fully expressed, the compatible allele does not interfere with the incompatibility reaction between a haploid grain and the style. In *Physalis ixocarpa*[9], in which incompatibility is controlled by 2 series of S alleles, when 2 alleles act independently, the occurrence of only one common allele in the pollen and the style is enough to produce incompatibility. These observations support the 'oppositional inhibition' theory[10] of physiological mechanism of incompatibility reaction.

KAMLA KANT PANDEY

Crop Research Division,
Department of Scientific and Industrial Research,
Lincoln,
New Zealand.

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MISCELLANEOUS

Plastic/Carbon Replication for Electron Microscopy

THE use of plastics, such as 'Bedacryl 122X' (ref. 1), 'Triafol', etc., in the preparation of carbon replicas for examination in the electron microscope is well known. A massive plastic replica is made and vacuum-coated with carbon; the plastic is dissolved in a suitable solvent, leaving a carbon replica of the surface of the original specimen. The carbon replica can be mounted on a copper grid, shadowed, and examined in the microscope.

However, the plastics tend to swell as they dissolve, occasionally causing the carbon replicas to buckle and fold over or break. This is particularly true of replicas of rough surfaces. Sometimes a coating of paraffin wax can be used to stabilize the carbon replica while the plastic is being dissolved, the wax coating being afterwards dissolved in petroleum ether. This technique can be applied to 'Triafol'/carbon replicas; methyl acetate saturated with paraffin wax is used to dissolve the 'Triafol'.

At best, however, this method is somewhat unsatisfactory. First, the wax does not adhere very well to the carbon replica and may become detached from it during dissolution of the plastic. Secondly, unless precautions are taken, evaporation of the wax-saturated solvent precipitates wax on to the plastic, which consequently is incompletely dissolved. The technique is anyway inapplicable to 'Bedacryl'/carbon replicas because of the high solubility of paraffin wax in solvents used for 'Bedacryl'.

A method free from the shortcomings of the paraffin-wax technique has been developed in this laboratory. It involves the use of gelatin (which is insoluble in most organic liquids but soluble in water), and can be used with 'Bedacryl' as well as with other plastics. The procedure recommended is as follows:

A carbon-coated plastic replica is prepared by the usual method. A thin layer of a warm, freshly prepared, 20 per cent aqueous solution of gelatin is then spread over the carbon-coated surface and allowed to dry. The plastic/carbon/gelatin film is cut into small pieces which are transferred to a bath of solvent to dissolve the plastic. During the dissolution the carbon replica adheres well to the gelatin and does not buckle. When all the plastic has dissolved, the carbon/gelatin films are removed from the bath, allowed to dry, and then floated, gelatin downwards, on the surface of a bath of warm water. After the gelatin has dissolved the replicas are picked up on grids, rinsed with water, and allowed to dry. The replicas can now be shadowed (if this has not already been done prior to carbon coating) and examined in the microscope.

By this method, clean, whole carbon replicas, large enough to cover a standard grid, can be made.

N. J. HULMSTON

Unilever Research Laboratory,
Port Sunlight, Cheshire.

[1] Bradley, D. E., *Brit. J. App. Phys.*, **5**, 65 (1954).

A Solvent Trough for Paper Chromatography

DR. M. D. GWYNNE has described an easily made solvent trough for use in descending paper chromatography[1].

In this laboratory we have also found the glass troughs costly, difficult to make, and easily broken. For several years we have, therefore, made troughs from commercial plastic knife-boxes. In these boxes there are compartments of different sizes for the storage of knives, spoons and forks. A compartment of suitable size is cut out as shown in Fig. 1 (top). Two glass rods of suitable length are held together by means of two plastic pieces also cut out of the knife-box (Fig. 1).

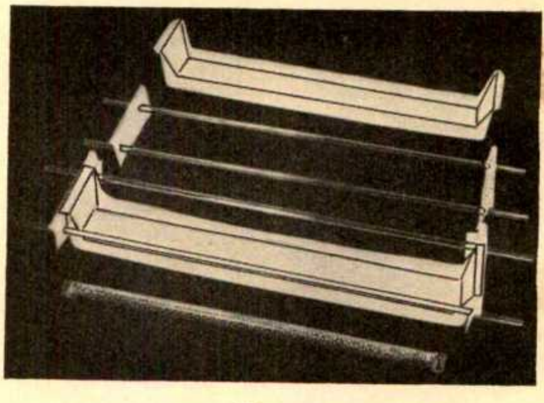

Fig. 1

When the trough is to be used, it is held between these two plastic parts (Fig. 1, bottom). The detail in the foreground of the illustration is a glass tube, filled with lead shot and sealed in both ends. It is used for keeping the papers in position when the trough is used.

NILS NYBOM

Balsgård Fruit Breeding Institute,
Fjälkestad, Sweden.

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FORTHCOMING EVENTS

Tuesday, July 17—Thursday, July 19

Society for Applied Bacteriology (at the School of Agriculture, Sutton Bonington, Loughborough, Leics.)—Summer Conference.

Thursday, July 19

Royal Society of Tropical Medicine and Hygiene (at the Hospital for Tropical Diseases, St. Pancras Way, London, N.W.1), at 7.30 p.m.—Clinical Meeting. Chairman: Sir George McRobert.

Friday, July 20

Nutrition Society (at the Central Veterinary Laboratory of the Ministry of Agriculture, Fisheries and Food, New Haw, Weybridge, Surrey)—Symposium on "The Effect of Changes in Nutrition on the Host-Parasite Relationship".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Librarian—The Registrar, The University of York, The King's Manor, York (July 20).

Lecturer or Assistant Lecturer in Applied Mathematics—The Registrar, The University, Sheffield (July 20).

Demonstrators (with good honours degrees and approximately one year's research experience) in Chemistry—The Registrar, University College of Wales, Aberystwyth (July 21).

Junior Research Fellow (with a good honours degree, or equivalent, in chemistry, biochemistry or agricultural chemistry) in the Department of Soil Science, for chemical studies on changes in forest litter resulting from nitrogen fertilizer treatments—The Secretary, The University, Aberdeen (July 21).

Lecturer (with research experience in experimental nuclear physics) in Physics—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (July 22).

Assistant Lecturer in Applied Mathematics—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (July 23).

Assistant Lecturer (with a good honours degree in zoology and preferably a special interest in genetics) in Zoology—The Registrar, King's College (University of London), Strand, London, W.C.2 (July 23).

Assistant Lecturer (with a good honours degree in botany) in Botany—The Registrar, King's College (University of London), Strand, London, W.C.2 (July 23).

Junior Research Fellow (with a first- or second-class honours degree in chemistry or biochemistry, or equivalent qualification, plus at least two years postgraduate experience, preferably in a field involving biochemistry), to work under a microbiologist on the problem of chemical compounds associated with the development of mould growth on oilseeds and related foodstuffs, including the biochemical aspects of the investigation—Tropical Products Institute, D.S.I.R., 56–62 Gray's Inn Road, London, W.C.1, quoting Ref. YE.728/2 (July 23).

Lecturer or Assistant Lecturer (preferably with experience in the field of nuclear magnetic resonance) in Inorganic Chemistry—The Registrar, The University, Leicester (July 24).

Lecturer and an Assistant Lecturer (men or women graduates) in Zoology—The Secretary to the Council, Westfield College (University of London), Kidderpore Avenue, London, N.W.3 (July 27).

Assistant Lecturer or Demonstrator in Pathology—The Registrar, The University, Manchester 13 (July 28).

Junior Research Fellow in Botany—The Registrar, The University, Sheffield (July 28).

Chair of Biochemistry; and Chair of Pharmacology in the University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, Senate House, University of London, London, W.C.1 (July 30).

Senior Lecturers or Lecturers (2) in Anatomy; and a Senior Lecturer or Lecturer in Physiology at the University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, Senate House, University of London, London, W.C.1 (July 30).

Evans Medical Research Fellow (with an honours degree in physiology or biochemistry) in the Nutritional Aspects of Hæmatology—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 31).

Lecturer in Psychology at the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, July 31).

Assistant in Research (with an honours degree in engineering, agricultural engineering, or the equivalent) in Agricultural Engineering—The Secretary, School of Agriculture, The University, Cambridge (August 2).

Lecturer (with an honours or higher degree in physics, and preferably previous teaching and research experience) in Theoretical Physics at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 18).

Fellow (with experience of research in New Guinea in social anthropology) in the Department of Anthropology and Sociology, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 24).

Ferranti Fellow (with the degree of Ph.D. or similar research experience) to carry out research in any field connected with electronic computing machines—The Director, University Computing Laboratory, 9 South Parks Road, Oxford (August 31).

Lecturer in Parasitology (with special reference to flatworms)—Head of the Department of Zoology, Imperial College of Science and Technology, London, S.W.7 (August 31).

Lecturer/Senior Lecturer (with experience and research interests in inorganic chemistry, and preferably with a special knowledge in the application of visible and ultra-violet absorption spectroscopy, magnetism and theoretical chemistry to co-ordination compounds of the transition metals) in Inorganic Chemistry at the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

Lecturer (preferably with a special interest in economic geography, particularly in regard to location theory, transport, and/or resource development) in Geography at the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

Arthur Jackson Chair of Human Biology and Anatomy—The Registrar, The University, Sheffield 10 (September 20).

Official Fellow and Tutor in Mathematics—The President, Magdalen College, Oxford (October 22).

Chemist, Biochemist or Microbiologist, to study the aspects of cellular radiobiology—The Director, Experimental Radio-pathology Research Unit, Hammersmith Hospital, Ducane Road, London, W.12.

Engineer (with Higher National Certificate or proven practical ability, and preferably a working knowledge of transistor technique) in the Statistics Department, to assist with maintenance of electronic digital computers and peripheral equipment—The Secretary, Rothamsted Experimental Station, Harpenden, Herts.

Experimental Officer or Assistant Experimental Officer (with a pass degree, H.N.C., or equivalent, or, if under 22, G.C.E. "A" level or equivalent) in the Chemistry Department, to help with research on mineral metabolism in cattle, particularly the forms of combination of magnesium and calcium in cattle diets and ingesta, and the development of the analytical methods to be used—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 61/15.

Head of the Mathematics Department (Grade E)—The Headmaster, Bradford Grammar School, Bradford.

Lecturer or Senior Lecturer (with good academic qualifications, and preferably research experience to doctorate level or equivalent) in the Department of Electrical Engineering—Dr. J. H. Calderwood, Royal College of Advanced Technology, Salford.

Lecturer (preferably with interests in the mechanics of continuous media) in Applied Mathematics—Head of the Department of Mathematics, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

Lecturer (with experience and interest in electrical measurement, and preferably special qualifications in electronic and/or high frequency techniques) in the Department of Electrical Engineering—The Academic Registrar, Loughborough College of Technology, Loughborough, Leics., quoting Ref. 15/G.

Mathematical and Science Teachers (fully qualified) at the Imperial Ethiopian Naval College, Massawa—Cdr. I. Desta, Imperial Ethiopian Embassy, 17 Princes Gate, London, S.W.7.

Research Assistant (honours graduate in botany) in Quaternary Research—The Registrar, The University, Leicester.

Research Assistant (with experience and ability in organic chemistry), for the preparation of compounds related to bile acids for biological assay—Prof. G. A. D. Haslewood, Biochemistry and Chemistry Department, Guy's Hospital Medical School, London Bridge, London, S.E.1.

Research Fellows (2) (preferably with a Ph.D. degree in engineering or physics, or equivalent research experience) in the Department of Electrical Engineering, for work in the field of millimetre wave techniques, under Prof. A. L. Cullen—The Registrar, The University, Sheffield 10.

Senior Geologist (with a first- or good second-class honours degree in geology or engineering geology with at least seven years postgraduate experience in water supply geology, and preferably experience in electric logging of boreholes) in the Geological Survey Division of the Federal Ministry of Mines and Power, Lagos, Nigeria, for duties which will include the compilation of water supply records, the preparation of water supply papers for publication, and the supervision of water supply exploration drilling programmes—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. J.4/7.

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Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

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ALLOCATION OF SCIENTIFIC RESEARCH IN BRITAIN

IN January 1961, the Council of the Institution of Chemical Engineers set up an Exploratory Committee under the chairmanship of Prof P V Danckwerts to review the role of chemical engineering as applied to all manufacturing processes It was expected that one result would be the recognition of new fields for the application of chemical engineering principles and practices Specifically, the Committee was to survey selected fields and report on recent developments, trends and gaps in knowledge, assessing their chemical engineering significance, to recognize the major advances and to indicate how the deficiencies might be made good On occasion the Committee might indicate the existence of unnecessary, or duplication of, effort in chemical engineering research, and from time to time it might form *ad hoc* sub-committees as study groups for particular aspects of their work

This Exploratory Committee, the report of which was published in the April 1962 issue of *The Chemical Engineer*, covered a rather narrower field than the Committee on Chemical Engineering Research of the Department of Scientific and Industrial Research which reported early in 1951 That Committee, of which Mr H W Cremer was chairman, was appointed to review the needs for research in chemical engineering and the extent to which they could be met by existing facilities, but it also indicated a number of fields in which industry considered there was further need for chemical engineering research The main conclusion of the Cremer Committee was that national requirements for chemical engineering could only be met if existing facilities for research were supplemented by a central organization specially adapted or created for the purpose No such organization has yet emerged, and although the colleges of technology may now be able to make a more substantial contribution to research in this field, in the view of the Danckwerts Committee, requirements for research remain as stated by the Cremer Committee

The Danckwerts Committee has therefore considered the contribution which existing Government research institutions, such as the National Chemical Laboratory, the National Engineering Laboratory or the Warren Spring Laboratory, might make in this field While close contact with industry is desirable, the Committee considers that these research stations should remain free to carry out autonomous programmes of a general and fundamental nature There is much work which is unlikely to be done by industry and which is unsuitable for universities Research institutes, with their continuity of work, large technical staff, and freedom from the need to shape projects to degree requirements, are better suited than universities to make systematic measurements of chemical and physical quantities of the kind which look into chemical engineering design calculations It is suggested that consultation between industry and the universities could lead to the formulation of a worth-while programme on these lines

Besides this, the Committee points to sub-technologies which enter into a wide variety of industries, but are not studied adequately in their own right, because they do not form the primary interest of any one industry Powders and granular materials are given as one example and one for which the Warren Spring Laboratory might be appropriately used More information is also needed on heat transfer in situations of particular interest to chemical engineers It is suggested that work here on viscous and non-Newtonian fluids, condensing and multi-component vapours, and packed and fluidized beds, for example, would be a logical extension of the work of the National Engineering Laboratory on heat transfer It is also suggested that the Department of Scientific and Industrial Research could provide a valuable service to industry generally by making critical surveys of the literature of selected subjects Examples are to be seen in those on crushing and grinding and on industrial drying which have already been published, and that on solid–liquid separation which is in hand, but the point is made that such surveys should be regarded as a public service rather than as something to be sponsored by particular industries

The Danckwerts Report suggests that the Institution of Chemical Engineers should play a greater part in influencing the activities of the Department of Scientific and Industrial Research along the lines indicated Industrially sponsored projects should not be allowed to dictate the programme, and after discussion with the Secretary of the Department, the Committee recommends the establishment of a Research Committee of the Institution to formulate detailed proposals, and it would seem that what the Exploratory Committee has in mind is the formation of a Research Council such as has recently been established by the Institution of Civil Engineers in collaboration with industrial associations and the Department of Scientific and Industrial Research. In the present report the activities of the research associations are only mentioned, although the Exploratory Committee is obviously aware of their potentialities However, they are concerned with activities which seek to further the dissemination of ideas and not the raising of funds for research, stress is laid on the need for constructive thinking if satisfactory proposals for an effective organization are to be formulated

The Exploratory Committee was naturally enough concerned particularly with the possible role of the Institution of Chemical Engineers in encouraging, promoting or guiding research in chemical engineering and allied fields, the proposal for a research committee or council is only one of the recommendations of the Report. The Committee thought that there were four requirements First, to help industry to formulate and implement explicit research programmes in fields of chemical engineering in which fundamental research is needed Secondly, to help the universities and colleges of technology to maintain lively and realistic research programmes Thirdly, to suggest speculative and exploratory fields of research detached from the present-day preoccupations of industry; and fourthly, to make recommendations in the appropriate quarters as to the part which Government research institutes might play in chemical engineering

A professional institution is clearly at an advantage over industry in approaching the universities and colleges of industry in the way suggested, and the whole Report appears to be inspired by a conception of the functions of the professional institutions in the structure of research in Britain This is in striking harmony with the views which Lord Hailsham has expressed to the Parliamentary and Scientific Committee and which the Parliamentary Secretary for Science outlined in an address "A Policy for Science" to the Reading and District Council for Education in World Citizenship on May 25 The Exploratory Committee stresses the importance of giving due weight in research to the development of concepts and theories, and is considering how best the practical problems of industry can be grouped into separate fields in such a way as to prevent overlapping and to emphasize those features which are common to apparently different problems The appointment is recommended of a number of investigators, who would be called Industrial Research Fellows, to report on one specific field (see p 229 of this issue)

Each Industrial Research Fellow should endeavour to establish what are the real industrial problems, what additions to our knowledge might mitigate these problems, and what programme of fundamental research might provide the knowledge required Admittedly, these questions are difficult to answer with any precision, and it is arguable whether any one man could always be expected to provide the answers However, the Exploratory Committee cited the report of the Distillation Panel of the Association of British Chemical Manufacturers and the British Chemical Plant Manufacturers Association as an example of the degree of detail which might reasonably be expected Judgment and common sense are clearly as important as intellectual ability, and the Committee looks to the scheme to promote association with the staffs of universities and colleges of technology as well as with industry The resultant reports might serve as the primary agenda of a working party or form the basis of a co-ordinated scheme of research

The working parties visualized by the Exploratory Committee are likewise regarded as a means of bringing together those in industry, in the universities, in research associations and research institutes who are working on, or interested in, specific topics Careful thought must obviously be given to both the size and functions of any such working parties and to their relations with other bodies, though it appears to be of the essence of the scheme that the topics will represent fields which are not at present receiving attention An actual example is given in the working party on fluidization which has already grown up through the influence of a member of the Institution, Prof J F Richardson, and any such working parties are visualized as sponsored specifically by the Institution, which would appoint chairman and honorary secretaries and provide secretarial assistance and accommodation as required

This proposal for Industrial Research Fellows is designed to cover research in fields which represent established industrial activities, but the Committee recognizes that it is equally necessary that there should be speculative or exploratory research aiming ultimately at new techniques, substances, processes, inventions, design procedures, control mechanisms and such like Such research, it points out, is less likely to be inspired by the day-to-day problems of industry and is more likely to arise from an appreciation either of industrial requirements in the widest sense. or of the possibilities inherent in some phenomena or idea. Another problem of wide scope is the recognition of new fields of chemical engineering, and like speculative or exploratory research, this cannot be stimulated in such a systematic way as research directed towards the solution of present industrial problems

Here, as the Danckwerts Committee recognizes, something is being dealt with which cannot be organized It is a matter of providing the conditions which foster creative work and seeing that men of the requisite quality are given the opportunity to develop their ideas There is indeed a matter of communication and seeing that the channels for the communication of ideas are free and adequate However, on this aspect of chemical engineering research it is interesting to note that the Danckwerts Committee is not looking solely to the universities either for investigators or facilities

In a recent broadcast talk on the Third Programme, published in *The Listener* for May 3, Dr Dennis Chapman pointed out that there was already a marked trend for experimental research in the physical sciences to move away from the universities, and he thought that this trend would continue Although the actual expenditure on research in the universities had increased, relative to industry, expenditure on university research had dropped remarkably Much nuclear research that would at one time have been the sole prerogative of the universities is now also carried out, for example, at the National Physical Laboratory.

Admittedly, this trend can be attributed at least in part to the high cost of much modern equipment coupled with the fact that a university department is seldom well placed to provide the organized team with a variety of scientific talents which is so often essential for an organized attack on complex problems. Quite apart from the Government's rejection of the University Grants Committee's advice on university finance for the next quinquennium, concern has been expressed about the financing of fundamental physical research in the universities

However, Dr Chapman rightly points out that the financing of university research to-day presents a far too complex problem to be solved merely by increased funds It calls for clear thinking about the functions of the universities in regard to research and whether they should be just training centres or something much more Nor, as Dr Chapman fully recognizes, can such questions be answered without reference to what is being done elsewhere If we are satisfied that the trend away from the universities is reasonable, are we, for example, also satisfied with the quality of research carried out elsewhere ?

Dr Chapman does no more than touch on the problems involved He recognizes that industry, at least large-scale industry, is seeking to find a reasonable balance between the more fundamental type of research and the more directly applied, as well as the purely empirical, work This is true, too, of the research associations, while, as he also notes, a good deal of university research could, with every justification, be called applied research Work correctly described as applied research in one field may yield facts and conclusions that are fundamental to another, and while Government and industrial establishments are now responsible for much that can properly be termed fundamental research, it is so easy to ignore research of this type that special care must be taken to foster and protect it

Dr Chapman suggests that to counteract the deleterious effects of the present trend of research away from the universities, the universities must either modify their organization and appoint many full-time research workers, with perhaps loosely linked research institutes for advanced study, or sponsor research centres for co-operative work At the same time, research in Government and industrial laboratories requires expansion in scale and improvement in quality, and, like the Zuckerman Report, he urges greater mobility of personnel between Government, industrial and university laboratories The first suggestion obviously involves a considerable expansion in the funds at the disposal of the universities for research, and if, in fact, the ceiling imposed by the Government on university expenditure is valid for economic reasons, there may well be sound reasons why, in the national interest, the universities should allocate a larger proportion of their funds for research purposes instead of for expansion of the numbers of students The second manifestly is a matter of administration and may well be for consideration by the Treasury Committee which is reviewing the organization for the promotion of civil service by Government agencies

What seems important is that the policy suggested by the Exploratory Committee of the Institution of Chemical Engineers in regard to chemical engineering and by Dr Chapman in discussing research in transition should have so much in common with the views which both the Minister for Science and his Parliamentary Secretary have freely expressed They are in full accord with the research council philosophy, with the idea that the first task of the Government in fostering research to support first-class people with promising ideas, and to refuse to support the bogus or second-rate Administratively, the prime need is an organization headed by a Minister, capable collectively of isolating the right questions, conducting the proper discussion of them with the right people both inside and outside the Government organization, making certain that the real problems are discussed and that the scientific answers are given by those who alone are capable of giving them

It is, of course, a help for the administrator to have a knowledge of scientific principles and even some scientific training But the vital need is to ensure a proper and effective relation between the scientist and the administrator and between the two of them and the responsible Minister In this connexion, in his address on May 25, Mr Freeth stressed the fact that the Research Councils are not research monopolies The effectiveness of the present system in Britain derives largely from this fact, from its flexibility and from the care which is taken to ensure that private foundations, professional bodies and the independent research institutes have their full opportunity to contribute There may be room for discussion and examination as to the respective roles of these bodies, the universities, the colleges of technology, Government institutions, the industrial research associations and industry, and chemical engineering is probably not the only field in which arrangements for co-operation could be improved

Lord Hailsham is clearly determined to re-value thoroughly the whole Government organization The Zuckerman Report is already being used as a working document for this purpose, including some inquiry into the organization of the Scientific Civil Service Besides the inquiry into the research associations instigated last autumn, the Committee set up by the Treasury under the chairmanship of Mr B St Trend, to review the organization of civil science under Government agencies, is expected to give the Minister some guidance on the handling of radio-astronomy and large projects in nuclear physics which involve the question of university grants in the physical sciences as against the life sciences It is also hoped that this Committee will assist the tidying of the organization of science under Government generally and the removal of such apparent anomalies as the responsibility of the Admiralty for the Astronomer-Royal or of the Board of Trade for the National Research Development Corporation

The Treasury Committee's terms of reference are comprehensive and its membership, including Sir Keith Murray, Lord Todd and Prof C H Waddington, guarantees that the mechanism of advice to the Minister for Science and functions of the various agencies for civil science and their relations with other bodies will not be considered from a narrow angle Its existence indeed should encourage the Institution of Chemical Engineers and other professional institutions to proceed with any constructive proposals for strengthening Britain's organization for research The next report from the Advisory Council for Scientific Policy will be awaited with special interest.

THE ALGAE

The Algae
By Prof V J Chapman Pp viii+472 (London Macmillan and Co , Ltd , New York. St Martin's Press, Inc , 1962) 35*s* net

THE purpose of this book, which follows the general layout of the author's earlier work (1941), is to provide a survey of all aspects of the algae such as may be required by a university undergraduate, with selected parts marked for the use of schools and first-year students The author emphasizes that this work is not a second edition of his 1941 book but a new work incorporating recent advances in phycology

Some chapters—algal ecology, ecological factors and algal physiology—have been rewritten and two new chapters on the history of the algae and the economic uses of seaweeds have been added However, a large portion of this book has been taken direct from the 1941 book without any attempt to recast the text A survey of the algae is certainly given, but whether the balance the author has adopted with regard to the subject-matter will give the student a true picture of the algae is doubtful It is also debatable whether the type method of presentation that the author has used will be welcomed by the majority of university teachers

The first two-thirds of the book is devoted to morphology and taxonomy, and the introductory outline classification might, with advantage, have been a little fuller to give continuity to the rest The bulk of the morphological section is devoted to Euphycophyta (Chlorophyceae 99 pp , Phaeophyceae 93 pp , Rhodophyceae 42 pp), the Chrysophycophyta (Xanthophyceae, Bacillariophyceae, Chrysophyceae) occupying a bare 16 pages, the Pyrrophycophyta only 3 pages and the Myxophycophyta 16 pages The classes Euglenophyceae and Chloromonadophyceae are omitted from the taxonomical section although the genus *Euglena* is referred to in later sections The omission of these classes is regretted, particularly as *Euglena* is a type for many university entrance examinations also there is no certainty that filamentous (or pseudofilamentous) forms, equivalent to those in the Chrysophycophyta and Pyrrophycophyta, do not exist

The rest of the book considers fossil forms, reproduction, evolution, marine, salt-marsh and fresh-water ecology, ecological factors, physiology, symbiosis, geographical distribution, life forms and utilization In the space available this part of the book gives a considerable amount of useful information It is a pity however, that the author did not take the opportunity to incorporate into the appropriate sections the results from important recent researches, such as the work on fossil Dinophyceae and the recent studies on marine and fresh-water phytoplankton

A subject and author index is provided, and selected references for further consultation are given at the end of each section The work is profusely illustrated, and while this will be of considerable help to students, the general effect of the illustrations is very displeasing The lines are too heavy and the figures too crowded together Where figures have been redrawn they are usually inferior to those in the 1941 book and in one instance (Fig 29) the legend written for the 1941 book has not been corrected to fit the revised figure

As a result of utilizing text from the 1941 boo without any revision there are many inconsistencie in terminology which will be very confusing t students, for example, the use of Myxophyceae an Bacillariophyceae in the new parts and Cyanophycea and Diatomaceae in the text taken from the earlie book It is even more confusing when the autho uses "cilia" to mean flagella on page 9 and the sam word to mean mastigonemes (hair-like processes on flagellum) on p 259 Further, the genus *Botryococcu* is still left in the Xanthophyceae instead of bein moved to the Chlorophyceae. In addition, erroneou statements such as "the Chrysophyceae are nearly a freshwater", that "leucosin is a protinaceous substance" and that *Polysiphonia fastigiata* (*P lanosa* is "always found on one particular host, *Ascophyllum*" cannot be excused Neither can the incorrect statement (p 126) that Manton found only 9 fibrils in th posterior flagellum of the phaeophycean autherozoi be allowed to stand

We are very disappointed that the publicatio of this, the most recent algal text-book published i English, was not delayed until the whole text ha been thoroughly recast and brought into line wit modern trends Then much more of the interestin recent work could have been included in the tex and the result would have been a somewhat large but much more useful student algal text-book

Mary Parke
G T Boalch

CELL BIOLOGY

International Review of Cytology
Vol 9 Pp x+424 13 dollars Vol 10 Pp xiv+409 13 dollars Vol 11 Pp xxi+356 88*s* Edited by G H Bourne and Prof J F Danielli (New York Academic Press, Inc ; London Academic Press, Inc (London), Ltd , 1961)

THE sails of histology, after dominating the physiological seas in the nineteenth century and in the early years of the twentieth, fell limply into the doldrums in the period between the two World Wars But now, once again, they are filled and, indeed, fresh winds are blowing so strongly from several different directions that it becomes increasingly difficult for the cytologist to preserve his balance as his vessel veers hither and thither on new courses of discovery

In these three volumes of the *International Review of Cytology* many new approaches toward the study of cells come under expert scrutiny by investigators of many nationalities, though preponderantly from the United States Moreover, the cells may be bacterial, protozoan, annelid, arthropod, echinoderm, molluscan, piscean, amphibian, avian, mammalian or human, all come under review, and the reader must take them all in his stride He must either be a real biologist already, or become one

Naturally, all the articles are not uniformly good or interesting, for, in any event, what is interesting depends partly on the taste and idiosyncrasy of the reader For this reason it would be invidious to select particular articles on which to comment Some are almost purely chemical (for example, "The Chemistry of Schiff's Reagent"), some are embryological and functional (for example, "The Mechanism and Mechanics of Cleavage"), others are studies of detailed structure made mostly with the electron microscope (for example, "The Fine Structure of

Insect Sense Organs", or "The Electron-microscopic Analysis of Secretion") Incidentally, this last article emphasizes the difficulties of gaining any real concept of the sequence of events by studies of sectioned material only In spite of much excellent micro-cinematography of cells (see the article by C M Pomerat on "Cinematography, Indispensable Tool of Cytology") the concept that granules in cells are a necessary part of the secretory process dies hard Such activities as pinocytosis and the temporary storage of raw materials and the accumulation of metabolic by-products tend to be forgotten by many investigators of secretory processes when they are attempting to interpret the photographs of fixed sections Similarly, if the vast array of surfaces and membranes, belonging to various parts of the endoplasmic reticulum, mitochondria and the like are really the sites for the production of secretions by chemical syntheses, the final products might never accumulate anywhere, but could be dispersed molecule by molecule into the tissue-fluids, ducts or blood-stream, just as and when they were produced In a factory, storage is only necessary when production exceeds demand Fleet Street can daily saturate the breakfast tables of Britain with only the minimum of storage in the printing works

Books of the kind under review make invaluable reading for all biologists New horizons are inevitably opened up to the reader, and the exploration of uncharted seas provides stimulating competition for the old-established and perhaps obsolete trade routes Though some of the articles are more condensed than others, all are genuine reviews and not just annotated lists of references As such they should appeal to all biologists who are concerned with how things work and not only to cytologists in the narrow sense

E N WILLMER

FACT AND SURMISE ON VIRUSES

Advances in Virus Research

Vol 8 Edited by Kenneth M Smith and Max A Lauffer Pp ix + 414 (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1961) 86*s*

THE three main components of a science are the methods used to make observations, the observations, and the interpretations based on them The last merge smoothly into surmises, and the zone to which one label rather than the other is attached is clearly a matter of opinion In the early days of virus research there was so little genuine information that interpretation made up an unusually large proportion of the whole This seems to have established a tradition, and it is piously maintained by Kellenberger and by Mahler and Fraser in the articles on bacteriophage in this book Each contains much valuable information, and the authors, as a rule, distinguish observation from surmise but there is a risk that many readers will not keep the distinction continually in mind, in the face of such admirably persuasive presentations, and will get a false impression of the amount that is definitely known

To redress the balance there is an article by Valentine on the enhancement of contrast in electron microscopy This explains what is done, why it is done, and why alternative methods would be ineffective After a general section on photographic contrast, the merits of different methods for varying the electron-stopping power of the specimen are discussed. This is followed by a comparison of the results achieved by 'staining' viruses with heavy atoms, by surrounding them with a film containing heavy atoms so that the viruses show up in 'negative contrast', and by Muller's technique of evaporating metal across the dry preparation from one side so that the viruses and their surface features cast shadows

Cooper contributes an article on plaque assay of animal viruses A thin layer of living susceptible cells is immobilized on the wall of a suitable vessel, either by allowing the cells to become attached or by embedding them in agar, the virus suspension is then spread over the surface Plaques of infected cells develop and can be recognized either by direct observation or after staining The method is formally identical with the plaque assay of bacteriophage on a plate covered with susceptible bacteria It has now been applied to more than 50 viruses or virus strains and permits a precision and rapidity of work that was impossible with intact hosts or host tissues

Mumps virus is in many ways similar to the influenza viruses, Newcastle disease virus, and the para-influenza viruses Like these viruses it is absorbed by erythrocytes and agglutinates them, but its enzymatic activity, leading to release, is feeble. Cantell describes its biological properties, the difficulties met in attempts to purify it, and the variability of the response of hosts to it There are surprising, and still unexplained, differences in even such a simple host as the egg allantoic membrane so that, at some seasons, it is almost impossible to work with the virus This is experimentally inconvenient, but it is a salutary reminder that, in spite of the importance of information gained by physical methods, virus research remains a branch of biology

There are three purely biological articles, McNair Scott discusses poliomyelitis, Coxsackie, and other viruses that characteristically attack intestinal and respiratory tissue Bang and Luttrell discuss the nature of pathogenesis—that is, the relationship between host and parasite—in an extensive group of animal viruses No generalizations emerge from this except the not unexpected one that any treatment which affects the metabolism of the host will affect the behaviour of some virus in it Pereira has a similar theme he is concerned with changes in individual infected cells and the light they throw on the mechanism of virus multiplication and on the reasons why this is so often harmful to the host

This volume maintains the high standard of the series It could be argued that the absence of an article dealing specifically with plant viruses makes the book unbalanced, but this should not lead those primarily interested in plant viruses to disregard it for there is much here that, either by extension or analogy, will be of interest to them N W PIRIE

EVERYDAY USES OF ATOMIC ENERGY

The Release and Use of Atomic Energy

By Dr T E Allibone (Based on the Royal Institution Christmas Lectures) Pp xii + 158 + 20 plates (London Chapman and Hall, Ltd , 1961) 25*s* net

THIS book is based on the Christmas Lectures for young people which Dr Allibone gave in 1960 at the Royal Institution It follows the admirable tradition of those lectures in giving a clear and well-illustrated outline of the principal discoveries and concepts in atomic and nuclear physics, and of their application to electrical power generation. The first three chapters carry the reader from Neanderthal man to Calder Hall in a series of leaps from peak to peak in the curve of discovery over the period. Chapter 4 summarizes the leading features of the principal power-producing fission reactors in use or under development in Britain and the United States. In Chapter 5 some of the applications of radioisotopes are described. Finally, Chapter 6, one of the best in the book, is devoted to the work on controlled thermonuclear reactions, with a comment on the possibility that such reactions can be utilized to generate electrical power in the future.

In the course of the lectures Dr. Allibone tried to reproduce most of the crucial experiments in atomic physics over the past sixty years. These demonstrations must have fascinated his young audience. A hint of the fascination, and of the care with which the demonstrations were prepared, is given by the brief descriptions of the special experiments devised to illustrate principles, for example, the experiments to show the Doppler effect and the pinch effect in parallel conductors carrying a large current. The illustrations are clear and helpful and some are unusually vivid in the impression they give of classic experiments—the diagram of Mosely's apparatus is an example.

In one or two places one would have liked a little more detailed or exact description. The account of neutron slowing-down on p. 43, for example, leaves the reader uncertain as to why the neutron's speed is reduced. On p. 61 control of a reactor is described so briefly as to leave the impression that power output is varied simply by pulling control rods in or out and leaving them so. But these are minor defects in a book that should interest laymen of all ages and be of considerable use to teachers.

It is pleasant to see the cautionary tale of Frederick Worms (whose parents were not on speaking terms) preserved between hard covers.

K. E. B. Jay

THE WRITING OF AFRICAN GEOGRAPHY

The Republic of the Sudan

A Regional Geography. By K. M. Barbour. Pp. 292+42 plates. (London: University of London Press, Ltd., 1961.) 42s. net.

Africa

A Social Geography. By Anthony Sillery. Pp. xii+244+101 plates. (London: Gerald Duckworth and Co., Ltd., 1961.) 35s. net.

SOCIAL anthropologists often produce large volumes concerned with individual tribes, sometimes with single villages. Geographers perhaps have a different purpose, certainly a broader sweep; but if they study in sufficient detail and write at sufficient length they should manage to avoid those generalizations which the alert traveller or astute politician so often makes. Prof. Barbour, at one time a lecturer in what has become the University of Khartoum, and now head of the Department of Geography in University College, Ibadan, shows in his book on Sudan how this may be done. He has drawn extensively on his own field-work to bring out the significant regional variations in a country that, with an area of nearly a million square miles, is the largest independent State in the African continent.

This book is not a handbook of a vast territory, but it does provide a most useful introduction to it. A little less than half the book deals with Sudan as a whole, in terms of its physical and social geography and of its particular concern for the control and use of the Nile waters. There then follow chapters describing the six regions recognized by the author, who emphasizes for each its special characteristics and peculiar problems. Finally, there is an analysis of the factors contributing to the growth of a modern State in Sudan, and a conclusion that looks, with tantalizing brevity, at 'the way ahead', with the author's comment—applicable to so many countries besides Sudan to-day—that "the white man set in motion revolutionary changes in Africa, but responsibility for the future lies in the hands of the Africans themselves".

When more countries have volumes like this one on the Sudan and that on Nigeria in the same series by K. M. Buchanan and J. C. Pugh, or atlases such as those of Kenya or Tanganyika, both the white man and the African will be helped towards a deeper and more realistic understanding of the continent's present problems and future prospects.

Mr. Sillery, who was for many years a member of the Colonial Service and has latterly made some studies in African history, has tried to provide such understanding for the general reader who, in his own words, wants "some idea of Africa and of the people who live in it". It is a mine of up-to-date information about various parts of the continent, ranging from relief and climate to ethnology, agriculture, population, race relations and politics; it is written most readably and is very adequately illustrated by more than a hundred well-produced and usefully captioned photographs. Why the publishers and the author should call all this "social geography" is never made clear. Much, it is true, is basic material for the geographer (and many other students), but only rarely is it analysed geographically. It is significant, for example, that the book has only three maps, one of which shows relief by the "caterpillar method" associated with the map-making of more than a century ago. Furthermore, much of Mr. Sillery's information goes far beyond the limits suggested by the adjective "social". The titling of the book is indeed most unfortunate. Most geographers will almost certainly agree with my comment; other readers may understandably fight shy of a useful general work that has "geography" in its title. I may, of course, be wrong and the publishers right; they have had experience in this respect, since some years ago they produced a volume on the Far East with a similar title, also by an author who is not a geographer, and this book has recently gone into a third edition. It would be satisfying, however (certainly to geographers), if the world at large would accept that geography is no longer what it was once thought to be—capes and bays, products and tribes, and a general mass of miscellaneous and often unrelated information—and that real integrated geography of the kind generally taught to-day is most effectively written by trained geographers. Prof. Barbour's book is good evidence of what can be done if geographers are given the chance to travel, to observe and to analyse.

R. W. Steel

THE FUTURE NATURAL SCIENCES PROGRAMME OF UNESCO

By Dr HILLIARD RODERICK

Deputy-Director, Unesco Natural Sciences Department

THE United Nations Educational, Scientific and Cultural Organization (Unesco) was established in 1946 as one of the United Nations specialized agencies. (The specialized agencies of the United Nations are 15 intergovernmental bodies with wide international responsibility and specialized competence which have been brought into relationship with the United Nations by formal agreement.) As its name implies, it is devoted to education, culture and science, particularly fundamental science. Other members of the United Nations family also deeply engaged in science, usually of an applied nature, are Food and Agricultural Organization (FAO)—agricultural and fishery science, World Health Organization (WHO)—medical science, World Meteorological Organization (WMO)—weather science, International Telecommunication Union (ITU)—communication science, and International Atomic Energy Agency (IAEA)—peaceful development and application of atomic energy. Unesco is located in Paris and employs about 1,200 persons.

The Natural Sciences Department represents about 8 per cent of the professional staff and about 11 per cent of the regular budget of Unesco. In the 1963–64 regular budget of 39.0 million dollars, the substantive departments share as follows: Education (24 per cent), Culture (14 per cent), Natural Sciences (11 per cent), Mass Communication (11 per cent) and Social Sciences (8 per cent).

The Unesco charter states that one of the major objectives of Unesco is to increase and diffuse knowledge—in the case of the Natural Sciences Department, scientific knowledge.

What should Unesco, an intergovernmental agency devoted to science, do to increase and diffuse scientific knowledge? Primarily, it should serve as the agent of its 106 Member States in those international scientific matters which they cannot or do not wish to handle alone. Since most Member States have as yet no domestic policy towards science, it is unrealistic to expect them to have a foreign policy towards science and to know what they want done internationally. The result is that Unesco gets few specific requests and instructions concerning science from Governments. (However, it does receive many demands from the scientific community.) In a sense, Unesco was born before its time, but in another sense, it is already useful because it encourages Governments to co-operate in science and thereby to develop the healthy habit of participating in co-operative enterprises of mutual benefit.

Unesco's main method of action in science is arranging meetings to bring people of different nations together. Last year, the Natural Sciences Department held sixty meetings—seminars, conferences, working parties, panels of experts, etc. These meetings had widely varying purposes: to exchange scientific information, to survey a scientific field and plan what should be done in it by scientists and Governments, to found an international non-governmental scientific organization, or to create a regional or international research centre. But Unesco's job was almost the same in each case: it provided the initiative for the holding of the meeting and all the administrative services such as location, language interpretation, documentation as required, including agenda, working papers and commissioned reports; travel services for delegates to meetings, including visa arrangements, and in many cases payment for travel. Much of Unesco staff work requires a combination of creative imagination, scientific judgment, and diplomatic skill if international meetings are to present clearly scientific issues requiring action and possible alternatives for such action, and to arrive at a compromise which quickly leads to practical implementation.

Other types of activities which Unesco undertakes to increase and diffuse scientific knowledge are holding training courses, providing fellowships and equipment, supporting the preparation and publication of reports, sending survey missions and serving as the secretariat of a commission of Governments engaged in concerted oceanographic research.

To carry out these activities, the Unesco Natural Sciences Department has a professional staff of thirty-nine scientists, engineers and administrators from twenty nations, under the direction of Prof. V. Kovda, on leave from the University of Moscow, whose scientific speciality is soil geochemistry. Staff costs amount to about 30 per cent of the regular budget of the Department.

Outside its regular budget, and with much larger funds made available under the U.N. Technical Assistance and Special Fund Programmes, Unesco administers the provision of equipment, fellowships, salaries and travel of professors and scientists, to meet the needs of research and training institutions in developing countries.

Determination of Programme

Unesco tries to find out from the world scientific community what it believes should be done internationally. It does this by means of advisory committees and through four other means (Fig. 1). First, through the Unesco National Commissions: these are the channels which have been specifically created in each Member State for bringing the wishes of individuals and groups to the attention of Unesco.

Secondly, through the Government itself, in some nations, there exist ministries for science, councils for scientific policy, academies of sciences, etc. Some of these bodies have among their responsibilities the making of foreign policy in science. Where they exist, they are a useful means of making the scientist's opinions known in Unesco.

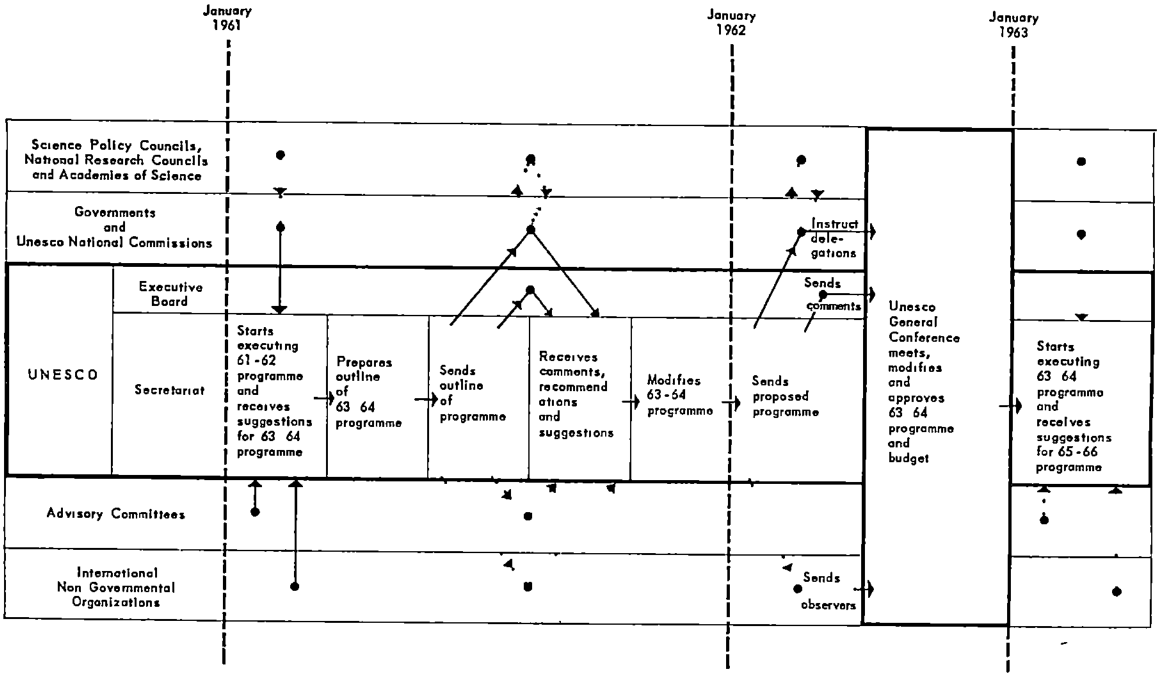
January 1961
January 1962
January 1963
Science Policy Councils, National Research Councils and Academies of Science
Governments and Unesco National Commissions
UNESCO
Executive Board
Secretariat
Advisory Committees
International Non Governmental Organizations
Starts executing 61-62 programme and receives suggestions for 63 64 programme
Prepares outline of 63 64 programme
Sends outline of programme
Receives comments, recommendations and suggestions
Modifies 63-64 programme
Sends proposed programme
Instruct delegations
Sends comments
Sends observers
Unesco General Conference meets, modifies and approves 63 64 programme and budget
Starts executing 63 64 programme and receives suggestions for 65-66 programme

Fig. 1. Initiation and determination of Unesco natural sciences programme

The third way is up the pyramid of scientific non-governmental organizations. Here a suggestion brought to the attention of the national scientific society is forwarded to the appropriate international union, and then, through the mechanism of the International Council of Scientific Unions (ICSU), the Union of International Engineering Organizations (UATI) and the Council for International Organizations of Medical Sciences (CIOMS), it is presented to Unesco.

Finally, there is the direct approach to Unesco by letters and personal contacts with the Unesco secretariat. Using the information received through these four channels, Unesco prepares a draft programme about eighteen months before it is to become operational. The cycle of programme initiation, modification, and legislative approval is shown in Fig. 1. In this cycle, the 1963–64 proposed programme, presented here, has already passed the Executive Board (a supervising committee made up of representatives of twenty-four nations) and is being modified slightly for presentation to the Unesco General Conference in November 1962. (The General Conference is a legislative assembly at which representatives of 106 Member States make known the wishes of their Governments and approve the programme and budget. It meets once every two years to determine the programme for the following two years. Often, new projects originate at the Conference.) Although it is almost too late to suggest major alterations, it is hoped that scientists and scientific organizations will offer criticisms and suggestions to permit a better detailed formulation of the 1963–64 programme. Suggestions for important changes should reach Unesco by mid-1963 to be considered in preparing the 1965–66 draft programme.

A ten-year science programme. In 1960, Unesco developed a ten-year plan in science (approved by the Unesco General Conference in November 1960) which has as its objectives (*A*) to promote the advancement of science through international co-operation, (*B*) to encourage the development of certain fundamental scientific activities which can be foreseen as being directly related to the future welfare and economic progress of mankind, (*C*) to assist Member States in improving their level of science and widening its applications to their economic growth.

The 1963–64 Programme

With the above objectives in mind, the proposed 1963–64 programme has been constructed along three main lines: (*A*) promotion of international co-operation in science, (*B*) co-ordination of research in earth sciences and natural resources, (*C*) aid for national scientific development.

It is made up of 21 individual programmes, projects and continuing activities gathered into nine broad fields (Fig. 2 and Table 1). The annual regular budget for 1963–64 is set at 2·15 million dollars, which is an effective increase of about 6 per cent over the period 1961–62. In 1963–64 the extra-budgetary funds to aid the developing nations are 2·5 times the regular budget and will be distributed as shown in Fig. 3.

(*A*) Promotion of International Co-operation in Science

Under this very general heading are activities (i) to support the most important international structures for the exchange of scientific information—the international non-governmental scientific organizations, (ii) to improve scientific documentation by integrated co-operative planning, (iii) to strengthen international co-operation for the advancement of research in the life sciences and chemistry.

(i) *Support for international non-governmental scientific organizations.* Since the arranging of international meetings to exchange scientific information has been done traditionally and most efficiently by the non-governmental scientific organizations,

Table 1. Summary of Unesco Natural Sciences Programme 1963–64 Regular Budget

Field	Project	Year started	Funds for 1963–64 (*b*)	Stage (*c*)	Present objectives (*g*): Co-ordination of research	Present objectives (*g*): Concerted research programmes(*e*)	Present objectives (*g*): Training of scientist
(*A*) International Co-operation in Science							
1. Subventions to non. gov. sci. organiz.	1 (*a*)	1947	541	cont.			
2. Co-ordination of sci. documentation	2 (*a*)	1949	170	plan.	×	×	
3. Advancement of laboratory research	3 Brain research	1959	70	act.	×		×
	4 Cell biology	1955	90	plan.	×		×
	5 Chemistry	1963	30	stim.			
(*B*) Co-ordination of Research in Earth Sciences and Natural Resources							
4. Scientific studies relating to natural resources	6 Scientific surveys	1961	45	stim.			
	7 Making of scientific maps	1959	100	cont.	×		
	8 Scientific hydrology	1963	50	study	×	×	
	9 Soil biology	1961	30	study	×		
	10 Arid zones studies	1951	290	act.	×	×	
	11 Humid zones studies	1954	120	plan.	×		
	12 African conferences	1961	85	stim.			
5. Geophysics and space sciences	13 Seismology	1961	45	study	×	×	
	14 Geophysical data	1957	30	study	×		
	15 Space sciences	1961	60	stim.			×
6. Marine sciences	16 Intergov'tal Oceanogr. Comm.	1961	70	cont.	×	×	
	17 Int. Indian Ocean Expedition	1961	50	act.	×	×	
	18 Marine sci. regional progr.	1954	343	act.	×		×
(*C*) Aid to National Scientific Development							
7. National science policy	19 (*a*)	1961	80	cont.			
8. Aid to technological research	20 Standards and testing labs.	1963	30	stim.			
9. University education in sci. and technol.	21 (*a*)	1961	204	cont.			×

(*a*) Field and project have same title; (*b*) In thousands of dollars; (*c*) Cont. = continuous activity; stim. = stimulation; plan. = planning act. = action; (*d*) Co-ordination of research is obtained by means of committees, working parties, surveys, special review studies, etc.; (*e*) By concerted research is meant research co-ordinated by agreements *before* or *while* being undertaken, rather than after as is usual in (*d*); (*f*) By fellowships, training courses, etc.; (*g*) All projects aim at increased exchange of information.

Governments have continued to support Unesco subventions to these organizations. Unesco provides financial support in the form of subventions to three of the largest international non-governmental scientific organizations—ICSU, UATI and CIOMS—which, annually, hold more than 100 scientific meetings and bring together more than 10,000 scientists. The funds provided by Unesco pay for about one-half the organization's costs in holding these meetings. Unesco will increase its total funds for these subventions in 1963–64.

(ii) *Co-ordination of scientific documentation at the international level.* For a number of years, Unesco has studied various facets of the problem of enabling the individual scientist to find out rapidly and easily if others have already done what he is trying to do and if their results are relevant to his own work. Unesco's role will now be to promote an integrated approach to scientific documentation bringing together the international scientific and professional organizations such as the International Federation for Documentation (FID), the ICSU Abstracting Board (ICSU–AB), the International Federation of Library Associations (IFLA), the International Organization for Standardization (ISO) and government bureaux concerned with the different aspects of scientific information at the national level. Unesco will set up an Office of Scientific Information, somewhat similar to its Office of Oceanography, to carry out Unesco's programme in documentation and to serve, if required, as secretariat in carrying out a ten-year plan for the co-ordination, on a world basis, of scientific documentation.

In 1963, five working parties will consider the problems in: primary scientific publications, abstracting activities, translation and terminology, mechanization and coding, information on and proceedings of congresses and scientific meetings.

After the working parties have met, there will be a summary planning meeting in 1964 of governmental and organizational representatives and experts in the aforementioned fields. The summary planning meeting will determine, on the basis of reports from the earlier working parties, what steps must be taken to improve the availability and usefulness of scientific information and what the responsibilities of the various existing organizations should be. On the basis of this, a ten-year plan for action, including the appropriate mechanisms, will be drawn up and agreed to by the responsible parties.

(iii) *Co-operation for the advancement of research in the life sciences and chemistry.* Because knowledge of the biology of the cell and the function of the brain has been increasing at a remarkable rate in recent years and may be opening an era as fruitful in biology as the discovery of the structure of the atom was in physics, Unesco has singled out these two related fields for support. In brain research, further progress is more than ever dependent on the collaboration of scientists from the different disciplines (as far apart as communications engineering and neuro-surgery) working together on common problems, and on an increase in the number of well-trained scientists. Unesco has therefore developed with the International Brain Research Organization a joint programme to organize interdisciplinary research teams to sponsor fellowships and to conduct a survey of existing institutions for research and training. A long-term co-ordinated research project on the multi-disciplinary aspects of brain growth and development will also be started.

Within the past five years there have been rapid advances in cell biology, particularly towards clarifying mechanisms of genetic control at the molecular level; however, progress on a world-wide basis is being limited by the lack of sufficient communication

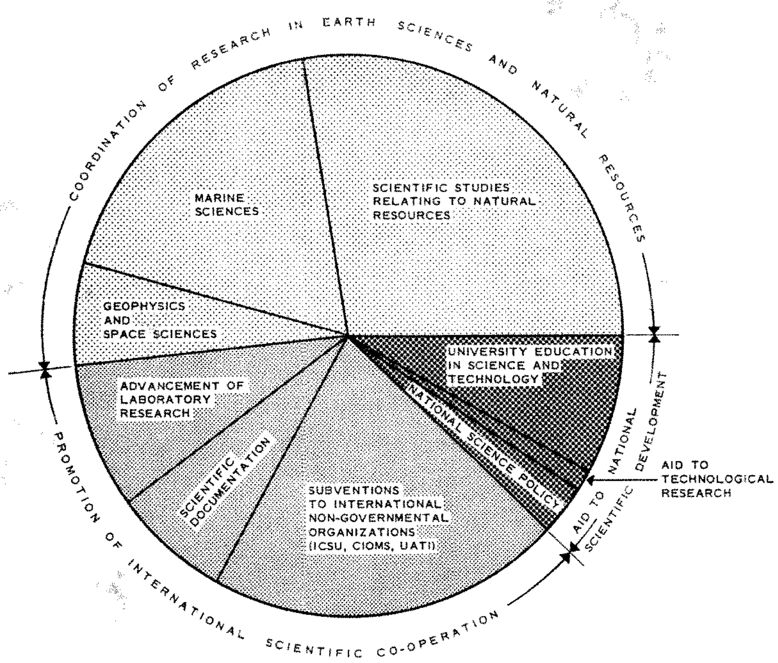

Fig. 2. Proposed programme in natural sciences for 1963–64. Distribution of regular budget 4·3 million dollars for two-year period

of new ideas and techniques in experimentation, and lack of co-ordination between research programmes being developed at the more active cell biology research laboratories. To enable research centres, as well as individual cell biologists, to improve their communication and to co-operate in the planning of research programmes, Unesco will undertake both a pilot project to associate a small group of research laboratories, and a project to help the cell biologists organize an international cell biology organization (ICRO). It is expected that the directors of the associated research laboratories will meet to co-ordinate their research planning and Unesco will help them through fellowships to provide for the exchange of research scientists. The proposed ICRO will concern itself with voluntary concerted research planning between a large number of associated cell biology research centres, and with the problem of raising the scientific level of cell biology research throughout the world.

In chemistry, Unesco will aid the International Union of Pure and Applied Chemistry in holding a series of specialized small meetings to increase the exchange of information on the theoretical aspects of catalysis.

(B) Co-ordination of Research in Earth Sciences and Natural Resources

The promotion and co-ordination of earth science research make up the largest portion of the regular programme of Unesco Natural Sciences Department and are undertaken because the need for international co-operation in these in studies is both obvious and great; these fundamental studies are required before the exploitation of natural resources for human welfare and can be effectively undertaken. Major activities to be continued include: (i) standardization of earth science surveys and map making; (ii) improvement in the handling and diffusion of geophysical and seismic data; (iii) marine sciences; (iv) arid zone studies; (v) humid tropic zone studies.

(i) *Standardization of earth science surveys and map making.* Unesco will hold in 1964 an international inter-disciplinary conference on survey techniques, particularly photo-interpretation and other aerial methods, used for natural resources exploration in soils, botany, geology and hydrology. The purpose of this conference is to bring together both makers of surveys and those who use their results such as geologists, botanists, hydraulic engineers, land-use planners, etc., in order that future surveys may be arranged to produce the maximum amount of integrated scientific information possible for the users.

Furthermore, the scientific bodies which are preparing international small-scale geological, metallogenic, tectonic, soil, hydrogeological and vegetation maps with universally accepted legends and nomenclatures will receive administrative services and financial aid from Unesco.

(ii) *Improvement of geophysical and seismic data handling.* Eleven international non-governmental services which receive geographical data on a routine basis from a total of 1,600 observing stations, and then reduce, analyse and distribute them to interested astronomers, astrophysicists, geophysicists, oceanographers and other scientists, from the Federation of Astronomical and Geophysical Services (FAGS) (these services are the following: International Time Bureau, International Latitude Service,

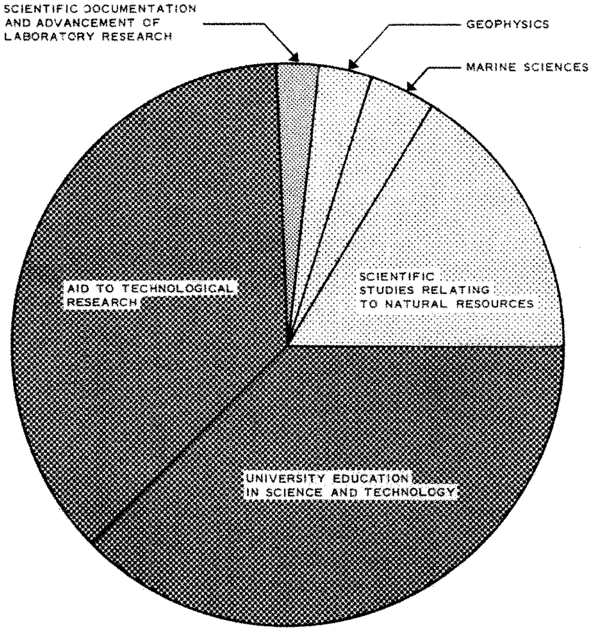

Fig. 3. Aid for national scientific and technical development. Distribution of extra-budgetary funds for developing nations 10·8 million dollars for two-year period 1963–64.

International Gravimetric Bureau, International Seismological Bureau, *Quarterly Bulletin of Solar Activity*, Ursigram Bureau, International Seismological Summary, Permanent Service of Geomagnetic Indices, Permanent Service of Mean Sea Level, International World Days Service, International Centre for Earth Tides) and receive financial support from both ICSU and Unesco

As a start towards providing FAGS with the facilities that it wished and needs to meet the modern demand for new data in a form convenient for immediate use, Unesco proposes to bring together interested Governments and organizations in working parties to agree on how to improve the handling of seismic data Presumably a form of international centre for the treatment of seismological data having appropriate electronic computing facilities will be required

Unesco will also hold an international conference in 1963 on seismology and its applications to earthquake protection This conference will review the results and recommendations of the Unesco–IUGG survey missions undertaken since 1961 to the various seismic belts of the Far East, Andes Mountains of Latin America, the Mediterranean, the Caribbean and East African regions by professional teams of observational seismologists, seismo-geologists and earthquake engineers This conference, with the goal of a more complete understanding of seisms and their origin, is expected to determine what can be done to improve the operation of the existing seismic stations, where new stations should be set up, and how to integrate the flow of data dispatched from the various stations in the most efficient way

In addition, it is expected that the conference will suggest action that should be taken at the governmental level to improve the protection of populations against effects of earthquakes

(iii) *Marine sciences* Unesco will expand its activities in support of marine sciences These have two major aspects, namely (1) co-ordination of concerted national efforts in marine sciences through the Intergovernmental Oceanographic Commission (IOC), (2) aid to oceanographic research and training in selected regions of the world In addition, there is a world-wide competitive fellowship programme for both shipboard work and study abroad, as well as support for the exchange of information through a *Unesco Marine Sciences Newsletter*

The Unesco Office of Oceanography serves as the secretariat of the Inter-Governmental Oceanographic Commission and functions as a liaison body in carrying out the agreements and recommendations of the Commission The role of the Secretariat of the IOC in the co-ordination of the International Indian Ocean Expedition (IIOE) will increase significantly in 1963 This expedition has been organized by the Scientific Committee for Oceanic Research (SCOR) of ICSU, with the aid of Unesco

During 1963–64, peak activity will be reached with the operation of between 10 and 15 survey ships in the Indian Ocean Although the Expedition has been envisaged as a loose combination of individual scientific expeditions, in order that the maximum flexibility be realized, it is necessary that certain activities be agreed on in advance Working parties from the various ship-operating organizations are thus brought together by SCOR and Unesco to co-ordinate tracks and to standardize methods and equipment In physical oceanography, for example, it is desirable to agree on methods of measuring oceanic currents and the salinity of sea-water if comparability of results is to be attained. In chemistry, methods for determining dissolved gases and trace elements involved in biological cycles are necessary In marine biology, methods of collecting plankton and determining biological productivity must be agreed on and adopted In addition to arranging the above working parties with funds provided by Unesco, the Secretariat will later convene similar groups to appraise the results obtained and to prepare joint reports for publication of atlases of the region

Unesco will aid the IIOE by providing funds and expert advice for the creation of the Indian Ocean Biological Centre at Cochin, India It is expected that each ship will send a portion of its plankton obtained with standard nets to Cochin, where the samples will be sorted into major groups The curator of the international collection will send samples on a world-wide basis to those experts most interested in them Finally, the classified holotypes will be deposited in the international reference collection and will serve the entire Indian Ocean Region

To stimulate national efforts in oceanographic research and training, Unesco will help develop a number of marine science activities on a regional basis The Indian Ocean, South-East Asia, North Latin America, and West Africa regions are four regions of the world where projects are already in existence or planned, but where limited staff and facilities are restricting their execution Unesco's activities will reinforce these projects in a way to increase the long-term growth of local marine sciences

In each region, it is hoped eventually to establish a regional training centre equipped with a suitable research vessel, laboratory facilities, class-rooms and instructional staff During the next few years, Unesco will encourage the development in each country of at least one centre of size and variety sufficient to ensure the rapid growth of national activities in marine sciences Finally, for each region, Unesco will help local institutes to co-ordinate their research and training activities and possibly to develop an overall regional research programme For example, in the Indian Ocean region, Unesco's activities will be organized to fit the activities of the IIOE Support will be given to both India and Pakistan for their participation in the Indian Ocean Expedition by providing equipment to one oceanographic ship from each country

(iv) *Arid zone studies* The arid zone research programme of Unesco has among its objectives, an increased systematic study of the scientific problems arising in the arid and semi-arid zones of the Earth Of particular importance to these climatic regions are salinity, ground-water hydrology, plant–soil–water relationships and, of course, climatology Unesco's programme of the past ten years has been to stimulate greater interest in these and related problems by scientists of the arid zone regions and by those in other parts of the world The programme consists of four parts (1) exchange of information through meetings and publications, (2) training courses and fellowships, (3) co-operative planning of surveys and research through special advisory committees, (4) support of special studies, reports and maps

During 1963–64, the project will be extended to the arid zones of Latin America A large international conference will be held to review present-day scientific knowledge of the arid regions of the various Latin American States and to stimulate such studies, in relation to these nations, as surface hydrology, geology and ground water, meteorology, soil sciences, plant and animal ecology and physiology. Finally, interdisciplinary problems such as geo-morphology and the integrated approach to arid zone research, efficient uses of water, and optimum grazing land use, will be discussed.

Also, the nations of North Africa will be encouraged to make a co-operative survey of their artesian water basin and to prepare international maps of it.

(v) *Humid tropics studies.* Similarly, the humid tropics programme has, among its objectives, an increased systematic study of scientific problems of the humid tropic regions of the Earth. Of particular importance of these climatic regions are laterization of the soils, conditions controlling extension of the savanna into the tropical forests, and equilibrium conditions between land, sea and fresh water in the delta areas. One should mention also the biology, functions and control of termites in the tropics and the collection, preservation and taxonomy of tropical flora and fauna.

During 1963–64, three symposia will be held on humid tropics problems dealing with laterization, delta areas, and relationships between the savanna and the tropical forests.

Also included under the programme to co-ordinate and promote earth sciences research are two small continuing projects to provide modest support for exchange of information in space research and soil biology through the appropriate professional societies, and a new project to initiate a long-range programme of international co-operation in scientific hydrology. This project will have as its objective a world-wide study of water cycles and will provide a scientific basis for an evaluation and use of world water resources.

(C) Aid for National Scientific Development

Although much of the regular programme of Unesco in science provides assistance to Member States, there is a group of activities where direct aid to Member States for their scientific development is the prime motive. These continuing activities are: (i) comparative studies relating to national science policy, (ii) a project to provide information and material for the improvement of university science education, and (iii) a small project to aid nations in the establishment of testing and standards laboratories.

(i) *National science policy.* Unesco will continue its comparative studies relating to the national science policy of Member States, with particular emphasis on the administrative structure required for the organization of research at the national level, on scientific manpower and financing problems and on national scientific research policy. Unesco also sends specialists to the developing nations, on Government request, to help them set up their own national research academies and science councils or fundations.

(ii) *Improvement of university science education.* Comparative studies of curriculæ, laboratory practice and teaching methods at university-level will be undertaken with the aid of the international scientific unions in physics and chemistry and will eventually be started in biology and geology. Information provided should aid universities in the process of remodelling their educational structure to provide better and more modern scientific education.

Unesco source books will be prepared in physics, chemistry, mathematics, biology and geology as teaching aids and as handbooks of modern practical information not readily available in a single volume, for students and teachers in the first and second years at the university. The manuscripts will allow for cheap production in local languages.

Furthermore, Unesco will support international postgraduate training courses and schools established in the developed nations in selected disciplines such as experimental physics, mathematics, chemistry, cell biology, plant biology and soil sciences. This programme will help developing countries to acquire a nucleus of highly trained scientists, for both teaching and research work.

Field Science Co-operation Offices

To implement its programme and serve local scientists, Unesco operates four Field Science Co-operation Offices at Djakarta, New Delhi, Cairo and Montevideo. The work of each Office includes: aiding the formation of regional scientific centres, such as the Latin American physics and mathematics centres, holding annually four or five scientific seminars and training courses of specific regional interest as, for example, the symposium on the vegetation and soil of limestone formations in South-East Asia planned to be held in Malaya in 1964, and acting as a clearing house for scientific information and material. In 1963–64, the Offices will emphasize the improvement of university science teaching.

Although Unesco does not yet have a science office in tropical Africa, it is developing a programme of local interest to that area. A large conference will be organized there in 1963 on research and training relating to African natural resources.

Extra-budgetary Aid for National Scientific Development

Unesco, using funds of the U.N. Technical Assistance Programme, will provide for about a hundred professors and scientists to go to forty developing countries. These experts will teach at the university, improve curricula and laboratory practice and, in some cases, advise ministries on scientific matters. Also under this programme, about a hundred fellowships will be provided, enabling young scientists from developing countries to train abroad. Using the funds available from the Special Fund of the United Nations, Unesco will continue to build up six institutions for research and technological education and will start six new projects. (It is expected that 7.4 million dollars will be provided by the Special Fund for the operation of these twelve projects during 1963–64.)

Discussion

Twenty-one projects, programmes and continuing activities (Table 1) of greatly varying size and objectives have been chosen for Unesco's attention from the broad spectrum of modern science. Concerning them, three questions immediately come to mind: (I) Why this number of projects? (II) Why this distribution of funds among the projects? (III) Why these projects and not others?

(I) *Number of projects* It is evident that Unesco must develop its programme as a compromise between almost unlimited requests for projects from scientific and Governmental groups in 105 nations and the limited resources at its disposal The proposed programme, made up of twenty-one carefully chosen projects, aims at a satisfactory compromise with the following characteristics

(i) Those activities have been chosen which appear most suited for international action Thus, projects in support of international co-operation for the exchange of scientific information utilize approximately a half of the total resources of the natural sciences regular programme, another quarter will be spent to meet the needs of research co-ordination at the international and regional levels, while the remainder of the budget is to support the training of scientists and to stimulate research at the national level

(ii) The programme has been planned to ensure its concentration in three broad fields (actually, as noted in Table I, five large projects account for two-thirds of the regular budget)

(iii) The programme has been planned to be of interest to all nations in an equitable manner Thus, 56 per cent of the regular programme is of general scientific interest, another 10 per cent is of particular interest to the developing nations, while the remaining 10 per cent is aimed at the advanced nations

(II) *Distribution of funds* It has been argued that with such limited funds and with so many requests for programmes, Unesco has dispersed its efforts by starting a large number of projects relatively weakly supported financially To answer this argument, it is necessary to observe that projects usually evolve through four stages study, stimulation, planning and action, and in each stage require different amounts of expenditures

To illustrate this concept, the marine sciences project may be presented as an example In 1954, when this project was launched by the General Conference, only a general objective had been set and neither specific goals nor the means of attaining them were clearly defined

In the course of the following seven years, the project evolved through four stages

(1954–55) A study-stage during which working parties met and decided on preliminary requirements

(1955–59) A stimulation-stage during which marine science was stimulated by an increased flow of information supplied by seven Unesco-supported conferences

(1955–59) A planning-stage during which the International Advisory Committee in Marine Sciences was created in 1955 After a series of meetings, the Committee advised the Organization in 1958 to carry out a concrete programme of concerted international action in marine sciences

(1959–) An action-stage during which Unesco took measures to establish an Inter-governmental Oceanographic Commission which met for the first time in 1961 and initiated plans for joint scientific expeditions operated by interested Member States to eleven separate regions of the world ocean

The foregoing example demonstrates how a general idea may develop in seven years into specific international action on a world-wide scale, with a ten-fold increase of financial resources, if circumstances are ripe for such evolution

The time-scale for this chain of project evolution is long because of the varying world outlook of nations, the slowness with which scientists and Governments become informed and arrive at opinions about a project, the multiple consultations required for decision making, and because of modest funds which limit the rate of activity Thus it requires at least two years to get even a small project started as part of the programme, it usually takes another four to six years to reach the action phase, and perhaps four to six more years to complete the action, depending on its character

In this process, one of the limiting factors which could be changed is money Unesco's annual regular budget in science is of the order of magnitude of 0 1 per cent of the sum total of funds spent on basic research by all the nations International money is scarce, but it can be very useful, it can usually pay for things which national money cannot, such as the expenses of international meetings or publications If wisely spent for stimulation or for a concerted programme, it should cause much more national money to be spent in the selected field and in a more effective manner

(III) *Selection of projects* The priority given to projects has been determined by the following considerations (1) Unesco's ten-year plan in the field of natural sciences, (2) the stage of evolution and age of continuing projects, (3) the maturity of new projects with respect to international action, and (4) the desire and the ability of Member States to provide appropriate investment of their own funds and scientific resources in joint activities co-ordinated through a Unesco project the funding of which is necessarily limited

The question of whether the proposed selection of projects in 1963–64 makes the best use of the available funds for the international promotion of science must also be considered in the light of the following basic questions

(1) Should Unesco support—and for how long—projects which stimulate a field of science but the results of which cannot be easily measured ?

(2) In the light of other immediate needs in international science are concerted action projects such as marine science or scientific hydrology proper types of activity for Unesco considering their long gestation period ?

(3) What should be the ratio of new to continuing projects, considering the great length of time required for projects to mature ?

(4) Are staff studies and clearing house activities such as the programmes on information about the national science policy and university science education more suitable for Unesco than projects to co-ordinate or promote research, considering their low costs and predictable rate of activity ?

Since the 1963–64 programme has evolved under the guidance of scientific committees of national Commissions and with the advice of international advisory committees, these questions have obviously been considered The resulting programme thus represents a practical compromise of different points of view Nevertheless, the need for reconsideration of these questions is a recurring one, their formulation is intended to aid deliberation about the future programme of Unesco

One of the reasons for reviewing Unesco's role in science and its proposed programme for 1963–64 is to solicit suggestions and criticisms, only through them can future programmes of Unesco truly represent the needs of scientists and be really effective in making for international co-operation in science

One may ask about the value of Unesco's programme to the individual scientist, to the development of science, and to mankind As an individual, one is most likely to get direct benefit from Unesco only if he happens to hold a Unesco fellowship or attend one of the 150 international meetings annually supported by Unesco

Although the development of science proceeds through individual efforts supported by local expenditures which are larger than Unesco can provide even to the poorest of nations, Unesco's aid has often greatly stimulated local scientific growth Furthermore, in the earth sciences Unesco's role of co-ordinator of national efforts has proved essential, and the benefits to science have been indeed large

Finally, there is the ultimate value to mankind of Unesco's efforts, the fact that scientists and Governments of many nations can work together through an intergovernmental agency for the common advancement of human knowledge should be both an inspiration and an example to statesmen and laymen faced with the diversity and dangers of competitive ideologies and nationalisms

INDUSTRIAL CHEMISTRY SURROUNDING GOLD*

By The Right Hon Lord FLECK, K B E , F R S

I HAVE been asked to discuss some definite theme in the history of science and technology in the nineteenth and twentieth centuries, particularly a theme which might illustrate the action and interaction between developing technology and economic conditions and where industrial needs and purely scientific developments have suggested (or failed to suggest) problems for each other It is perhaps difficult to fill this specification fully, but if the sequence

enlarged demand
leading to
better process
leading to
industrial production of new materials

is of interest by its reactions and interactions, then I think the study of gold is worthy of some consideration In the latter decades of the nineteenth century the ever-present demand for gold increased There was then the invention of the cyanide process—a much better process in every way than had been available before then That had to be accompanied by better processes for cyanide manufacture, thus leading to what in itself is a sizeable chemical industry So therefore I propose to review gold production and some of the aspects of industrial chemistry associated with it I am happy to do what I can with this subject for two reasons first it is a subject in which I have been much interested over many years, although I must disclaim any suggestion that I can approach the subject as an economist My interest is decidedly amateurish Secondly, it so happens that I was brought into personal contact with some of the personalities, particularly two, John Stewart McArthur and George Thomas Beilby, who played some decisive part in the industrial part of the story, and I would like, before it is too late, to put on record some personal information That personal information is, I am afraid, largely undocumented, but nevertheless it may be helpful in understanding the course of industrial events

The subject of gold has excited the interest of mankind through countless years There is reason for believing that native gold and native copper were the earliest of mankind's attempts at working metals Gold was widespread, and in the ancient world it was distributed through, and worked in, for example, Arabia, India, Persia, Caucasia, Asia Minor and the Balkans It was in Egypt that there appears to have been the most regular production and *The History of Technology* gives 30 kgm per annum as the regular output from the Nubian mines I shall be using tonnes as my units for gold production, so that this figure of 0 03 tonnes per annum is not at all large compared with the figures that I will presently put before you Rameses II in the thirteenth century B C was concerned about the number of gold washers who died in the Nubian desert

There is very little that can be usefully said about the quantities of gold produced up to the time of the discovery of America The situation in the years after that date, however, is very different, and we are indebted to the researches of Adolph Soetbeer of the University of Gottingen for bringing up much quantitative information, writing in the 1890's His information is shown in Fig 1 extended up to 1961

Gold production is usually given in so many thousands or millions of fine ounces, but I have transferred my units into tonnes to make it easier to make mental comparisons with quantities of production in the chemical industry

After the discovery of America, owing to the strength of the Spanish situation, most of the gold

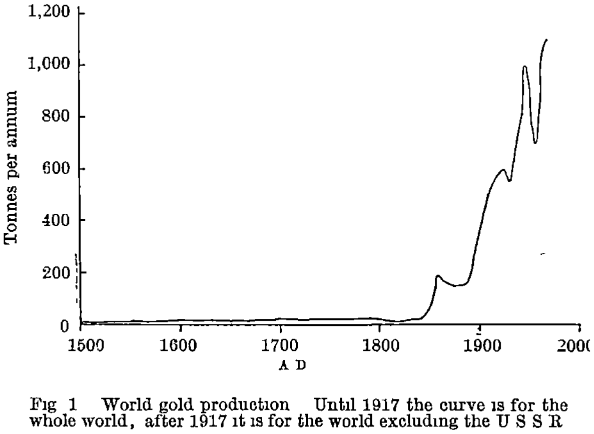

Fig 1 World gold production Until 1917 the curve is for the whole world, after 1917 it is for the world excluding the U S S R

transferred from America to Europe was channelled through Madrid for many years, and thus it can be understood why we can place a measure of some accuracy on the early figures of gold production. Certainly, so far as I am aware, Soetbeer's figures have not been challenged.

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* Substance of a lecture delivered in the series "Science and Economics in Chemical Technology" at All Souls College, Oxford, on May 2

Our figures start at the beginning of the sixteenth century when the annual world production was an amount in tonnes that could be counted on the fingers of the two hands—something of the order of 5–8 tonnes per annum. By the beginning of the eighteenth century these figures had increased to double figures, largely from Brazil and then in the middle of the nineteenth century, owing to the Californian and Australian discoveries, it rapidly increased into hundreds of tonnes per annum.

"For many years after the discovery of America, the output of new gold there was small, and much of the gold which was exported to Europe in the sixteenth century seems to have been obtained by looting from the natives the gradual accumulation of centuries. The first important source of new gold was discovered in New Granada (Colombia) in 1537, and throughout the seventeenth century that country remained the world's largest producer with about 40 per cent of a world annual output of under 9¼ T. Towards the end of the seventeenth century the discovery of the deposits of Brazil brought a new sharp rise in world production, and in the eighteenth century Brazil alone is estimated to have produced 45 per cent and South America as a whole 80 per cent of the world output of about 18½ T. a year.

"During the first 40 years of the nineteenth century South American production gradually declined, and world output fell off despite some increase in Russian production. But the discovery of gold in California in 1847, followed by the discoveries in Australia in 1851, brought a rapid increase in world output from 15½ T. to 180/190 T. a year. In the 1860's the alluvial deposits began to show signs of exhaustion, and the gradual increase in lode mining was not sufficient to prevent a fall in world output from 200 T. a year in the late 1850's to under 155 T. in 1886.

"This proved to be the lowest point reached. South Africa had already been producing small quantities of gold from alluvial deposits and lode mines, but the discovery of the Barberton gold field in 1882, and of the infinitely more important Witwatersrand field in 1884, opened the way for a new great expansion in world output. . . ."

We see from Fig. 1 that after the 1890's there was a surge forward interrupted as was natural by the Boer War troubles until the depression after the First World War. The upward trend was resumed and reinforced by the Second World War to a maximum about 1941. This was followed by a drop which was then counteracted by the incoming of the Free State gold and to-day we are again having record productions, essentially of course dominated by South African production.

We are now in the position that the output of gold for 1961 is at a record level, essentially due to the output from South Africa at 1,100 T. per annum. This record is not great over 1942 period, but it is definite although the margin is small.

The figures I have quoted are to the best of our knowledge the global world figures until 1917, the date of the Russian Revolution, since when the figures exclude U.S.S.R. production. The U.S. Bureau of Mines has published estimates for the U.S.S.R. and their latest figures are estimates for present-day production at between 280 T. and 315 T. per annum. This will give us a probable global production of gold for the world, including the U.S.S.R., of the order of 1,400 T. per annum. I have only two additional comments of a general nature to make. First, it is worthy of acknowledgment that South Africa has made a remarkable achievement in raising its gold output from 410 T. in 1954 to 625 T. in 1959—a 50 per cent increase when various rumblings have been heard that the price of gold should be raised. The other production figure which should be noted is the increased production from Ghana, 21 T. in 1954 to 28.5 T. in 1959, a 35 per cent increase, which brings Ghana nearly to the same level as Australia. There is no reason, so far as I know, to suggest that there are vast quantities of gold in Ghana awaiting exploitation, nevertheless, if the gold resources there can be increased over the present, or even maintained at the present relatively high level, there may be much significance to the economic prosperity of Ghana in the gold situation there.

That is the story of gold production in a simple form up to the present day.

Looked at in the cold light of industrial production of a thousand and one commodities in the context of world tonnages, 1,400 seems a very small figure indeed for a commodity which is so much in the eye of peoples all over the world. But no one can deny that, small as it is, the gold production has many actions and reactions in all sorts of quarters.

The first one that I would mention was an economic one. In many books there is reference to the economic consequences of the influx of gold in the sixteenth and early part of the seventeenth centuries to Spain, and then the outward spread of gold from there to cover all Europe. It had a great effect in pushing up prices for many commodities. The situation was very fully examined, for example, by Jacob, writing in 1832 in London. I can also quote Moncada, writing in Madrid in 1619: "Before the discovery of the Indies he who possessed 100 reals was as rich as he who now enjoys 500 for with the abundance of gold and silver its value has fallen".

No doubt the earliest gold production was native gold which could be comparatively easily rolled and worked into gold vessels and other objects. To collect the small particles of gold would, however, be a slow and tiresome business, and so it is easily understood that alternative techniques would be looked for, thus we have the introduction of amalgamation somewhere around about the early years of the Christian era; certainly it was well established by the Middle Ages, and amalgamation has continued down to recent times. Amalgamation, however, that is, the process of picking up small gold particles by mercury, filtering the liquid amalgam away from extraneous material and then distilling off the mercury to leave as a residue relatively pure gold, was never a very satisfying process to either a trained engineer or to a trained chemist. To begin with, the process could not be applied to ores relatively poor in gold. It was applicable to ores containing at least 4 oz. of gold per ton only.

When it is remembered that many of the South African ores which to-day are being worked economically contain only some 5 dwt. it can be seen that the industrial application of amalgamation processes has a limited field of application. Then again, the operation of amalgamation processes is very inefficient—40 per cent recovery is often spoken of, seldom did it reach 60 per cent. That, however, was the main process of gold recovery until nearly the end of the nineteenth century. Then there was increasing demand for gold as that century moved towards its end, no doubt stimulated by the economic forces at work throughout the world. The gold standard was a very real thing with us, and trade and commerce, both nationally and internationally, were developing and important factors and required increased quantities of gold for their operations.

So we arrive at the 1880's, when important things began to happen in the gold industry. As I have already stated, in 1882 gold at Barberton in the Transvaal was discovered, and then in 1884 came about the discovery of gold on the Rand. These, of course, were not alluvial deposits, and in the case of the Rand, particularly, the gold was disseminated through the rock in a very fine state. There was therefore very considerable pressure on the mining engineers and mining metallurgists to have a better way of isolating the gold than the crude amalgamation process in operation up to that time.

And now I would say a few words about chemical industry in Great Britain at that time. In 1881 the Society of Chemical Industry was formed in Britain, so, apart from the dyestuffs industry, there was a vigorous heavy chemical industry based essentially on salt and sulphur.

Signs of this vigour in Europe as well as in Great Britain were the arrival from America of H. Y. Castner in 1886 with a process for the chemical production of aluminium, and the patenting in 1886 in France of a process for the electrolytic manufacture of aluminium. In 1888 there was patented an electrothermal process for phosphorus manufacture.

Chemical processes using electricity were very much in fashion, and so it is not surprising that another American, Henry Renner Cassel, had landed in Britain and proclaimed that he had an electrolytic process for the isolation of gold. That again was in 1884. It was in a climate of broad chemical industrial activity that he interested that very astute and courageous Glasgow chemical manufacturer, Sir Charles Tennant, in his venture. The process turned out to be a bogus one, and Cassel took advantage of a wave of unjustifiable optimism in his process to sell out his founders shares and go back to America with his ill-gotten gains. But quite a lot was saved out of the wreck of the Cassel Gold Extracting Co. John Stewart McArthur was a chemist employed by the Tharsis Copper Co., of which Sir Charles Tennant was chairman, and in his private time McArthur was interested in gold. Many years later, during 1910–20, I got to know McArthur very well, in connexion with work quite unconnected with gold, and have heard from him some of the details of the development of the McArthur–Forrest cyanide patent, which was the sole and essential means which enabled the world's gold production to soar as it did after the 1890's when the cyanide method really got to work on South African gold.

McArthur was a chemist and founder member of the Society of Chemical Industry. The Forrests were two Glasgow medical men who had a Glasgow shop converted into consulting rooms, and to those rooms there was an underground basement where the three of them conducted chemical experiments. McArthur was a chemist of the highest integrity, and he described to me how he would not consider any experiment worthy of further consideration unless he was able to place the recovered gold as gold available to be weighed as such. At this time the chemistry of gold and all its complex salts was not fully and exhaustively worked out, and if hydrogen sulphide did not pick up the gold it is not surprising that McArthur would miss it. McArthur himself described to me how in their treatment with cyanide solution they were aware that they had lost gold from the mineral, but they were not able to obtain it back by any precipitation method then known. It was two years later before their attention was directed to the potential use of zinc, which is now the standard method of recovering gold which has been dissolved by cyanide.

Sir Charles Tennant, who had very considerable interests in the Indian Mysore gold mines, was, of course, very much involved in the breakdown of the alleged Cassel electrolytic process, and he sent down McArthur to investigate the Cassel experiments, and McArthur reported adversely on them. The next step was that the Cassel Gold Extracting Co. obtained the rights of the McArthur–Forrest process and proceeded to develop them. The first full-scale plant was in New Zealand and was a complete success, and then South Africa was tackled. The Cassel people and the South African mines failed to come to agreed terms on the use of the process the result was litigation in which the Cassel people were finally —in 1896—very distinctly the losers. The McArthur–Forrest patent was invalid in South Africa. The losing situation quickly made itself effective all round the world, so that in general the Cassel Co.'s income from royalties quickly melted away. I will spend no more time moralizing on the ingratitude of mankind to industrialists who have contributed great skill in developing technologies—the pressure of events led to a great and effective discovery, the failure to come to agreed rewards led to there being no reward at all, and in the end, although he had been the effective cause of a far-reaching technical and economic revolution, McArthur lived and died with only a modest financial competence. The development of gold-winning techniques owes a great debt to Sir Charles Tennant for the logical and persistent way he stuck to the need to get technical solutions to the various problems that the physical side of the gold business required.

The Cassel Co. were clearly, after the legal defeat in 1896, not going to be able to maintain an existence by supplying tanks and other relatively simple mechanical appliances. There remained, however, a big chemical question cyanide was wanted in industrial quantities which, even in the period 1890–1900, could be seen to be potentially great. Until the rising use of cyanide for mining purposes became a definite factor, it was used industrially only for electro-plating purposes, and the supply came mainly from ferrocyanide, a by-product of the gas industry. The total cyanide world production at that time was estimated at 50 tons per annum. It is at this point that Beilby enters the picture and McArthur fades out.

The method which McArthur, as an executive of the Cassel Gold Extracting Co., attempted to use for the large-scale deliberate manufacture of cyanide was based on barium, and this process met infinite difficulties.

Beilby in the '80's was an Edinburgh chemist who was employed by the Oakbank Oil Co. in mid-Scotland, the basis of which was distilling oil shale to yield oil and ammonium sulphate. Cyanide in small quantities was also obtained in the distillate, and Beilby's consideration led him to believe that if the temperature of distillation was increased the yield of cyanide would be significantly raised, even at the expense of a smaller yield of ammonia The oil company did not agree, and Beilby resigned to devote all his energies to the development of a direct process to manufacture cyanide from potassium carbonate, ammonia and charcoal This process was successful and supplied substantial quantities of cyanide to meet the growing demand for gold solution

Processes for cyanide manufacture were of very general interest to industrial chemists as a whole, and among those who studied the matter was Hamilton Young Castner—a vigorous American who was attracted to Great Britain, as already stated, by the possibility of manufacturing aluminium by a chemical process This failed because an electrolytic process established itself Castner's interest was thus roused in electrolytic processes, and he then studied and was commercially successful with two electrolytic processes, the electrolysis of brine to give chlorine and caustic soda and the electrolysis of anhydrous caustic soda to yield metallic sodium Castner and his colleagues were able to show that the use of metallic sodium plus charcoal plus ammonia could yield a very pure sodium cyanide which had many advantages over the Beilby potassium carbonate process But Beilby was a wise and shrewd technologist, he saw quicker than anyone, possibly even before anyone else, that the Castner process was a more economical process than the Beilby one, and so without hesitation the Castner process was installed in the Cassel works and proved to be the basis of the development of the Cyanide Industry The Cassel Gold Extracting Co left the scene and became the Cassel Cyanide Co, and most, if not all, of the cyanide to-day in Britain is sold as Cassel-brand cyanide

It is not often that the name of a man who tried to develop a bogus process finds a permanent place in an industry of considerable economic importance—for that is what I think we can describe cyanide manufacture as to-day Different ores require markedly different quantities of cyanide per ton of ore treated, thus Rand ores are low in cyanide demand per ton of gold, while Canadian ores, I believe, require more than ten times the amount used in South Africa In a paper which I gave some fifteen years ago I made an estimate of 75,000 tons per annum of sodium cyanide made by the Castner process for gold extraction purposes, and so far as I am concerned, this estimate, or something of this order, holds to-day, and that does not take into account various other industrial uses of cyanide, such as manufacture of dyes, case-hardening and pest control work, which have all sprung up largely because of the availability of cyanide following the economic need which was caused by the desire to win quantities of gold by steady industrial methods

This sequence of increased demand for gold for general industrial and commercial reasons, and the solution to that problem found by the very well-founded technological advance in the discovery of the cyanide process, which in its turn brought about a scientifically well-founded addition to the chemical industry, is a story which I believe helps towards the understanding of action and reaction between economics, technology and science

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RADIO AND THE IONOSPHERE*

By Sir EDWARD APPLETON, G B E, K C B, F R S

Principal and Vice-Chancellor, University of Edinburgh

THE ionosphere is a realm of the universe which was both invented and discovered The physicist Balfour Stewart, for example, invented the ionosphere, in 1882, as the hypothetical location of an overhead atmospheric system of electrical currents In his own words, he was driven to postulate that the upper atmosphere was an electrical conductor because he could find no other satisfactory explanation of the daily rhythmic changes in the Earth's magnetic field, which had been the subject of much speculation since their discovery, by George Graham, in 1722 A E Kennelly and Oliver Heaviside also invented the ionosphere as an ingredient in their theoretical explanations of Marconi's successful communication by radio, across the Atlantic Ocean, in 1901 However, it was not until 1924 that the existence of the ionosphere was demonstrated by direct radio experiments and its height above the ground determined The methods used for locating the ionosphere would, nowadays, be called frequency-modulation radar and pulse radar

Once the ionosphere had been identified and located it was possible to devise all kinds of radio experiments calculated to disclose its electrical content and its structure Using pulse radar, for example, it was discovered that the ionosphere is a doubly refracting medium, due to the influence of the Earth's permanent magnetic field From a study of echo-doubling it has been possible to identify, by radio means alone, the nature of the particles which confer electrical conductivity on the upper atmosphere Such particles are found to be negatively charged and to have the charge-to-mass ratio of the electron Radio exploration of the ionosphere has therefore been, to a large extent, the determination of the way the electron density varies with height above the point of observation With a sufficiently close network of such exploring stations, distributed over the Earth's surface, it has been possible to derive a global picture of the morphology of the ionosphere and its variations, which, again, has stimulated much theoretical work on upper-atmospheric atomic physics

The determination of the way the electron density varies with height in the ionosphere is usually accomplished by vertical radio sounding leading to the production of an ionogram This is a graphical portrayal of the relation between the equivalent height of reflexion, measured in kilometres, and the exploring radio-frequency, measured in megacycles per second From an ionogram there can be read off the critical penetration frequencies of the various

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[*] Substance of one of the lectures delivered at King's College, London, on February 5, to mark the centenary of the publication of Clerk Maxwell's papers on electromagnetic radiation The lectures will be published in book form in due course

strata into which it is convenient to divide the ionosphere From such critical penetration frequencies the maximum electron densities of such strata can be calculated using well-known magneto-ionic dispersion formulæ Ionospheric electron densities, when measured by the ionogram method, are found to be in the range of a million electrons per c c A confirmation of such values has, more recently, been derived from rocket sounding flights conducted in the United States

As soon as the method of measuring maximum electron densities—or peak electron densities, as they are sometimes called—had been devised, it was natural to make ionogram studies of the way the two main strata of the ionosphere, the *E*-layer and the *F*-layer, varied with the hour of the day, with the season of the year and with the course of the sunspot cycle There was thus inaugurated the long-term exploration of the ionosphere which has continued, in a steadily expanding fashion, since 1931 to the present day

The earliest ionogram study of the *E*-layer was made at King's College, London, during the year 1931 It was then at once discovered that the peak *E*-layer ionization was subject to a marked solar control, for the electron density was found to increase round about sunrise, to reach a maximum round noon, and to wane at sunset The continuation of such measurements on a routine basis, which was taken over by the Radio Research Station, Slough, showed that the *E*-layer ionization was greater in summer than in winter, a further illustration of solar control However, the same series of measurements led, in due course to the discovery that the peak *E*-layer ionization varied in sympathy with the trend of the sunspot cycle This has led to the conclusion that the solar radiation which causes the *E*-layer, now thought to be X-rays, varies directly with sunspot activity This is in striking contrast with the visible light waves and the heat waves received at ground-level from the Sun, for they do not vary at all with sunspot activity

The discovery of the sunspot-cycle control of *E*-layer ionization at once recalled the suggestion of Balfour Stewart that an upper-atmospheric conducting layer might be the location of the currents responsible for the small changes of the Earth's magnetic field which are to be noted all over the world, especially just before local noon A critical examination of geophysical events during the last two sunspot cycles, made at the University of Edinburgh, has shown that there is a close quantitative parallelism between the magnitudes of the *E*-layer ionization and the small daily changes of the Earth's magnetic field This has afforded strong support to the view that the overhead current system, the existence of which was postulated by Balfour Stewart, is in fact located at the atmospheric level of the *E*-layer

Since the electron density in the *E*-layer does not increase without limit under the influence of solar irradiation, there must be some electron dissipative process concurrently operative Now, undoubtedly, the first thing noted about atomic processes in the *E*-layer was that electrons seemed to disappear far more rapidly than was expected according to the simple theory of radiative recombination between electrons and positively charged atoms The rate of electron disappearance is, in fact, 10,000 times as great as simple theory predicts The recognition of this discrepancy led D R Bates and H S W Massey to propose that recombination in the lower ionosphere takes place by way of the union of an electron and a positively charged molecule, the latter being dissociated in the process The elaboration of this theory of dissociative recombination has proved notably successful in explaining, in quantitative fashion how electron capture-rates vary with height in the atmosphere

Turning to consider the *F*-layer of the ionosphere we may note its special importance from the point of view of practical long-distance radio communication This arises from the fact that it is the most strongly ionized stratum of the ionosphere and therefore reflects the shortest radio waves, and, as simple ionospheric theory demonstrates, the shorter the wave-length the less is the attenuation of the waves during the process of reflexion Because of this importance of the *F*-layer in facilitating short-wave radio communication there has been a practical objective in the study of its global morphology and the variation of that morphology with time The practical objective is that of forecasting the optimum radio wave-length for use in any part of the world at any particular time For this can be done, by way of appropriate calculations, provided we know the maximum electron content of the *F*-layer, and the atmospheric height at which that maximum is reached, for ionospheric conditions along the track of the radio circuit in question

During the Second World War many ionospheric sounding stations were instituted in different parts of the world to serve the needs of the Allied Forces in forecasting optimum wave-lengths for long-distance transmission by short waves However, when the War was over and practical requirements had less urgent priority, it was possible to subject these war-time measurements to more leisurely scientific study At once a remarkable feature of the *F*-layer was discovered, in that its peak ionization was found to be subject to a geomagnetic control The morphology of the *F*-layer maximum is, in fact, more closely associated with magnetic latitude than with geographical latitude Actually, it is thought that the electrons and ions in the *F*-layer are initially produced by electromagnetic solar radiations and therefore, to that extent, there is a control according to geographical latitude But it is now generally accepted that such ionization does not remain where it is originally produced, and soon moves, possibly by diffusion or electric drift, into what may be called its geomagnetic position

Much attention is at present being given to the theoretical study of the *F*-layer of the ionosphere, but it is clear that more experimental information is needed particularly about that part of the *F*-layer which exists above the level of its peak Such information is steadily being acquired, by rocket and satellite studies, as well as by other means, largely under American auspices One notable discovery has been made by studying the orbital decelerations of satellites due to atmospheric drag, namely, that there are very large variations of the actual air density and temperature at high atmospheric levels Such variations are found to be subject to diurnal, seasonal and sunspot-cycle control Undoubtedly, these remarkable air density variations at high levels must have a profound effect in determining the variations in *F*-layer morphology and so probably will provide the clue for explaining many of the *F*-layer anomalies previously identified—and for which no satisfactory theoretical explanation has been, so far, forthcoming

EUGENICS IN EVOLUTIONARY PERSPECTIVE*

By Sir JULIAN HUXLEY, F.R.S.

THE 'knowledge explosion' since Darwin has given us a new evolutionary vision, in the perspective of which eugenics must be viewed. Man, we now know, has become the latest dominant type to be produced in the nearly three billion years of biological evolution. Barring accidents due to his own stupidity, he has at least an equal space of time before him. He owes his dominant position to the development of a new mechanism of inheritance and transformation, operating by cumulative transmission of experience. With his arrival on the scene, evolution has passed from its biological to its very different psychosocial phase. His destiny is necessarily that of directing (or misdirecting) the future course of evolution on this planet.

In spite of his astonishing improvement, brought about by natural selection in the past, from a sub-microscopic to a cellular, a metazoan, a vertebrate and a hominid stage, he is still very imperfect as a psychosocial type of organism, and is capable of great genetic improvement. The aim of eugenics is to effect such improvement of the human type.

Some critics of the eugenic movement assert that large-scale eugenic improvement is impossible until we have a much more detailed scientific knowledge of human genetics. This is not so. Relying on our knowledge of the course of pre-human evolution and present-day species-formation, and on the results of pre-scientific animal and plant breeding, we can be sure that the general level of human performance and genetic capacity could be markedly raised, simply by encouraging the differential reproduction of human beings exhibiting generally desirable characteristics such as health, physical beauty, manual dexterity, longevity, athletic ability, intelligence, general mental ability, mathematical, aesthetic and other special aptitudes, and capacity for leadership and for co-operative effort. What is more, since outstanding individuals are a major source of social efficiency and cultural advance, the level of human performance could be markedly raised by the production of quite a small additional number of men and women exceptionally endowed with outstanding mental qualities.

Others claim that large-scale eugenic improvement could only be brought about by rigorous authoritarian methods. This again is not true. Experience shows that radical social changes can occur by consent, and can be effected by a combination of persuasion and good leadership, especially when the level of general education is high. Accordingly, better education, notably concerning biology, reproductive physiology, genetics and evolution, is needed before we can embark on a eugenic programme. Another prerequisite for successful eugenics is the control of the present excessive rate of increase in population.

Negative eugenics aims at reducing the frequency and preventing the spread of genetic defects due to undesirable genes or gene-combinations, including such conditions as hæmophilia, schizophrenia, proneness to diabetes, various types of mental defect, including very low intelligence quotient, and certain temperamental deficiencies, and, of course, the great majority of radiation-induced mutational defects. The need for negative eugenics has become urgent in recent years, partly because of atomic and nuclear explosions and of the increase in man-made radiation, partly owing to the differential increase of lower-income groups, the effects of which cannot be eugenic and are probably dysgenic, but mainly owing to the progress of medicine, public health and social welfare, which permits the survival and reproduction of many types who would have died young in earlier periods.

We can be sure that early man's social organization, in the form of small competing groups, would have encouraged genetic improvement of characters like intelligence, manual dexterity and ability to communicate and to co-operate. To-day, however, with the increase in size and reduction in number of social groups, inter-group selection has become much less effective, intra-group co-operation has permitted the survival of genetically defective types and the spread of undesirable mutations, and there is every prospect of general genetic retrogression unless we amend our social organization. Accordingly, for negative eugenics to succeed, medical and public health services must be enlarged to include family planning and eugenic advisory services and the regulation of population and reproduction in general. Taxation and family allowance systems should be modified to discourage excessive reproduction.

In general, eugenists must not disregard the environment, but must help to plan social environments which will promote genetic improvement as well as cultural advance. Simpler methods of birth-control are needed, whether oral or by injection, or by voluntary sterilization. Especially in combination with sterilization, artificial insemination by sperm from a non-defective individual (A.I.D.) is strongly indicated.

Positive eugenics is more inspiring but also more difficult. It aims at the progressive genetic improvement of the human species, in full consciousness that the possibilities of such improvement are virtually unlimited. It does not aim at the creation of a single uniform breed of man, but at a diversity of excellence, based on a general raising of the level of desirable genetic capacities, coupled with the provision of fuller outlets for their effective exercise. It does not envisage the dogmatic application of rigid rules as to desirable and undesirable qualities, nor the employment of dictatorial or authoritarian methods. It prefers to rely on education, persuasion and intelligent leadership, acting on the good will and good sense of humanity at large.

To succeed, positive eugenics can no longer rely on such slow and marginal methods as the financial or social encouragement of the reproduction of 'superior' couples. It must yield obvious and reasonably rapid results.

Luckily the necessary techniques are already available, or can be readily perfected. These are (1) safe and simple methods of conception-control or prevention, (2) artificial insemination from donor males (A I D), and (3) the preservation of mammalian sperm for indefinite periods by deep-freezing Furthermore, it can only be a matter of time before we arrive at the complementary method (4) of assuring a long-term supply of female gametes by deep-freezing immature female germ-cells

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* Substance of the Galton Lecture given before the Eugenics Society on June 6.

The first step towards large-scale positive eugenics would be the decision by a few enlightened couples to have recourse to what has been called eutelegenesis—A I D by means of sperm from some admired donor to 'father' their children Deep-freezing of sperm will make it possible to utilize a smaller number of more outstanding donors as parents, even after their bodily death

Doubtless this would be met with abuse and with various legal difficulties at the outset but the certain success of the experiment, in the shape of outstanding and happy children, would soon be decisive in inducing an increasing number of couples to adopt similar methods, and eventually in making it an accepted and approved practice

Meanwhile, the immediate provision of shelters for banks of deep-frozen sperm is desirable as a protection against the genetic effects of atomic blast and fall-out, and would, of course, facilitate positive eugenics by eutelegenesis

It is frequently objected that such practices will never be generally adopted, as they run counter to deep-seated human 'instincts' I do not believe this Certainly there will at first be widespread and often violent opposition, just as there has been to birth-control and is now to legal reforms concerning homosexuality But the opposition will spring from tradition and prejudice, not from instinct Human instincts are few and very plastic During human history, the operation of the sexual instinct has been modified in a number of surprising ways There has been acceptance of polygamy, polyandry and monogamy, with or without divorce, there has been approval of clerical celibacy and female virginity there has been tolerance of man's promiscuity, or pre-nuptial sexual partnership and of male indulgence I see nothing against an eventual acceptance and approval of eutelegenesis, in the shape of vicarious parenthood by outstanding donors After all, it would have the highest ethical motivations, in promoting the welfare and fulfilment of the children, of society and of the species

So we return to the evolutionary perspective Once it is grasped that man's destiny is to direct the future evolution of himself and his planet, the fuller realization of desirable possibilities will become man's strongest ethical motive To achieve this, he must improve not only his social system but also his genetic endowment This means eugenics and it is inevitable that eugenics will eventually play a key part in the future world society

Meanwhile, the immediate tasks of the eugenic movement must include much increased research on human heredity and reproduction and their control, together with the promotion of better education in the facts and implications of evolutionary and social biology, and of public understanding of the practical possibility, the social value, and the ethical importance of positive eugenics

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

RETIREMENT OF SIR CHARLES HARINGTON, K.B E., F.R S.

On July 31, Sir Charles Harington retires from the directorship of the National Institute for Medical Research after having held this important post for twenty years At the beginning of his directorship the Institute had a scientific staff of thirty-five and was housed in an old converted hospital at Hampstead, at the end it had a scientific staff of some 125 and, since 1950, had been accommodated in the new Institute at Mill Hill as well as in the original building at Hampstead

Sir Charles received his scientific education at Cambridge, and then, in 1920, went as research assistant to the Department of Therapeutics in Edinburgh Two years later he became lecturer-in-charge of the Pathological Chemistry Department at University College Hospital Medical School and successively reader in 1928, and professor in 1931, with which post, from 1937, he combined the directorship of the Graham Research Laboratories In 1931 he was elected to the Royal Society, being its Croonian Lecturer and its Royal Medallist in 1944 During this period he rendered outstanding service as editor of the *Biochemical Journal* and later played an important part in founding the International Union of Biochemistry

In respect of his personal contributions to research, Sir Charles's name will always be identified with his elucidation of the structure of thyroxine, and later, in conjunction with Barger, the synthesis of this compound in its racemic form Out of this developed an impressive mass of work on other iodinated compounds and the biochemistry of the thyroid gland, which introduced a new dimension to the biochemistry of the endocrine glands But his interests were not confined to those fields A second line led to the synthesis of glutathione, which was important not only in itself but also because of the new synthetic methods for peptides that he introduced In a third, he pioneered the field of synthetic immunochemistry, preparing protein complexes of tyrosine, thyroxine and aspirin and investigating their biological and immunological behaviour Most of this work took place before the outbreak of the Second World War, and later, with his increasing responsibilities, the opportunity for Sir Charles to work with his own hands lessened The influence, however, of his early interests in the chemical approach to biology was reflected in the encouragement and development of related work at the National Institute for Medical Research

In the second period of his career, Sir Charles Harington's contributions to the advancement of scientific knowledge centred on his directorship of the National Institute By personality and experience he was admirably equipped for this task. Even before he became director the breadth of his knowledge on medical and biological research was impressive, and this great ability to assess the importance and potential value of particular research programmes became even greater while he was in charge of this large Institute with its many and varied activities Combined with this was exceptional administrative ability and a capacity to take responsibility and objective decisions These attributes, although essential to a man in such a post, would not, in themselves, have been enough To have their full effect it was necessary for them to be combined with an ability to win confidence not only as a scientist but also as a person There must to-day be many research workers who have occasion to thank him for his help and advice while they were in their formative years It was Harington's intellectual integrity, his unshrinking but generous capacity to deal with a case on its merits, and his willingness to take endless trouble not only on scientific but also personal matters, that accounted for the value that was placed on his opinion and the frequency with which his advice was sought—and accepted—both by his staff and by outside bodies

The dilemma that faces any director of a large research institution is that of ensuring a synthesis of interests while preserving the intellectual initiative of individuals Failure in one direction leads to the institution becoming merely a collection of isolated individual sub-departments, failure in the other to a stereotyped team of deteriorating quality The responsibility for resolving this dilemma rests on the director Its solution lies in wide scientific knowledge and ability and a capacity for objective judgment that commands confidence outside the range of the director's personal experience

It was inevitable that Sir Charles Harington's services should have been sought in increasingly wide fields Throughout his directorship he had been very aware of the need to integrate research being undertaken by a body like the Medical Research Council with that done in the universities and by the practising profession It was, therefore, particularly appropriate that he should be chairman of the Council of University College Hospital Medical School and take a prominent part in the work of the North-West Metropolitan Regional Hospital Board In the day-to-day application of medical and related biological research to practical problems his services are too numerous to mention

It will be a source of gratification that, on his retirement, Sir Charles's services to medical research will not be lost He will continue in his relationship to the Medical Research Council as consultant adviser to the Council's secretary

[As announced in *Nature* of July 30, 1960 (p 374), Sir Charles is to be succeeded as director of the National Institute for Medical Research by Prof. P B Medawar, Jodrell professor of zoology and comparative anatomy in University College (University of London) An appreciation of Prof Medawar's scientific work was published in *Nature* of September 17, 1960 (p 991)]

NEWS and VIEWS

Industrial Research Fellows

The Institution of Chemical Engineers has appointed two Industrial Research Fellows, Dr J R Bourne and Mr R Jackson These appointments follow the first report of the Exploratory Committee of the Institution of Chemical Engineers on Chemical Engineering Research which was published in *The Chemical Engineer* in April 1962 (see also p 209 of this issue) In that report, the Exploratory Committee recommended that a number of investigators, to be called Industrial Research Fellows, should be appointed, each to report on one specific field Each Fellow so appointed is to establish what are the real industrial problems, what additions to our knowledge might mitigate these problems and what programme of fundamental research might produce the knowledge required

Dr Bourne, who is a member of the chemical engineering staff at the University of Nottingham, is concerned with the mixing and agitation of non-Newtonian fluids, powders and pastes, and Mr Jackson, who is at present working in the chemical engineering laboratories of the University of Edinburgh and Heriot-Watt College, is to investigate the field of formation and coalescence of drops and bubbles in liquid systems It is estimated that the Industrial Research Fellows will require from six months to one year to complete their task and produce a report The report may be used as the primary agenda for a working party in the field and/or it may be published by the Institution in order to stimulate individual research workers and sponsors Alternatively it might form the basis of a co-ordinated scheme of research Further information can be obtained from the general secretary, the Institution of Chemical Engineers, 16, Belgrave Square, London, S W 1.

Mental Health at Bristol : Prof. D. Russell Davis

The new Department of Mental Health in the University of Bristol has been established out of private endowment Dr D Russell Davis, the newly appointed professor and head of the Department, will be particularly welcomed His teaching and research experience as reader in medical psychology and director of the Medical Psychology Laboratory in the University of Cambridge will find appropriate fields for development in the University of Bristol and in the United Bristol Hospitals Undergraduate teaching in clinical psychiatry will be increased and closely co-ordinated with other clinical departments in the Medical School A fully equipped unit has been prepared in the Bristol Royal Infirmary so that close functional relationship shall exist with the rest of the hospital and give medical students ample opportunities to pursue the study of the physical and mental aspects of illness and disease A unit has been provided in a nearby Regional Board Hospital for the care and study of mental illness requiring longer periods of hospital care Dr Russell Davis will develop research into the various aspects of mental health, including the ætiology of mental disease on which community and social factors may have an important bearing Opportunities will arise to encourage and develop co-operation between the University and the Local Authorities and the South-Western Regional Hospital Board in connexion with the Mental Health Services In this newly established chair and Department, the University is responding to a long-felt need to reinforce and extend the range of research and teaching in the Medical School and to contribute towards the promotion of mental health and the understanding of mental illness in the community throughout the south-western region of Britain

The Vernon Prize of the National Institute of Industrial Psychology : Prof H Kay

Prof H Kay, professor of psychology at the University of Sheffield, has been awarded the Vernon Prize of the National Institute of Industrial Psychology for his research into industrial training, which has included the use of teaching machines The Prize, founded by the late Dr H M Vernon, of the Industrial Health Research Board, is awarded in alternate years to the British investigator under forty-five who, in the opinion of the Institute, has done the most valuable research work in industrial psychology and physiology Between 1948 and 1951, Prof Kay worked with the Nuffield Research Unit at Cambridge on problems of ageing and also went as psychologist on the Naval Arctic Expedition of 1949 From 1951 until 1959 he was lecturer in experimental psychology in the University of Oxford, but spent a year on research into ageing at the National Institute of Health in Washington, D C His psychological work at Sheffield, where he has been professor since 1960, has been financed partly by the Department of Scientific and Industrial Research

Science in Parliament.

The Royal Society Research Professorships

In a written answer in the House of Lords on June 28, the Minister for Science, Lord Hailsham, stated that the President of the Royal Society had proposed to the Chancellor of the Exchequer that the Society should establish, with Exchequer support and with the approval of the universities concerned, a number of research professorships in British universities The purpose of this scheme would be to meet a need, not at present covered, to provide opportunity for research scientists who are worthy of appointment to a chair but for whom one is not available because they work in a borderline field or in one in which scientific developments are exceptionally rapid, or for similar reasons Lord Hailsham stated that the Government had agreed to provide further direct financial support to the Royal Society to enable them to sponsor the creation of five such professorships, and it was hoped that the first appointments would be made early in 1963 The conditions of tenure would be settled with the universities concerned It was intended that the grant-in-aid to the Royal Society for 1963–64 should contain provision for a new grant covering the salaries of the professors and their immediately supporting staff, and that the scientific investigations grant to the Royal Society should be increased to provide the necessary equipment The cost of this scheme was expected to be about £35,000 next year and £44,000 in the first full year of its operation

Emigration and Immigration of Scientists

In a written answer in the House of Commons on July 2, the Parliamentary Secretary of Science, Mr D Freeth, stated that the Committee on Scientific Manpower had made an estimate (published in Cmnd 1490) based on general population statistics of the total number of scientists who left the United Kingdom annually up to 1959 Figures for Commonwealth citizens travelling by long sea routes only were available up to 1961, and Mr Freeth gave the following figures physicists—emigrants, 1959, 72, 1960, 64, 1961, 101, immigrants, 1959, 34, 1960, 40, 1961, 58 . for chemists, the corresponding figures are —emigrants, 232, 246, 282, immigrants, 176, 208, 161 for biologists—emigrants, 56, 50, 67, immigrants, 48, 32, 41 for mathematicians—emigrants, 8, 8, 12, immigrants, 8, 8, 8 for other scientists—emigrants, 192, 220, 238, immigrants, 140, 154, 155

Education Research in Scotland

In a written answer in the House of Commons on June 29, the Secretary of State for Scotland, Mr J Maclay, stated that Exchequer grants to the Scottish Council for Research in Education were expected to be about £5,000 in the current financial year, compared with £3,000 last year New projects which the Council had recently decided to undertake covered studies in teaching mathematics and of teaching by television His Department had also suggested to the Council investigations of the age of transfer of pupils from primary to secondary education and the use of attainment tests, the optimum size of class and of school, and of the validity of examinations other than the traditional external written examinations and the comparability of examinations in various subjects

Production of Enriched Uranium

In reply to a question in the House of Lords on June 27, the Minister for Science, Lord Hailsham, said that supplies of enriched uranium available for the United Kingdom production were now such as to permit some reduction in the output of the Atomic Energy Authority's plant at Capenhurst Supplies were fully adequate for the present nuclear deterrent policy, which had not changed, and Lord Hailsham said they were also adequate for years to come in view of possible changes of requirements of the civil nuclear energy programme The numbers employed at the Capenhurst plant, which started operations in 1953, would fall by some 500 by about the end of this year and some redundancy would be unavoidable, although some of the reductions would be achieved by wastage and redeployment The greatest possible warning would be given to those concerned and every effort made to assist them in finding alternative employment The Authority would immediately discuss these matters with the staff associations and trade unions concerned

The *Telstar* Communications Satellite

The communications satellite *Telstar* was successfully launched from Cape Canaveral, Florida, at 08 35 U T on July 10 by a *Thor-Delta* rocket vehicle The satellite, which is designated 1962αε1, was placed in an orbit inclined at 44 8° to the equator, with an orbital period of 157 8 min Initially the height above the Earth varied between 950 and 5,640 km, with the apogee about 10° south of the equator when the satellite was going north the apogee is moving northwards round the orbit at a rate of about 2 degrees per day, so that the satellite will be well placed for communication in the northern hemisphere during the next few weeks The satellite weighs 81 kg (178 lb) and is roughly spherical in shape, except that it has 72 facets, 60 of which carry solar cells

Its diameter is 86 cm (34 in) *Telstar* is an active communications satellite—designed to amplify and re-transmit the signals sent to it—and has operated with notable success The first trans-Atlantic television pictures by satellite were transmitted from the station at Andover, Maine, and received at Goonhilly Down, Cornwall, and Lannion, Brittany, on the sixth revolution of the satellite, within 16 hr of launch

Tenth Pugwash Conference

FURTHER to the announcement of the tenth Pugwash Conference to be held in London during September 3–7, which appeared on p 126 of the July 14 issue of *Nature*, the following information has been received

British participants at the Conference will include Sir John Cockcroft, Master of Churchill College, Cambridge, Sir Howard Florey, Master of Queen's College, Oxford, and president of the Royal Society, Sir Julian Huxley, Lord Russell, Sir Charles Snow, and Sir George Thomson, Master of Corpus Christi College, Cambridge The chairman at the opening session of the conference on September 3 will be Sir Nevill Mott, Master of Caius College, Cambridge, and director of the Cavendish Laboratory Among Russians attending the conference may be included Academician Blagonravov, head of the Russian programme of space research, Prof Emelyanov, head of the department responsible for atomic research in the Soviet Union and Russian delegate to the International Atomic Energy Agency in Vienna, I V Federov, secretary of the Moscow Academy of Sciences and at one time the leader of the Russian delegation to the test-ban talks at Geneva, Academicians N Semenov and I Tamm, Nobel prizewinners in chemistry and physics, and Academician A V Topchiev, executive vice-president of the Moscow Academy of Sciences The group from the United States will include Dr D G Brennan, president of the Hudson Institute of New York, and an adviser to the U S Government on defence, Prof Margaret Mead, the anthropologist, Dr J Oppenheimer, director of the Institute for Advanced Study at Princeton and once head of the Los Alamos laboratory of the Atomic Energy Commission, Dr Linus Pauling, Dr Alfred Sabin, and Dr Victor Weisskopf, at present the director of the European Centre for Nuclear Research (CERN) at Geneva

A discussion is to be included in the programme of the Conference of the suggestion that West Berlin should be made the site of an international centre for scientific research

The Journal of Catalysis

THE phenomena of new journals devoted to a single aspect of a science or technology have been of frequent occurrence in the past few years While the prospect of the increasing number of publications one could reasonably be expected to have to scan has become somewhat alarming, it is nevertheless of great value to specialists to have available journals specific to their own field of work It is on such grounds that one can welcome the new *Journal of Catalysis*, edited by J H de Boer and P W Selwood and published by Academic Press (1, No 1, March 1962 Pp 92 New York and London Academic Press, Inc , 1962 Vol 1 (6 issues), 1962 18 dollars) The importance of the subject is one that merits this kind of treatment, but it is to be hoped that the editors will be able to encourage in their journal the publication of relevant papers on surface and solid-state properties, for it seems to be generally true that the sophistication of kinetic studies in catalytic processes has rarely been matched or accompanied by equivalently advanced investigations of the surface structures of the catalyst It would be of great value if specialists in these fields should consider the new *Journal* a suitable medium for the publication of particularly relevant papers in these fields and in heterogeneous processes in general

Bulletin of the International Computation Centre

THE first number of the *I C C Bulletin* has just appeared This continues the functions performed by the Bulletin of the Provisional International Computation Centre (*PICC, Bulletin*), and, in addition, is to act as the organ of the International Federation of Information Processing (IFIP) and will contain reports of the committees of that body (1, No 1 (April 1962) Pp vi + 78 Supplement Repertory of Computation Laboratories Pp 18 Rome International Computation Centre, 1962) In addition to a new set of loose-leaf sheets describing the equipment and facilities of various computer laboratories the latest issue contains an interesting paper, by Prof L Collatz, on the applications of functional analysis to numerical calculation An article on teaching machines and their simulation by a digital computer gives a picture of an automatic classroom of the future, which goes beyond the usual ideas of elementary rote instruction to a view of the way in which teaching administrators can be educated It is an interesting and controversial essay Among the descriptions of new machines and equipment is one of the 'IBM 1301' disk storage unit, its capacity of up to 280 million alpha-numeric symbols with a maximum access time of 0 18 sec is likely to have important implications in the field of information storage and retrieval

European Atomic Energy Research

THE last three issues of *Atom*, the monthly information bulletin of the Atomic Energy Authority, contain some articles of exceptional interest The April issue reports Sir Leonard Owen's re-examination of nuclear power costs and Sir Roger Makin's address to the Institution of Chemical Engineers on nuclear energy in 1962 The May issue includes a long article by V W Eldred and K Saddington describing the post-irradiation examination facilities at Windscale works, while the June issue contains a paper by J A. Williams and P C Davidge of an effluent at Windscale and the effect of the civil power programme given to the British Nuclear Energy Society It also includes a report by Dr M L Smith on the symposium organized by the Central Treaty Organization Institute of Nuclear Science on the role of science in the development of natural resources with particular reference to Iran, Pakistan and Turkey (the Central Treaty Organization countries)

Lasers

ONE of the outstanding technological developments of the second half of the twentieth century is undoubtedly the use of stimulated emission for coherent generation of micro-wave radiation and light The maser has been rapidly followed by the laser (or optical maser), and during the past two years the published literature on the latter has grown to vast proportions The Central Library (*TIL*3) of the Ministry of Aviation has accordingly performed a most valuable service to those interested in this rapidly growing field of research by issuing a bibliography with brief abstracts covering the period December 1958–May 1962 (Bibliographical Series, No 4 Lasers Compiled by K J Spencer Pp i+39 London Ministry of Aviation, 1962) The abstracts are short, but give an indication of the contents of most papers Some indication of the rate of growth of the subject may be gathered from the number of references quoted 1958, 2, 1959, 10, 1960, 19, 1961, 168, 1962 (January–May), 129 About eighty more references from symposia proceedings are also given The references deal not only with the physics of laser action, but also with their achieved, proposed and dreamed of applications

Foundations of a Flora

MANY of those who have rightly admired Sir Joseph Dalton Hooker's great achievement in producing his *Flora Tasmaniae* (London, 1860), with its important introductory essay on plant geography and its bearing on evolutionary fact and theory, may perhaps not have appreciated how much he was helped by a long series of reports and collections of specimens made to his father, Sir William Jackson Hooker, and to himself, by local Tasmanian naturalists and collectors In fact, as it now appears, he received an astonishing amount of information from local sources T E Burns and J R Skemp, and the Queen Victoria Museum, Launceston, Tasmania, are therefore to be congratulated on having made available some notable correspondence between R C Gunn—one of the great Tasmanian travellers and naturalists—R W Lawrence, Jorgen Jorgenson, Sir John Franklin and other botanists, and Sir William Jackson Hooker (one-time Regius professor of botany in the University of Glasgow and, later, director of the Royal Botanic Gardens, Kew), covering the period 1827–49 Not everyone has a taste for these by-ways in the history of botany, but for those who wish to understand something of the pioneering work that made possible the compilation of an important Flora, and of the cumulative experience that afforded substantial and timely support to exponents of evolution at a critical time, a perusal of the present volume is likely to prove rewarding (*Records of the Queen Victoria Museum, Launceston* (New Series) No 14 Pp xix+142 Launceston, Tasmania Queen Victoria Museum, 1961)

Influence of Mental Tension on Diabetes Mellitus

HEREDITY plays an essential part in the development of diabetes mellitus States of mental tension, no matter how severe, cannot cause diabetes without an inherited predisposition to the disease Mental stress can result in the appearance of overt diabetes among previously latent diabetics It can also aggravate existing diabetes mellitus temporarily, and sometimes permanently, especially in states of prolonged mental tension This is so much the case that, in mild cases previously controlled by diet alone, acidosis and even pre-coma may result In eight cases described by Dr S Sukiennik, Central Clinic, "Zamenhoff", Tel Aviv, diabetes appeared in four following severe mental tension (*Dapim Refuim*, 21, No 2, February 1962) As the nervous tension decreased, the diabetes regressed to its latent state or became mild and stabilized again. In the other cases, diabetes grew worse during mental stress, being temporary so long as the stress was temporary, but persisting while the stress continued States of mental stress cause the over-secretion of steroids of the 11-oxy-steroid group These substances lead to excessive catabolism of proteins, and to the overproduction of glucose by means of gluconeogenesis in the liver They also result in the decreased utilization of carbohydrates by the tissues, as well as changes in electrolyte balance With steroid therapy, diabetes becomes manifest only among those with an inherited predisposition to the disease Glucose tolerance tests with steroids show that even the temporary increase of steroids can result in temporary changes in carbohydrate metabolism This is seen also after excitement or following fits of anger in diabetes

The Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme: Awards

AWARDS under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows Mr J K Coulter, head of soil research and survey section, regional research centre, University College of the West Indies, to assist him to study the work on classification and fertility of tropical and sub-tropical soils being carried out by the Commonwealth Scientific and Industrial Research Organization in Australia, from December 1962 to February 1963, Dr A T Cowie, Department of Physiology, National Institute for Research in Dairying, Reading, to assist him to collaborate in research on the physiology of lactation at the Department of Animal Husbandry, University of Sydney, from April to June 1963, Dr E J Denton, principal scientific officer, Marine Biological Association Laboratory, Plymouth, to enable him to visit New Zealand and New Caledonia and related islands in order to study marine animals in environments different from those found in the English Channel and North Atlantic, for 3–4 months from mid-October 1962, Mr J R Dunstone, lecturer in biochemistry, University of Queensland, to enable him to pursue research into the physical aspects of biologically important mucopolysaccharides at the Lister Institute in 1963, Prof J F Heard, professor of astronomy and director of the David Dunlap Observatory, University of Toronto, principally to assist him to study methods of stellar spectroscopy at other Commonwealth observatories, including Mount Stromlo, for about 2 months during 1963, Dr J I Hubbard, Department of Physiology, Australian National University, Canberra, to enable him to study electron microscopy techniques at University College, London, from September 1962 for 5–6 months, Mr R A Jarvis, member of Soil Survey of England and Wales, to enable him to visit New Zealand at the end of 1962 for 3 months in order to make a critical comparison of red and yellow podzolic soils occurring there with the relevant English soils, Dr T F Neales, senior lecturer in botany, University of Melbourne, to enable him to study techniques in the culture of excised plant roots at the University College of Swansea, in 1963, Prof E L. Yates, professor of physics, University College of Rhodesia and Nyasaland, to enable him to discuss problems of solid-state physics at Melbourne, from May until September 1962

Physiology and Psychology in Arid Lands

A SYMPOSIUM on "Environmental Physiology and Psychology in Arid Conditions", organized by the United Nations Educational, Scientific and Cultural

Organization, is to be held in India during August 27–September 1 The programme will include discussions on medical climatology of arid zones, physiological anthropology, comparative physiology of arid lands, nutrition and heat, water and electrolytes, neurophysiology of heat exposure, significance of solar radiation in the heat balance, performance and comfort standards, psychological aspects of life in hot climates Further information can be obtained from the Department of Natural Sciences, Unesco, Place de Fontenoy, Paris 7e

The Night Sky in August

Full moon occurs on Aug 15d 20h 10m U T and new moon on Aug 30d 03h 09m The following conjunctions with the Moon take place Aug 4d 08h, Venus 4° S , Aug 14d 19h, Saturn 0 9° S , Aug 16d 23h, Jupiter 1° N , Aldebaran 0 9° S , Aug 24d 22h, Mars 3° N In addition to these conjunctions with the Moon, Mercury is in conjunction with Regulus on Aug 10d 05h, Mercury being 1 1° N , and Venus with Spica on Aug 31d 02h, Venus being 0 02° S There is a penumbral eclipse of the Moon on August 15, partly visible at Greenwich, where moonrise is at 19h 25m, mid-eclipse is at 19h 57m and eclipse ends at 21h 39m, and the penumbral magnitude is 0 62 Mercury is too close to the Sun for observation Venus is an evening star, visible low in the west after sunset and setting little more than an hour after the Sun Mars rises at 0h 10m, 23h 45m and 23h 30m on Aug 1, 15 and 31, respectively. It is at a distance of 164 million miles on August 15, and its stellar magnitude is +1 3 Jupiter rises shortly after sunset, and remains above the horizon all night It is in Aquarius, its stellar magnitude is −2 4 and its distance from the Earth on August 15 is 374 million miles Saturn is in Capricornus, setting at 4h 30m, 3h 25m and 2h 15m at the beginning, middle and end of the month, respectively Its stellar magnitude is +0 4 and its distance from the Earth in the middle of the month is 834 million miles Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich Aug 9d 21h 16 0m, 49 Lib (*D*), Aug 22d 00h 08 9m, 5 Tau (*R*), Aug 23d 01h 14 9m, 75 Tau (*R*), Aug 23d 01h 51 5m, 264 B Tau (*R*) *D* and *R* refer to disappearance and reappearance, respectively The Perseid meteors are active during the first half of the month, but conditions are rather unfavourable for observation

Announcements

Prof E B Chain, professor of biochemistry at the Imperial College of Science and Technology, has been elected an associate member of the French Academy of Science

Prof J E Harris, professor of zoology in the University of Bristol, has been elected a member of the Agricultural Research Council in succession to Prof P B Medawar, who retires from the Council on September 16

Mr J E C Bailey has been elected president of the British Scientific Instrument Research Association Mr Bailey, chairman and managing director of Baird and Tatlock (London), Ltd, Hopkin and Williams, Ltd, and W B Nicholson (Scientific Instruments), Ltd, and a director of Derbyshire Stone, Ltd, has for many years been prominent in the scientific instrument industry both as president of the Scientific Instrument Manufacturers Association during 1945–50, and since 1952 as chairman of the British Scientific Instrument Research Association

The Challenger Society is prepared to make small grants for research in marine biology or oceanography at a recognized laboratory during the year July 1962–63 Applications, accompanied by details of the proposed research, should reach the Honorary Secretary, Challenger Society, National Institute of Oceanography, Wormley, Godalming, Surrey, not later than August 4

A conference on "Gasification Processes", arranged jointly by the Institution of Gas Engineers and the Institute of Fuel, will be held at Hastings during September 10–14 Further information can be obtained from the Institute of Fuel, 18 Devonshire Street, Portland Place, London, W 1

The second congress of the International Council for Building Research on "The Influence of Changing Requirements and Developments in Materials and Components on Design and Construction" will be held in Cambridge during September 5–11 Further information can be obtained from the Organizing Secretary, CIB Congress 1962, Building Research Station, Garston, Watford, Herts

The third national conference of the British Computer Society will be held in Cardiff during September 4–7 Topics to be covered include business data processing, industrial data processing, data origination and transmission, developments in equipment Further information can be obtained from G J Morris, British Computer Society Conference Publicity Committee, I C T, Ltd, Putney Bridge House, London, S W 6

An international symposium on "Information Theory", organized by the Benelux Section of the Institute of Radio Engineers and the Belgian Society of Telecommunication and Electronics Engineers and sponsored by the Professional Group on Information Theory of the Institute of Radio Engineers, will be held in Brussels during September 3–7 Further information can be obtained from the chairman of the Organizing Committee, Dr F L Stumpers, Philips Research Laboratories, Eindhoven

A conference on "Low-Energy Nuclear Physics", sponsored by the Institute of Physics and the Physical Society, will be held at the Atomic Energy Research Establishment, Harwell, during September 12–14 Subjects under discussion will include the nuclear interactions of slow and fast neutrons, nuclear reactions induced by charged particles and γ-rays, nucleon–nucleon interactions, nuclear structure and nuclear models Further information can be obtained from Dr G C Morrison, Nuclear Physics Division, Atomic Energy Research Establishment, Harwell, Didcot, Berks

An international symposium on "Magnetoplasmadynamic Electrical Power Generation", sponsored by the North-Eastern Centre of the Institution of Electrical Engineers in collaboration with the North-Eastern Branch of the Institution of Mechanical Engineers, the North-Eastern Branch of the Institute of Physics and the Physical Society and the University of Durham, will be held at King's College, Newcastle upon Tyne, during September 6–8 Further information can be obtained from the Symposium Secretary, Institution of Electrical Engineers, North-Eastern Centre, c/o C A Parsons and Co, Ltd, Heaton Works, Newcastle upon Tyne, 6

THE MATHEMATICAL ASSOCIATION

THE annual conference of the Mathematical Association, held in London during April, attracted a record attendance This was, perhaps, in keeping with the growth of membership, the figure for which is now the highest in the history of the Association, having reached almost five thousand

Liveliest interest was aroused by the presidential address delivered by Mr J T Combridge, registrar of King's College, London, and by a discussion on the relationship between mathematics and industry

Mr Combridge himself introduced this subject, and major contributions were made by Mr F W Kellaway, principal of the College of Technology, Letchworth, and Mr P L Taylor of Associated Electrical Industries, Ltd Mr Kellaway dealt with the two-way problem of interpreting the needs of industry to mathematicians and explaining the uses of mathematics to industrialists He surveyed the supply of, and demand for, graduate and graduate-equivalent mathematicians and the need for near-graduate qualifications Already the Association had introduced the diploma in mathematics for teachers and intending teachers, the reputation and value of which had quickly become established "It was", said Mr Kellaway, "the duty of the Association to care equally for mathematicians in industry "

Mr Taylor expanded and supported this theme "If we, in this country, do not strive to foster the partnership between science and industry", he said, "we shall surely slip back from the front rank to the second, and the second to the third until we ourselves become an under-developed country Mathematics is central to science as a tool and as a discipline The challenge, therefore, is to produce the increasing numbers of technological mathematicians and mathematical technologists The life-cycle of the mathematician in the past—from school to university and so back to school—is not attractive enough for many young people to-day, when there are so many more interesting things to do in the world The task of members of the Association is to inform themselves of these exciting things and transmit their enthusiasm to pupils

"The second challenge is to determine both the matter and the manner of the teaching that will produce the sort of people that are needed in industry One good way of achieving this is to have some form of society or institute, to which the mathematician working in industry can belong His colleagues in physics, chemistry and the various branches of engineering and technology, all have their appropriate institutions The activities and benefits of these institutions were known to all The pointer to the Mathematical Association was obvious "

Following the discussion, the proposition that the Association should inaugurate a second diploma examination was carried by an overwhelming majority It is likely that the examination would be of a standard requiring part-time study extending over three years beyond General Certificate of Education, advanced-level, attainment Syllabuses would be appropriate to the needs of industry It was clear that a move towards the creation of an institute of mathematics might also be welcomed

In his presidential address Mr Combridge referred to the new outlook on mathematics resulting from the Bourbaki publications, and its effect on the content and orientation of university teaching Some of the newer concepts had applications in commerce and industry In this realm also some quite elementary mathematics was now applicable because of the development of machines able to deal with calculations that had previously been prohibitive

There was a danger that introducing the newer mathematical ideas too early might mean presenting pupils with abstract ideas before they were old enough to do more than cope with numerical applications Nevertheless, the increasing demand for competent users of mathematics was producing pressure on the Association which called for urgent consideration Thought and action in the mathematical world in the United Kingdom were hampered by lack of channels of communication within the Association and between the various mathematical organizations, and there was no one to speak for mathematics as a whole

The Association had not been inactive Its objects, which were "to effect improvements in the teaching of elementary mathematics and its applications, to provide means of intercommunication between mathematical students and teachers for this purpose, and to take such measures as may appear expedient to advance the views of the Association on any question affecting the study and teaching of elementary mathematics and its applications", allowed considerable development, and its appeal was becoming wider

Some acquaintance with the newer outlook on mathematics, and with applications of it and of more elementary mathematics in industry, should make the subject more meaningful for teachers and pupils alike, and thereby reduce the number of children being frightened off mathematics at an early age

The President believed that mathematics would not be endangered by placing itself at the service of the community He propounded the thesis that such action was in accordance with principles that underlie the development of the subject, and that these principles are intrinsic not only in the subject but also in nature itself, so that progress would only be achieved by working in harmony with them The mathematician who demands complete isolation, and the technologist who insists that mathematical development shall be governed by considerations of utility are both extremists who forget that one function of the community is to promote the proper development of each individual in it, and that the individual finds his true development in service to the community The development of mathematics exhibits this truth in action

The concept of mathematics as queen and servant of the sciences is a familiar one It did not imply, however, that pure mathematics is queen and applied mathematics servant Rather are 'queen' and 'servant' two interdependent aspects of mathematical development which are often to be seen in action simultaneously The proper distinction is perhaps the one that Hardy made between 'real mathematics' and 'trivial mathematics' In Egypt or Greece, land measurement and Euclidean geometry may well have developed side by side Centuries later, the theories of Bolyai and Lobatchevsky produced a major advance in abstract thinking, but Riemannian geometry provided Einstein with the tool for a similar advance in mathematical physics This in its turn led to extensive developments in the concepts of parallelism and of affine geometry

Just a century ago Clerk Maxwell, in the College where this address was being given, was formulating for the physical electromagnetic concepts of Faraday the mathematical equations which bear their author's name The development and applications of Fourier's work provided another example of the co-existent roles of queen and servant

So also Dirac introduces his delta function into Fourier theory for mathematical-physical purposes, Schwartz produces the rigorous generalized theory, Temple simplifies it, and Lighthill produces a text-book which might have satisfied Hardy and might even have been classified by him as 'real' mathematics

It is not, however, a question of mathematics alone Mathematics may be entirely an expression of human thought, it may be an objective reality awaiting investigation and discovery, the truth is probably somewhere between But the mathematical world consists of mathematics and mathematicians, and the total of these is something different from the arithmetical sum of the several parts It is not necessary to elevate mathematics to the category of personality, it has not the faculty of responsiveness in the way that human personality has But (as Henri Poincaré, for example, has shown) it does partake of the activities of the subconscious mind It calls for and responds to our questionings just as our body will demand and respond to alternations of exercise, work and rest It is not astonishing therefore to find that principle of simultaneous service and supremacy, which we discern in the development of mathematics, operating in the mathematical world—the world of mathematicians and their mathematics—as a whole

Moreover, this mathematical world is itself an intrinsic part of nature, and so, as a curve on a curved surface is also a curve in the Euclidean sp dimensions in which that surface may be as embedded, the laws of the mathematical wo laws of the nature that is intrinsic to all Progres will be achieved by working with them and not ignoring them The noteworthy feature of mathematical development, the constant interplay of service and supremacy, should also characterize the policy of the Mathematical Association The Association should refuse to give up freedom for itself or for others, the mathematical world must not be allowed to become merely a tool or a slave for the world of materialism But when it has freedom it should not disdain to serve, only those who are free can serve, and there is a service that is perfect freedom, and service—the service of one's neighbour as one's self—brings the intrinsic right to existence, to individuality, to personality, to continual freedom, and to the only kind of sovereignty worth having

Subjects of other papers read at the conference included "Matrices for the Million", by Dr G Matthews, of St Dunstan's College, Catford, and "Radar", by Dr E Eastwood of Marconi's A talk, with demonstrations, on mathematical films by Mr W M Brookes of the University of Southampton was widely acclaimed

At the business meeting Prof V C A Ferraro was elected president for 1962–63 Mr F W Kellaway was re-elected as honorary secretary for the thirteenth year and will serve as general secretary of the Association Miss R K Tobias was elected an honorary secretary in place of Miss W A Cooke tribute to whose services was paid Miss Cooke was elected a vice-president Prof R L Goodstein of the University of Leicester will continue as librarian and editor and Mr W M Brown was re-elected as honorary treasurer A number of posts of honorary assistant secretaries was also established Mr B J F Dorrington, Mr R E Green and Dr E Kerr were elected, Mr Dorrington will also serve as secretary to the Examination Board

VISCO-ELASTICITY AND THE WEISSENBERG RHEOGONIOMETER

THE meeting of the Cambridge Rheology Club held on May 7 was opened by Mr R G King (Farol Research Engineers), who spoke on "Visco-elasticity and the Weissenberg Rheogoniometer" He described the instrument as a rotating cone and plate viscometer which, while measuring viscous behaviour additionally recorded any normal forces which might be produced by a visco-elastic material Thus it was an instrument specifically designed for testing visco-elastic materials The upper cone was free to move about its axis against a torsion bar measuring tangential stress The lower platen was driven and the normal force on it measured From values of tangential stress and normal force the coefficient of viscosity and elastic modulus could be calculated Variations in shear-rate might affect both separately and to a different extent An alternative method of measurement was by oscillation about the vertical axis If the material was completely elastic, the input strain and resultant stress would be in phase, if purely viscous, the phase difference would be 90° Visco-elastic materials would exhibit intermediate phase differences

Mr King described the *R*14 rheogoniometer with particular reference to latest developments The instrument was fitted with two synchronous motors (one for oscillation, one for rotation experiments) each driving one 60-speed gearbox A shear-rate of from 10^{-4} to 4×10^{3} sec $^{-1}$ could be obtained In oscillation experiments a range of frequency from 25 cycles/sec to $25 \times 10^{-5\ 9}$ cycles/sec was available Tangential stress measurements could be taken from 1×10^{2} to $1\ 5 \times 10^{7}$ dynes/cm ^{2}, normal pressure measurements from 2×10^{2} to $2\ 7 \times 10^{6}$ dynes/cm ^{2} A servo system was fitted to maintain a constant gap between the plates during the experiment Five displacement transducers in conjunction with Pendeford multimeters were used for calibration and measurement The test could be conducted in an inert atmosphere and a temperature range of from $-40°$ C to $+400°$ C $\pm\ 0\ 5°$ C A small window and a light source in the walls of the chamber allowed observation of the sample during the test

Mr H G Muller (Spillers, Ltd) briefly described results obtained with the *R*7 type rheogoniometer on wheat flour dough A cone of 2 5 cm diameter with an angle of 2° had been used at 24° C and the lower platen modified so that the sample could be surrounded by an annulus of solution to keep it at constant humidity It had been found that rotation experiments could not be conducted as the dough immediately rolled out of the gap Using oscillation experiments the effect of relaxation between the plates had been studied The doughs (56 0 per cent absorption, 2 5 per cent sodium chloride) had been mixed on a farinograph under constant conditions and allowed to relax between the platens from 15 sec to 120 min They were tested at 6 cycles/min and an amplitude of 1° 15′ This was the largest amplitude at which no normal pressure was discernible Elastic modulus and coefficient of viscosity, both of the order of 10^4 c g s , dropped during 120-min relaxation from $6 5 \times 10^4$ to $1 5 \times 10^4$ c g s , the largest reduction occurring during the first 45 min When the frequency was altered from 4 to 15 cycles/min at a relaxation time of 60 min , the modulus remained unchanged while the coefficient of viscosity decreased from $3 5 \times 10^4$ to $1 5 \times 10^4$ c g s At 15 cycles/min vibrations were observed in the instrument It was proposed that exchangeable cone and plate assemblies should be designed for substances requiring longer relaxation times

In reply, Mr King pointed out that certain plastics showed cavitation during tests, but as yet no other material had rolled up making rotation experiments impossible Exchangeable assemblies had been considered, but technical difficulties had so far prevented a solution He also suggested that perhaps dough should be regarded as a readily deformable solid, and testing programmes be formulated accordingly, as this material does not exhibit viscous behaviour during rotational tests with a cone and plate

On the day following the meeting, samples of human blood plasma, provided by Drs D'A Kok and J Mehrishi (University of Cambridge), and bovine synovial fluid, submitted by Dr J Harris (Orthopædic Hospital, Stanmore), were tested With the former no readings could be obtained Using a 1° cone of 7 5 cm. diameter at 30° C and a shear-rate of 360 sec^{-1} with the latter, no normal force was observed The viscosity was 0 0104 poises At 9 04, 57 and 180 sec^{-1} and a 2° cone no stress readings could be obtained The fluid broke down rapidly during the test A drop of it spilt during the experiment immediately corroded the cast-iron base of the instrument

The next meeting of the club will be held on October 29 at the Technological Research Station, Spillers, Ltd , Station Road, Cambridge, when Mr J F Hutton (Shell Research, Ltd) will speak on "The Rheology of Motor Oils" H G MULLER

STANDARDS IN SCIENTIFIC PUBLICATION

THE Scientific Publications Council met at University College, London, on May 31, to discuss "What is a High Standard of Scientific Publication and how is it Attained ?" Prof G W Harris (Maudsley Hospital, London) took the chair

Prof W V Thorpe (Department of Physiology, Birmingham) introduced the subject As editor-in-chief of the *Biochemical Journal*, he confined his remarks to periodicals publishing original scientific work, as opposed to reviews or monographs High standards in such journals imply publishing clear and accurate accounts of original contributions to research on which others can build How is this achieved ?

First, Prof Thorpe suggested the editorial board must refrain from selecting topical and interesting papers in preference to unexciting routine work, for what is dull to a man working in one field, may be of vital importance to those working in others The editors must guard against rejecting poorly written papers for that reason alone, since it is their duty to help inexperienced or foreign authors to describe their work in an acceptable style Since science is an international endeavour, colloquialisms, jargon and abbreviations should be avoided and care taken that unfamiliar terms are explained so that the meaning may be clear to foreign readers unversed in the language of publication For the same reason it is good practice to adopt, so far as possible, internationally agreed usage with regard to nomenclature and units, and a standard form should be used for references The International Union of Biochemistry recently set up a Commission of Editors of Biochemical Journals to encourage international agreement on such matters

Verbose and repetitive styles must be amended, since clarity and conciseness not only make the text more easily intelligible but also save readers' time and, incidentally, reduce the cost of publication Experiments should be so described that they can be repeated without further research or correspondence with the authors An author can assist in reducing the delay between submission and publication by preparing his contribution carefully so as to reduce editorial processing to a minimum and to eliminate any necessity for revising the manuscript

Editors should be vigilant to the point of suspicion and should check all statements which are readily susceptible to testing, for example, that totals are correctly added and that theoretical values for elementary analyses are correctly calculated

Ultimately the Editorial Board is the custodian of a journal's standards That of the *Biochemical Journal* does not itself prepare papers for the press—this is done by the editorial office staff—but exercises a judicial and very critical selection, communicates suggestions to authors on how to improve their manuscripts and, where necessary, explains why one has been rejected Only 15 or 20 per cent of papers submitted have to be refused Members of the Board are all active scientific workers and selected so as to provide collectively a balanced and critical judgment in many fields Before a paper is accepted, it is normally submitted to the scrutiny of at least two members of the Board, one of whom is a specialist in the subject of the paper, while the other provides the point of view of the general scientific reader If necessary an independent referee is consulted High editorial morale is achieved by frequent discussion and self-criticism, including periodical scrutiny of the statistics of time-lag in publication so that causes of delay may be identified and so far as possible removed

Mr A L Bacharach (*British Journal of Nutrition*, etc) explained that the subject arose when the Council was framing the clause in its rules relating to its aims, namely, to promote and maintain high standards in the dissemination of scientific information and in particular of scientific books and journals At that stage it seemed natural to try to define and prescribe for high standards in scientific publication Mr Bacharach declared his interest by pointing out that he had served on the editorial boards or publications committees of the Royal Institute of Chemistry and the Society for Chemical Industry, and of the *Analyst*, of the *British Journal of Nutrition*, *Acta Pharmacologica et Toxicologica* and others

Whether the editorial policy of a journal is controlled by a learned society, by a commercial publishing house or, like the *British Journal of Pharmacology*, by a learned society and a publisher jointly, the aim should always be the same high standard

Mr Bacharach was in substantial agreement with Prof Thorpe, but wished to extrapolate the argument a stage further He thought that editorial boards should not over-stress the letter at the expense of the spirit by, for example, undue punctilio about conforming to internationally agreed conventions, for these are never so important as consistency and clarity

The ultimate aim of editorial policy should be to foster a relationship of confidence between author and reader For this it is of the first importance to establish a high level of scientific content Papers should contain new information couched in an interesting style, free from ambiguity and expressed with clarity so that the work will be intelligible in theory and repeatable in practice

Mr Bacharach defined referees as all those specialists charged with judging the scientific value of a paper's contents, while editors he defined as those concerned with the form of a paper and its preparation for the press, and this irrespective of the way the terms may happen to be used by any particular publishing body Often the same people may be involved, but their functions are distinct

Some journals have recently been started with the special aim of securing rapid publication by the reproduction of authors' own typescripts using a photolithographic process In this case the editors' function is reduced to a minimum, and the onus rests with the referees The first requisite for critical standards of refereeing is that it should be possible to send a paper to any suitable specialist anywhere in the world, and at least two independent opinions should be obtained, at any rate before rejection It is the duty of a referee to steer a careful course between the Scylla of academic orthodoxy and the Charybdis of extravagant novelty The editor is concerned to secure a high standard of presentation in style and syntax and to eliminate all obscurity or illogicality, and he is fully entitled to take all necessary corrective action Mr Bacharach did not agree with those who hold that what an author writes is his own affair alone To fail to obtain high standards of presentation is to abdicate from the editorial function and to connive at the reduction of a journal's status

Mr Bacharach then directed attention to the enormous proliferation of scientific publications in recent times and proposed a touchstone of justification for the appearance of a new journal The rapid advances in science are continually blurring old boundaries and creating new disciplines The test of a new journal is whether it will enable work lying across the boundaries of former traditional fields to come to the attention of workers on both sides of the division, whereas its publication in existing journals might bring it to the attention of one or the other only The same criterion might be applied to the creation of new learned societies

Mr Bacharach wondered what could be done about certain new journals, produced not under the ægis of learned societies but for commercial interests, relying for their circulation on what amounts to moral blackmail of librarians Their publishers know that the mere existence of a work creates a demand for it, and their activities are detrimental to the interests of science in general, of libraries with limited space and funds, and of other more scrupulous publishers *A fortiori*, the proliferation of technical books containing scientific information of an ephemeral or incomplete character represents a growing threat to the whole system of communication in science

Finally, Mr Bacharach remarked that *Nature* and similar journals exist for the rapid publication of short accounts of important new advances The publication of most researches should wait until a complete account of the work is available The insistence on speed in publication is a pathological development in science the influence of which is generally bad, being almost entirely at variance with the maintenance of high standards

Dr C C N Vass (St Thomas's Hospital Medical School, London) thought that the judicious selection of referees suitable for each manuscript submitted was one of the most important responsibilities of the editorial board and Mr J C Graddon (assistant editor, *Philosophical Transactions* and *Proceedings of the Royal Society*) felt that anonymity of referees might be a decisive factor in securing impartial advice Mr P C Williams (Imperial Cancer Research Fund) pointed out that referees have different standards, and it was agreed that anyone whose standards were known to be too low—or too high—should not be consulted Mr Williams wondered whether zealous editing might not even afford some authors an unjustified reputation for exposition and, moreover, might encourage people to submit slipshod manuscripts Mr N L Harris (General Electric Co , Hirst Research Centre) thought that editorial changes should be confined to grammatical corrections and should not aim at creating a drab uniformity of style However, Mr Bacharach maintained that this was a bogy, editors in general have no time to edit style because they are all too frequently engaged in grappling with its absence

Dr C A Hogarth (Brunel College, Acton) asked what should be done with manuscripts betraying signs of having been rejected by other editors Mr Bacharach replied that some journals require a declaration that a paper has not been submitted elsewhere, but Dr R K Callow (National Institute for Medical Research, Mill Hill) recalled the well-known fact that Sir Hans Krebs's announcement of his work on the citric acid cycle—for which he was later awarded the Nobel Prize—was refused by an eminent journal, there might be many reasons for the rejection by a particular journal of a paper without any reflexion on its scientific merits It is then good editorial practice to refer an author to an alternative vehicle for publication Mr Graddon remarked that the Royal Society is frequently asked to accept papers which are too long and expensive for others to publish It is a valuable custom that all manuscripts have to be communicated through a Fellow of the Society, who should be able to criticize the presentation and act, to some extent, as referee before consenting to act as sponsor

Mr P J Edmonds (Publishers Association, Technical Group) applauded Prof Thorpe's arrangements for periodic meetings of editors, production staff and printers, he thought that many of the problems with which the Council is concerned arise through imperfect comprehension between those responsible for the scientific, literary, technical and financial aspects of publication While appreciating Mr Bacharach's feelings about the proliferation of books and journals, he thought that the remedy lies with librarians and library committees, who should be more selective Mrs Olga Kennard (editor, *Crystal Data*) said that the publication of candidly critical reviews in reputable journals is the greatest safeguard, but Dr A J Evans (librarian, School of Pharmacy, London) complained that even though economic considerations force librarians to be selective, reviews often appear too late to be of much assistance to them

A V S DE REUCK

THE FULMER RESEARCH INSTITUTE

AN open day was held at the Fulmer Research Institute on June 7 The occasion marked the fifteenth anniversary of the Institute's foundation and the completion of laboratory extensions

The chairman, Mr W R Merton, introduced the principal guest, Mr Denzil Freeth, Parliamentary Secretary for Science, who emphasized in a short address that the Ministry did its best to persuade industry that scientific and technological research is not only worth while, but is in fact economical and vital for progress, expansion and prosperity The reply was often that it was too costly to set up extensive laboratories and engage specialized staff to carry out particular pieces of research This was true, but it was to overcome this objection and to meet these needs that establishments where confidential sponsored research could be undertaken had been set up Mr Freeth congratulated Fulmer on the high quality of its staff and specialized facilities and said that he envisaged a great future for sponsored research

The new extensions to the Physics and Physical Metallurgy Laboratories provide approximately 3,000 sq ft of additional laboratory space, about half of which is basement accommodation Provision is made in the extensions to the Physical Metallurgy Laboratory for handling toxic materials, and in the extensions to the Physics Laboratory for housing additional X-ray diffraction units, together with a new *EM*6 electron microscope

In recent years, a high proportion of the work of the Physics and Physical Metallurgy Sections has been concerned with metals of importance in the field of nuclear power, such as uranium, zirconium and beryllium In unalloyed uranium the orthorhombic α-phase is stable up to 660° C and the anisotropic nature of the α-phase can result in dimensional instability of fuel elements in the reactor One way of avoiding this is by quenching dilute alloys from the β-phase region to give a fine-grained structure, free from preferred orientation The mode and kinetics of transformation of the β-phase are being investigated at Fulmer using hot hardness, thermal analysis, X-ray diffraction and metallographic techniques, the structure of the β-phase is also being studied

Another approach for overcoming fuel-element instability is to alloy the uranium to a greater extent to give the cubic isotropic γ-phase at fuel temperatures Basic information pertaining to the processes occurring when fuel elements are cooled has been obtained at the Institute by quenching various alloys at different cooling-rates to give a sequence of transformation structures between γ and α The physical properties, kinetics and mechanism of transformation of these alloys have been studied

Zirconium and its alloys are important as canning and constructional materials in water-moderated reactors The recent announcement by the United Kingdom Atomic Energy Authority of the projected development of the steam generating heavy water reactor makes the Institute's work on zirconium of particular interest The reactive nature of zirconium has necessitated the design of a high-vacuum, high-temperature X-ray camera for structural studies at temperatures up to 1,000° C The embrittling 'omega' phase has been studied in a number of binary zirconium systems and the nature of the omega phase and its derivation from the high-temperature body-centred cubic structure of zirconium have been determined

Beryllium has important applications as a nuclear and structural material by virtue of its low neutron-absorption cross-section and high strength/weight ratio, but its present use is limited by its inherent brittleness The deformation characteristics of beryllium have been studied at Fulmer, and the present work is concerned with the effect of minute amounts of impurities in beryllium It is thought that some impurities form precipitation-hardening systems with beryllium and the mechanism of precipitation in beryllium having an artificially high iron content is being studied by X-ray diffraction A specially designed X-ray diffraction camera for studying the structure of liquid metals was demonstrated Recent work has shown that the structure of liquid tin can be regarded as consisting of tetrahedra of grey tin in a random matrix Work on alloy systems has shown that liquids of certain alloys, for example, gold–tin, copper–tin and gold–gallium, exhibit a high degree of order, and X-ray diffraction work on these systems is being supplemented by density studies

Chromium is attracting considerable interest as a high-temperature material, but suffers from brittleness at room temperature This brittleness is thought to be associated with the presence of impurities and the Institute's fluoride process for electrodepositing purity chromium (10 p p m nitrogen, 200 p p m oxygen and 80 p p m hydrogen) is an important contribution in this sphere Exhibits included a 4-in diameter chromium ingot prepared at Fulmer by melting electro-deposited flakes in a consumable arc furnace, and extruded chromium bars

Use of molybdenum as a high-temperature material is restricted because of the ease with which it oxidizes and the volatility of its oxide At Fulmer, molybdenum is being coated with boron and silicon in an attempt to overcome this problem The sample to be coated is placed in a graphite reaction vessel surrounded with small pieces of boron (or silicon) The vessel is heated to an appropriate temperature and a slow stream of boron (or silicon) halide is passed through the system A lower halide is formed and reacts with the surface of the sample to form a boron or silicon coating Work is also in progress on the coating of another high-temperature material, graphite, with tungsten The graphite is heated in a stream of hydrogen into which tungsten hexafluoride is injected and a tungsten coating is obtained

The results of work on the causes of 'feathering'—an extrusion defect—in lead–antimony cable sheathing were shown The defect was found to be caused by melting of segregates of low melting-point during extrusion and was overcome in the laboratory by use of a central chill during extrusion

Work at the Thornton Research Centre of Shell Research, Ltd , and at Fulmer has shown that the resistance of pearlitic cast irons of the type used for cylinder liners of marine Diesel engines to corrosion by condensed sulphuric acid is related to the silicon content This work has aroused considerable interest since corrosion of this type is thought to be a major factor in the wear of engines Recent work at the Institute has been concerned with the effect of copper and nickel additions on the corrosion resistance of these irons The Corrosion Laboratory exhibits included accounts of work on corrosion fatigue, stress corrosion and the effect of anodizing variables on the corrosion resistance of anodized aluminium The corrosion-fatigue properties of high-strength aluminium–zinc–magnesium alloys are being studied with electrochemical polarization of the specimen It has been shown that increased fatigue lives can be produced by electrochemical polarization and various stages in the potential/time curves can be related to different cracking mechanisms The work is valuable in contributing to an understanding of the influence of sacrificial anodic coatings in corrosion fatigue A 'Slipping-clutch' axial-load fatigue machine of the National Physical Laboratory type, designed and built in the Institute's laboratories, was an item of special interest among exhibits of apparatus, and arrangements have been made to build these relatively inexpensive machines under licence for sale to outside laboratories

The discovery several years ago of superconducting alloys which exhibit superconductivity at very much higher critical fields than had previously been achieved was an event of considerable importance These alloys are now being considered for such applications as power transmission and for achieving high magnetic fields for nuclear fusion experiments The staff of the Process Metallurgy Section have successfully fabricated the superconducting alloys Nb_3Sn, V_3Ga and Nb/Zr, which tend to be brittle and difficult to handle, into a variety of forms required for electrical measurements

The Physical Chemistry Section specializes in the determination of thermodynamic data of high accuracy, exhibits included accounts of calorimetric measurements of heats of formation of inorganic fluorides, chlorides, bromides, nitrides uranium alloys and various binary metal oxide systems Boron trifluoride is of interest as a rocket propellent because of its high heat of formation Boron monofluoride can also be formed in reactions involving boron and fluorine, and the stability of this radical is being studied At high temperatures, radiation from gases plays an important part in heat transfer and emissivity data are therefore important for the design of equipment An apparatus designed and constructed at the Institute for measuring emissivities of gases at temperatures up to 1,000° C was demonstrated in the Physical Chemistry Section

The wear resistance of thin metal films for use in potentiometers is being studied at Fulmer The films are produced by evaporating nichrome alloy on to a glass substrate Potentiometer conditions are simulated by mounting the film on a turntable and making contact with a sliding probe Results of the present work show that the mechanical wear depends on the current in the sliding contact and the design of the probe and may be reduced by limiting the maximum voltage at the contact

The Institute has an Associated Electrical Industries *EM*3*A* electron microscope and an *EM*6 instrument is on order Various types of replica techniques were demonstrated in the electron microscope section and emphasis was laid on the use of reflexion techniques for examining solids Recent uses of the microscope include a study of the build-up of scale in oil-fired boilers Electron micrographs showed how fine oxide needles are first formed on metallic surfaces These afterwards grow and entrap ash particles forming a basis for the build-up of scale Electron micrographs were also shown of razor-blade edges, evaporated metal films and metal fracture surfaces, and there were examples of the use of the electron microscope in the study of precipitation in thin films

Recent research on the mechanism of age-hardening in aluminium alloys, a field in which the Institute's work is widely known, has been concerned with the effects of quenching and of heating to ageing temperature on the ageing characteristics of high-purity aluminium–copper alloys containing small ternary additions such as indium and magnesium A study of the influence of small ternary additions in magnesium–lead, aluminium–magnesium and various copper-base alloy systems has indicated that their effects can be rationalized in terms of atomic size factor, particularly where they modify the distribution of precipitate rather than its structure, and where they influence grain boundary phenomena and over-ageing

Creep fracture is often associated with the linkage of grain-boundary cavities formed during creep Work on the influence of small binary additions on cavity formation was exhibited Addition of titanium to chromium results in a finer distribution of cavities than in unalloyed chromium, and vanadium additions appear to prevent cavitation Creep cavities are frequently nucleated at non-metallic inclusions and the effect of small solute additions on formation of cavities in copper is being studied using high purity copper free from non-metallic inclusions

In recent work on the deformation characteristics of copper–zinc alloys, yield stresses of alloys with varying zinc contents and grain sizes have been measured over a range of temperatures, the structures of slip lines in alloys of varying zinc content have been studied by electron microscopy The results indicate that the changes in slip structure and the solid-solution hardening which occur on addition of zinc to copper can be attributed to segregation of zinc atoms to dislocations J COLE

PHYSICS RESEARCH BY THE AUSTRALIAN COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

THE annual reports of the Divisions of Metrology, Physics and Tribophysics of the Commonwealth of Australian Scientific and Industrial Research Organization covering the year 1960–61 are issued separately*. The Division of Metrology reports a busy year in all sections, but restriction of activities because of difficulties encountered in staff recruitment and of available workshop facilities. The interferometry section concentrated its efforts on work associated with the optical definition of the metre, and precise studies have been made of the spectral quality of various radiations under consideration as possible primary optical standards of length, the measurement of wave-lengths and wave-length shifts, and the transfer from wave-length standard to mechanical lengths standards. A report on this work was made at the Eleventh General Conference of Weights and Measures in October 1960. The Division's mechanical length standards are now established in terms of the new fundamental unit of the wave-length of krypton-86 orange light adopted by the Conference. In the Electronics Section the application of a moiré fringe system to replace the mechanically contracting style and scale in the equipment for the measurement of periodic errors in machines has been considered and some experimental work has been carried out on electronic circuits to simulate sensory processes. No progress has been made in the applied mechanics section with the provision for a primary standard for force, but new proving equipment has been obtained to augment the Section's range of such devices as proving rings. The theoretical and experimental study of vibration isolation has continued, and much activity is reported in engineering design analysis, including the preparation of instruction manuals and the organization of lectures for design staffs.

In addition to its responsibilities for the maintenance and development of standards of physical measurement in the fields of heat, light, viscometry and hygrometry, the Division of Physics has made important advances in three major research activities. Using techniques developed in the Division of Electrotechnology for the inter-comparison of capacitors, investigations of thermal expansion of various solids have been made down to liquid helium temperatures, enabling the electronic contribution to be measured for the first time. A simple method of zero-field paramagnetic resonance spectrometry has been developed and applied to sapphire and a number of paramagnetic salts, and the $\frac{1}{8}$ Å birefringence filter has been used to study the vertical velocity distribution over extended regions of the solar chromosphere. Developments of new instruments have included a sensitive triode flame ionization detector for use in gas chromatography, a Fabry–Perot interferometer with plates which are servo-controlled for parallelism, and a stellar seeing-monitor.

The Division of Tribophysics had its origin as a group studying war-time problems of friction and lubrication, but it has extended its investigations to include fundamental studies in the physics and chemistry of surfaces of solids and in metal physics. In most of the experiments large single crystals are used, and since clean metal surfaces are highly reactive two ultra-high vacuum systems (to pressures down to 10^{-10} torr) have been constructed in association with equipment for following surface reactions by means of a mass spectrometer. The 'atomically flat' surface obtained when a crystal is cut by a plane consists of flat areas separated by monatomic steps. The distribution of such steps and of kinks on the edges of the steps for any crystal cut by a plane in an arbitrary direction has been calculated and models constructed of a surface. Two effects caused by bombarding metal crystals with inert gas ions are being studied. Work has continued on the determination of arrays of dislocations in metals and on the electrical resistivity of dislocations. The results of measurements of stored energy and electrical resistivity have been used to calculate the resistivity of a dislocation in aluminium, copper, nickel and alpha brass. The resistivity of a dislocation was found to increase as the stacking fault energy decreased, but the magnitude of the increase did not suggest that stacking faults make a major contribution to resistivity.

All three reports include lists of staff and of their published papers.

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* Commonwealth of Australia. Commonwealth Scientific and Industrial Research Organization. Division of Metrology Annual Report for year ending June 30, 1961. Pp. i+146. Division of Tribophysics Annual Report for year ending June 30, 1961. Pp. i+15. Division of Physics Annual Report for year ending June 30, 1961. Pp. iv+25. (Melbourne, Sydney, Canberra, Adelaide, Camden: C.S.I.R.O., 1961.)

THE NATIONAL INSTITUTE OF OCEANOGRAPHY, WORMLEY

THE annual report of the National Oceanographic Council* describes the work carried out by the National Institute of Oceanography during the year which ended on March 31, 1961. With improved recruitment of staff, the number in the scientific and experimental officer grades reached nearly 50, with about 60 supporting staff. The Institute's research vessel, R.R.S. *Discovery II*, remained in full commission throughout the year, working on a wide variety of projects, some cruises being joint ventures with other bodies, including the Universities of Birmingham, Bristol and Cambridge. *Discovery II* is rapidly approaching the end of her working life, and during the year under review much time was devoted to the design of a replacement. Since March 1961, the construction of the new ship has, in fact, been put in hand, and it is expected that she will be completed this autumn.

The research activities of the National Institute of Oceanography are aimed primarily at a better understanding of the physical and biological processes taking place in the sea. It is possible, in a review, to mention only a few highlights of the work being carried out. In pursuit of basic research on the generation of waves, a wave-recording buoy for use in the open ocean has been developed. It enables observations to be obtained by which the properties of the waves can be related to the structure of the wind flow over them. This work has a direct bearing on new theories of wave generation which have been put forward recently. Internal waves, which reach their greatest amplitude at an interface, or a region of steep density gradient some distance below the sea surface, are the subject of a new theoretical and observational study. Their presence becomes apparent in the vertical oscillations of isotherms, for example, or in movements of a sound-scattering layer.

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* Annual Report of the National Oceanographic Council, 1 April, 1960–31 March, 1961. Pp. v+41+6 plates. (Cambridge: At the University Press, 1962.) 5s. net.

Oceanic circulation continued to be one of the main fields of activity for the Institute, and a joint project was undertaken with the Woods Hole Oceanographic Institution, on deep-water movements in the Atlantic, near Bermuda. From observations extending over a number of months, it was found that the currents at 2,000 m. and 4,000 m. were both stronger and more variable than had been anticipated. The cause of such transient currents, with a time-scale of several weeks, has not yet been established, although possible theories are being considered. A variety of methods, including echo-sounding, horizontal echo-ranging and photography have been used in studies of the sea floor, in both shallow water and deep oceanic areas. In collaboration with the Department of Geodesy and Geophysics, University of Cambridge, the deeper structure of the ocean floor in the region of the mid-Atlantic ridge was investigated by magnetic and gravimetric surveys.

The increasing use of measurements of electrical conductivity as a means of inferring the distribution of density and salinity in the ocean has led to an international investigation being initiated of the relationship between these three properties. The Institute is taking part in this work, which also involves an examination of the relative proportions in which the major chemical constituents occur in sea-water from various regions.

On the biological side, the productivity of oceanic waters has continued to be one of the main fields of interest, and various instruments and techniques have been developed for studying light as a factor in production. One of the findings in the work on the distribution of organisms is that while some species appear closely dependent on the physical conditions in the water masses and on their movements, others show a marked independence of their physical environment. Research has continued on the life-cycles and population dynamics of whales and the application of the knowledge obtained to conservation problems.

The Institute continued to be directed by Dr. G. E. R. Deacon with Dr. N. A. Mackintosh as deputy director, in charge of the biological activities. Dr. J. N. Carruthers, well known for his work in physical oceanography, particularly on currents, retired from the post of assistant director on reaching the age limit, but continued to serve the Institute in a disestablished position.

Plans for the future growth of the Institute have been put forward in accordance with the recommendations of a conference held under the auspices of the Royal Society in November 1960. The National Oceanographic Council considers that an increase in real effort of 50 per cent during the quinquennium 1962–67 should be aimed at, involving the recruitment of 18 scientific staff and 40 in other grades. This would depend on the income being made available to meet a corresponding increase in the expenditure on the Institute and its research ship, which in the year 1960–61 amounted to £283,000. K. F. BOWDEN

A BIOLOGICAL PRINCIPLE AND ITS CONVERSE: SOME IMPLICATIONS FOR CARCINOGENESIS

By P. R. J. BURCH*

Health Physics Division, Oak Ridge National Laboratory†, Oak Ridge, Tennessee

SOMATIC mutations of one kind or another have often been suspected of contributing to carcinogenesis, and some recent evidence favours this view[1,2]. A variety of multi-stage mathematical models has been built on this basic supposition to account for the dependence on age of cancer mortality-rates[3].

Epidemiological evidence points to a marked inherited component in the ætiology of many malignancies, including leukæmia[4-6].

If the 'inherited component' can be equated with a (nuclear) gene mutation—and the commonest suggestion from the literature is of dominant inheritance with low penetrance—then if we assume a continuity of genetic information from the zygote to any nucleated somatic cell in the mature organism, it is difficult to avoid the following generalization and its converse.

(1) Where the ætiology of a given neoplasm involves somatic nuclear gene mutations, individual tumours of this kind will be found in which inherited mutations have substituted for somatic.

The converse principle should be valid.

(2) Where Mendelian inheritance of a neoplasm can be demonstrated, somatic mutation should also contribute to its genesis.

Exceptions to (1) can be imagined. Thus a particular allele present in a small proportion of cells may be tolerated in one type of tissue (to contribute to its ultimate malignant transformation), but distributed widely in another organ the same allele may have immediate lethal consequences. Inheritance of this specific mutation will therefore yield a non-viable karyotype, but arising *de novo* in an appropriate somatic cell, it could be carcinogenic. It is also conceivable that acquired immune tolerance[7] could develop to an inherited allele or alleles, whereas this would not be possible if the mutation occurred in a somatic cell of the immunologically competent individual

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* On one year leave of absence from the Medical Research Council Environmental Radiation Research Unit, Department of Medical Physics, University of Leeds, until August 1962.

† Operated by Union Carbide Nuclear Company for the U.S. Atomic Energy Commission.

Although (1) and (2), exceptions apart, are almost self-evident, they do not appear to have been previously enunciated or incorporated in theoretical arguments, nevertheless, their implications could be profoundly important

An analysis of the ætiology of human leukæmias has been attempted using (i) the above principles, (ii) a multi-stage mathematical model which takes account of growth and permits the derivation of a somatic mutation frequency, (iii) a wide range of epidemiological and cytogenetic evidence The following conclusions have been drawn, the detailed derivation will be given elsewhere

(*a*) Leukæmias in childhood and adolescence generally arise from one or more cells containing one inherited[6] and two somatic mutations, an additional, primarily non-genetic, factor is required for the malignant transformation of such cells This can be either the 'stress' produced by the stimulated proliferation of leucocytes by normal growth and certain pyogenic infections, especially pneumonia[6,8] or infection by a leukæmogenic virus More complicated inheritance occasionally occurs A similar ætiology probably applies to many other malignancies of childhood and adolescence

The theory accounts for (i) the frequency of childhood leukæmia in multiple sibs[6], (ii) the 'anomalous' frequency of childhood leukæmia in uniovular twins[9], (iii) the ratio of childhood leukæmia incidence in mongols (trisomic or effectively trisomic for chromosome 21), to that in non-mongols[6,10], (iv) the ratio of leukæmia incidence in Videbaek's[5] 'leukæmic' and control families, (v) the reported increased incidence of childhood leukæmias and malignancies[6,11] (in genetic carriers) consequent on small doses (~ 1–10 rem) of pre-natal X-rays

(*b*) Chronic lymphatic leukæmia in adults mostly arises from one or more cells containing one inherited and three somatic mutations A generally small proportion (increasing with age) is entirely post-zygotic[6] in origin—arising from one or more cells with four somatic mutations, this is probably an ætiological feature of most spontaneous malignancies in adults Hyperplasia (meaning an excessive but non-malignant proliferation) of pre-leukæmic cells occurs, and this phenomenon is probably exhibited in the ætiology of all cancers where, in adult life, the age-specific incidence, properly corrected for cohort effects and latent period, is proportional to a power of age higher than 2–3 The hyperplasia could be, but in general is probably not, produced through viral infection Excepting certain malignancies in childhood and adolescence, a multi-focal origin for spontaneous tumours should generally be restricted to those that are preceded by a hyperplastic condition

(*c*) Chronic myeloid leukæmia in adults generally develops from a cell containing one inherited and two somatic mutations, together with the Ph^1 chromosome[1], which is regarded as being equivalent to a gene deletion A mutant gene can sometimes substitute for the Ph^1 chromosome

(*d*) In adults, acute leukæmias of all histological types, and lymphosarcomas, generally develop from a cell containing one inherited and three somatic mutant genes There is little or no hyperplasia of pre-leukæmic cells

(*e*) For all the above ætiologies, the calculated somatic and germ-cell mutation frequencies are closely similar, from about $(1–5) \times 10^{-6}$ yr^{-1}, and comparable to the somatic mutation frequency in sheep[12] and to the known spontaneous germ-cell mutation frequencies at various loci in mice[13] and in man[14] These conclusions are based on the assumption (following the bacterial investigations and conclusions of Kubitschek[15]) that latent 'point' mutations arise in a formed gene (rather than at replication) and that their expression can in certain circumstances be independent of mitotic rate With this point of view, it is convenient to express mutation-rates in terms of the following unit specific mutations (or alleles), per specific gene, per cell at risk, per annum

From the work, mainly of Videbaek[5] and Stewart[6], it is suspected that the same alleles that predispose to acute lymphatic and some stem-cell leukæmias also predispose to lymphosarcomas[6], certain pernicious anæmias, and certain cancers[5], furthermore, a different set of common alleles may be involved in chronic lymphatic, chronic and acute myeloid, and some stem-cell leukæmias, together with certain pernicious anæmias and some cancers[5] For each set of alleles, it is estimated that the proportion at birth of genetic carriers in the population of England and Wales is about 1 per cent Given the predisposing dominant inheritance, the penetrance in the form of a specific leukæmia is typically of the order of 10 per cent for a normal life-span of 70 years The penetrance for acute lymphatic and stem-cell leukæmias during childhood (0–9 years) is about 2–3 per cent

(*f*) Viruses of the appropriate specificity should theoretically be able to induce or assist in the induction of malignancies by at least five distinctive mechanisms, however, their ætiological role, when it occurs in human malignancies is complementary, or additional to gene mutations

(*g*) There is usually a long and variable interval between the emergence of a cell (the 'carcinogenic nucleotype') the nuclear constitution of which confers malignant potentiality, and its conversion into an actively malignant cell For radiation-induced leukæmias at Hiroshima, for example, the median latent period was about seven years[16,17] Where the malignant conversion of the carcinogenic cell is not effected by viral or hormonal agents, it is most probably produced through an immune reaction[17,18]

(*h*) At Hiroshima, radiogenic non-leukæmic malignancies[19] were almost certainly induced by a mechanism in which ionizing radiation simulated the spontaneous mutation process Spontaneous somatic mutation frequencies relevant to leukæmic and non-leukæmic malignancies appear to be similar Radiogenic leukæmias at Hiroshima and Nagasaki were probably induced through a basically similar ætiology, although a very high value for α the radiation mutation coefficient is deduced for large (~ 600 rems) very acute doses This value is about 2×10^{-6} mutations per specific gene per cell at risk per rem—in contrast to the more normal value of $1\ 5 \times 10^{-7}$ rem^{-1} obtained for the numerically important non-leukæmic malignancies However, in the fractionated X-irradiation of ankylosing spondylitis patients, at much lower dose-rates, the average value of α applicable to chronic myeloid and acute leukæmias is about 8×10^{-7} rem^{-1} These conclusions suggest that the 'leukæmic genes' exhibit the same type of mutational dependence on dose-rate and absolute (acute) dose as the germ-cell genes in pre-meiotic cells of mice[13]

There is a hint of a similar dependence in the case of non-leukæmic malignancies, although the statistics are poor It is probable that chromosomal aberrations (for example, the Ph^1 chromosome) are associated with some radiogenic leukæmias, and this may help to account for the high value of α If the value of α relevant to chronic lymphatic leukæmia is similar to that for non-leukæmic malignancies, this will explain why this type of leukæmia is rarely encountered[20] in connexion with radiation exposures In human radiation carcinogenesis, it is necessary to distinguish between the short- and long-term responses in genetic carriers and in others In the 'short-term response' the nuclear carcinogenic changes are completed at the time of irradiation, in the 'long-term response' the ultimate nuclear change is made afterwards through spontaneous mutation With the exception of the highest age-range ($>$ 60 years), it is concluded that most of the victims of radiogenic malignancies at Hiroshima up to 1958 were carriers, through inheritance, of a predisposing mutation

Predicted dose-response relationships (in good agreement with those observed) are complex, dose-rate dependent, and strongly age dependent, but they tend to linearity at low dose If, in chronic lymphatic leukæmias and in non-leukæmic malignancies, the 'carcinogenic genes' mutate at low dose-rates in a way similar to the recessive genes of pre-meiotic mouse germ cells[13], it is estimated that background radiation of 0 1 rem yr^{-1} should be responsible for $\sim$ 0 2 per cent of the spontaneous incidence of these malignancies If, however, the mutational process is independent of dose-rate, an upper limit of $\sim$ 1 per cent is obtained With the more radiosensitive chronic myeloid and acute leukæmias, there is good evidence of a dose-rate dependence, and background radiation may be responsible for $\sim$ 3 per cent of the spontaneous incidence

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COMPLEX OF RIBONUCLEASE WITH A SUBSTRATE ANALOGUE, CYTIDINE 2'-PHOSPHATE

By DR. E A BARNARD and DR A RAMEL

Zoology Department, King's College, London, W C 2, and Physical Chemical Institute, University of Basle

THE direct examination of the complex of an enzyme and its substrate (true or virtual) is of interest in investigations of mechanisms of action Methods that have proved of value in particular cases include spectroscopy of complexes having characteristic absorption[1], equilibrium dialysis[2] and centrifugal stratification[3] This latter class was introduced by Velick *et al*, in examining complexes of coenzyme I with dehydrogenases[4] they sedimented the protein components in a preparative ultracentrifuge tube and analysed layers of the supernatants to deduce the binding We have made an examination, in the analytical ultracentrifuge *in situ* by use of absorption optics, of the migrating complex of ribonuclease (RNase) with a substrate analogue

Cytidine 2'-phosphate (2'-Cp) structurally closely resembles a substrate (cytidine 2',3'-phosphate), and is a powerful competitive inhibitor[5] Such inhibition does not necessarily imply specific binding at the active centre, but further evidence comes from the spectral change observed[6] near 260 mμ on mixing RNase and 2'-Cp This decrease in absorption is about maximal at equimolar proportions (at *p*H 5 6) (ref 6c) and may indeed indicate a unique interaction at the active centre, but to establish this the binding must be identified with the active centre region and the alternative excluded that other molecules of 2'-Cp are also bound without spectral consequences

A direct method of establishing the composition of the complex or complexes here is thus desirable The present availability of absorption optical systems (with the Spinco Analytrol or, as used here, the scanning system of Hanlon, Lamers and Schachman[7]) in the ultracentrifuge, offers the possibility of following, by boundary separations, specific enzyme-substrate type interactions, if the small component shows strong light-absorption (A use of absorption optics (Analytrol) for measuring weight average sedimentation coefficients of mixtures of methyl orange with serum albumin has been mentioned by Schachman[8]) The RNase case, as it turns out, is well adapted to such a measurement of complexes, in that the dissociation constants are such that free and bound molecules can readily be distinguished thus (although the protein absorbs in the same region) within the limited concentration range of nucleotide optically acceptable here, and without significant error from interaction boundary phenomena[9]

Fraction *A* was isolated from RNase (Armour) by chromatography[10] and exhaustive dialysis Carboxymethyl-ribonuclease (Cm-RNase) was prepared[11] at pH 5 6 with modifications[12b] in technique, and was homogeneous after chromatography[10] on '*IRC*-50' 2′-Cp was chromatographically pure on 'Dowex-1' (ref 13) The Spinco model *E* ultracentrifuge was used with the scanning ultra-violet absorption system of Hanlon *et al* [7] and a chlorine-bromine filter isolating lines near 260 mμ Scanner deflexion from a baseline is proportional to absorbance in the range (0–1) used here and was calibrated in each run by the increments given by known amounts of nucleotide The scan of the solution at zero time was checked in each experiment to avoid anomalous variations across the cell, and readings were corrected for the small 'background'

The protein in solvent (0 70 ml) in the cell was taken to 59,780 rev/min and the absorbance scanned then and at 16-min intervals thereafter After redispersion, known amounts of 2′-Cp were added in turn in very small volumes from an Agla micrometer syringe, each mixture being sedimented and serially scanned similarly (The slight sedimentation of 2′-Cp itself produced in time a shallow curvature of its boundary Adequate correction of the supernatant concentration of 2′-Cp for this lowering at the meniscus was obtained by utilizing the observation that beyond 80 min (when, also, the protein has sedimented well away from the meniscus) the height at the meniscus was a linear function of time in both the calibration (2′-Cp alone) and experimental runs The heights and slopes of these lines (fitted by least squares) were used to predict the height at the meniscus as corrected for 2′-Cp sedimentation, at a fixed time (112 min) in each run, by comparison with a calibration run with 2′-Cp, assuming proportionality to 2′-Cp concentration within this set)

For determination of $S_{20,w}$, sufficient accuracy for present purposes was given by determining the mid-point of each boundary at 11 intervals (to 176 min) and plotting the log of the displacement of this against time Good linearity was obtained for each run ($S D \leqslant 7$ per cent) and lines fitted by least squares were used in deriving (ref 14) $S_{20,w}$ For RNase, one run where the transport treatment[14] was simultaneously applied also gave a value for $S_{20,w}$ within the same range (Table 1)

Binding of 2′-Cp to RNase At a ratio of RNase to 2′-Cp of 1 1 ($3 4 \times 10^{-5}$ *M*), almost all the optically dense material sedimented together at a rate similar to that for RNase alone (Fig 1*A*) In contrast, at 1 2 ratio (Fig 1*B*) the supernatant showed distinct 260 mμ absorbance (that is, unbound 2′-Cp) corresponding to virtually all the added second equivalent At 1 3 ratio, again no significant increase was found in the ultra-violet-absorbing material migrating with the boundary (Fig 1*C*), while the supernatant concentration was doubled (Table 1) Hence only the 1 1 complex is formed appreciably in this range, and the binding is 90 per cent complete at 1 1 ratio even at these low concentrations A dissociation constant K_1 (see Table 1) for the first 2′-Cp molecule bound can, in principle, be readily obtained from such measurements, the accuracy here is determined by the error limits in reading the traces, the instrumental reproducibility and the sharpness of boundary of the protein studied, as well as by the strength of binding Analysis in terms of multiple alternative binding sites is not required here, any second binding is so weak, relatively, that only an order of magnitude of its lower limit can be estimated ($K_2 \geqslant \sim 10^3 K_1$)

Table 1 BINDING RATIOS, DISSOCIATION CONSTANTS AND $S_{20,w}$ FOR 2′-CP COMPLEXES AT 20°, pH 5 6

Protein	2′-Cp Protein	r	K_1 range (M)	$S_{20,w}$ (mean)
RNase	0	—	—	2 05 ± 0 19 *s*
($3 4 \times 10^{-5}$ *M*)	1 1	0 90	$2 0 \times 10^{-6}$ to $1 1 \times 10^{-7}$	1 72 ± 0 08 *s* (*P* > 0 05)
	2 1	0 99	$2 3 \times 10^{-6}$ to ~ 0	
	3 1	0 97	$4 5 \times 10^{-6}$ to $6 9 \times 10^{-7}$	
			Mean $K_1 \simeq 1 \times 10^{-6}$ (all readings, RNase)	
Cm-RNase	0	—	—	
($3 4 \times 10^{-5}$ *M*)	1 1	~0*		1 59 ± 0 09 *s*
	2 1	0 05†	$\geqslant \sim 10^{-3}$	

r, Moles 2′-Cp bound per mole protein *P*, Probability of difference (by *F* test) from $S_{20,w}$ value of RNase alone Ranges of K_1 values obtained from the limits of estimated uncertainty in the readings RNase-2′-Cp values are from means of 2 complete sets

* Exact value uncertain, due to change of background between runs, but r is small since certainly a very large part at least of the 2′-Cp did not migrate with the protein

† Precision rather low, due to the small difference involved

Binding of 2′-Cp to Cm-RNase Cm-RNase migrated very similarly to RNase, but with $S_{20,w}$ apparently rather lower (Table 1) At 1 1 ratio (Cm-RNase to 2′-Cp) virtually all the 2′-Cp remained in the supernatant, the behaviour being as in that illustrated by Fig 1*B*, and the same was true at 1 2 ratio (Table 1) Hence no binding of 2′-Cp was detectable and if any occurs *K* must be very much larger (estimated $K \geqslant \sim 10^{-3}$ *M*) than for RNase

Difference spectroscopy[6c] (using Cary model 11 and Beckman *DU* spectrophotometers) confirmed the difference spectrum[6c] of RNase mixed with 2′-Cp, relative to the sum of the components, and showed that RNase (0 08 m*M*) mixed with 4,5-dihydrouridine 2′,3′-phosphate[15] (2 0 m*M*, pH 5 8, 0 1 *M* sodium acetate, 25°) gave no such difference spectrum, in the range 250–290 mμ, neither initially nor after the hydrolysis Guanidinated RNase[16] (> 97 per cent inactivated, 99 per cent lysine guanidinated) gave the same difference spectrum (at 0 08 m*M*, pH 5 6 or 6 0) with 2′-Cp (0 09 m*M*) as RNase, and the same loss of it at pH 8 0 Fuller details will be reported elsewhere Similar measurements on our sample of Cm-RNase with 2′-Cp (each about 0 07 m*M*, pH 5 5, $I = 0 1$) have been made by Dr M Irie, who finds the maximal spectral decrease near 260 mμ is less than about 20 per cent of that given by RNase with 2′-Cp (Irie, personal communication)

Discussion It is clear that the boundary observed (Fig 1, *A–C*) represents, virtually entirely, a molecular species, the complex of RNase with the substrate analogue, containing one molecule of each K_1 is about 10^{-6} *M* and K_2 is negligible compared with this

In this complex sedimentation method, equilibrium might be disturbed by separation of the species[9,14], if the complex has an appreciably different *S* to

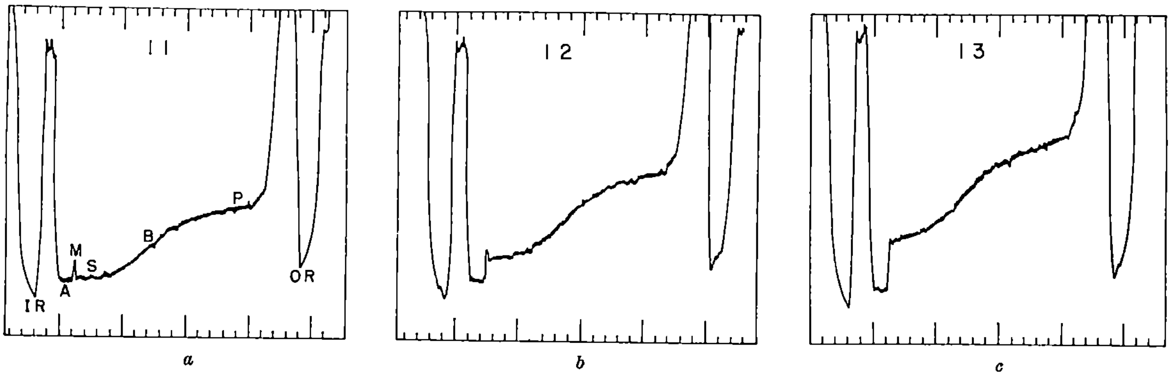

Fig 1 Scanner tracings (all at 112 min, speed 59,780 rev/min) Absorbance (heavy line) is plotted linearly (increasing upwards) by the scanner Distance from axis of rotation increases to the right (*a*) Sedimentation of RNase with 2′-Cp (one equivalent) *M*, meniscus, the short line (*A*) to the left marks absorbance level in air space above it *I R* , *O R* , inner and outer reference holes *B* is the boundary (broad, for RNase) of the sedimenting protein, plotted directly. *P*, plateau region Nearly all the absorbance, initially dispersed across the cell, is now accounted for behind the boundary, the trace is very similar to that for RNase alone, but with *B* and *P* higher here *S*, supernatant region, where residual absorbance (free 2′-Cp) is measured All readings are taken from a baseline (not shown) level, slightly below the level of *A* (*b*) With two equivalents of 2′-Cp About one equivalent is now sedimenting with *B*, as shown by absorbance in *S* region (*c*) With 3 equivalents About two equivalents are now accounted for by *S* height For Cm-RNase with 2′-Cp, at 1 1 the corresponding trace is very similar to that in (*b*), and at 1 2 to that in (*c*), but with *B* and *P* lower In all cases, protein is 3 4 × 10^{-5} *M*, solvent is 0 057 *M* sodium acetate (a compromise concentration, for stability in sedimentation without affecting binding), *p*H 5 60, temperature maintained at 20 0 ± 0 1°

that of the protein In the present case, the direct measurement of the free 2′-Cp and the simple stoicheiometry involved assure that the amount of free protein in a composite boundary must be small. The difference in *S* would need to be very appreciable for the conclusions to be erroneous here, especially at these concentrations well above K_1 $S_{20,w}$ for RNase and the complex (Table 1) showed no significant difference (at the 5 per cent level), if the small apparent difference was real, *S* decreases on complex formation and this would slightly inflate the 2′-Cp level in the supernatant[9,14], merely reinforcing the conclusion drawn and making K_1 slightly smaller

Technical refinements would be possible, for example, following the protein and the nucleotide by differential absorptions, comparison of *S* values more precisely (it would be of interest to know if the apparent slight decrease in *S* of the complex could be real, due to some configurational change), and more detailed investigation of *K*, but these results are adequate for the present purpose and establish a single strong binding as the basis of the spectral change, which may now be utilized, conveniently, for further study of the interactions (Confidence in the reliability of the measurements (which required only 330 μg protein per experiment) is reinforced by the reasonable agreement of the $S_{20,w}$ value with those found conventionally for RNase, extrapolated to similar protein concentration (close to 1 90), and by the contrasting distributions observed under identical conditions with RNase and with Cm-RNase, differing only at one residue and in activity) The usual Analytrol system can also be used similarly The value of K_1 agrees with that found from enzymic inhibition by 2′-Cp (ref 17) (for example, 4 5 × 10^{-6} *M*, at *p*H 5 6, $I = 0\ 1$ (ref 17*a*)) and by protection from urea unfolding[12], and is consistent with the spectral data at *p*H 5 6 (ref 6*c*)

Both observations made on Cm-RNase appear to disagree with that of Mathias *et al* [6b,18a], who reported unaltered interaction[18b], but support that of Hummel *et al* [6c], each based on the spectral criterion The derivative used here was homogeneous, inactive and contained one carboxymethyl group per molecule (by labelling with carbon-14) (A much weaker binding, only, might remain and might not be apparent in the sedimentation run, this must be investigated further)

Evidence available on the complex of 2′-Cp with RNase can be summarized (1) Only one molecule of 2′-Cp is bound with high affinity, as shown by complex sedimentation, by extent of spectral change[6c] or inhibition of urea denaturation[12], or by a recent, concurrent equilibrium dialysis measurement[17a,19] (2) This binding must be in the active centre region, from its loss in Cm-RNase (where, also, orthophosphate affinity is much reduced[12]) and from the strong competitive inhibition (3) The *p*H-dependence of binding[6c] is similar to that of substrate binding[20] The decrease in binding from *p*H 5·6 to 8 cannot be due to lysine groups, since this is also shown after guanidination At least one histidine appears to be involved, and the Cm-RNase evidence presented suggests histidine residue 119 (ref 11) is part of the binding site Unfolding evidence shows the 2′-Cp site is composite and across more than one chain[12] (4) Conversely, Cp binding prevents the attack of bromoacetate at histidine 119 (ref 11) (5) A specific absorption decrease near 260 mμ accompanies the binding of Cp or Up (ref 6c), but is absent in the hydrogenated Up compounds (which also bind well[20]), presumably it arises from an interaction of the ring with the enzyme In the guanidinated protein it is seen, significantly, that this binding can be separated from catalytic activity

Further results on and a molecular interpretation of the binding of inhibitors and of substrates to RNase will be presented elsewhere

Much of this work was performed at the Virus Laboratory, University of California, Berkeley, during the tenure of (E A B) a fellowship of the Rockefeller Foundation, 1960–61, and of (A R) a U S Public Health Service fellowship We thank Dr H. K Schachman for the use of the ultracentrifuge and scanner, Dr H Fraenkel-Conrat for the provision of facilities (to E A B), Drs M Irie and C A Dekker for providing the results of spectral measurements made on our sample of Cm-RNase, Dr J P Hummel for the communication of his results prior to publication and Mr D C Teller for assistance

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QUANTITATIVE EVALUATION OF HUMAN CHORIONIC GONADOTROPHIN IN PREGNANCY URINE

By P. G. LYNCH and HERTA SCHWABACHER

Group 9 Laboratory, Peace Memorial Hospital, Watford

A QUANTITATIVE evaluation of human chorionic gonadotrophin (HCG) would be most valuable Methods have recently been described by Brody and Carlstrom[1-3], McKean[4] and Wide and Gemzell[5] In our hands the hæmagglutination test developed by the last-named authors failed to give constant results and was abandoned

It seems worth while, because of its potential clinical applications, to record our findings based on a complement fixation test for the determination of urinary HCG

Rabbits received by subcutaneous injection up to 3,300 I U of 'Pregnyl' (Organon) admixed with sterile Ramon's adjuvant[6] The injections were spaced out over a period of 16 weeks

Titration of HCG antiserum was found to be impracticable if 'Pregnyl' was used as the antigen At low concentrations, no fixation of complement was obtained and if higher concentrations were used the material was found to be anti-complementary HCG as described in the addendum or HCG obtained from the Department of Biological Standards, National Institute of Medical Research, London, proved to be satisfactorily antigenic Complement was used at 3 minimal hæmolytic dose (MHD) strength in all tests HCG used as antigen was found to give satisfactory results if used at strengths of 24, 12 and 6 I U The HCG antiserum was found by complement fixation to have a final titre of 1 in 8 using 6 I U of standard HCG as antigen in the test

For titration of urinary gonadotrophin the urine, preferably a first-morning specimen, is concentrated according to Scott's technique[7] and the concentrate accurately neutralized before being used as antigen The urine extract so prepared is stored at ± 2° C before testing and there seems to be no appreciable loss in HCG activity in extracts kept at this temperature for as long as seven days

Throughout the test 0 2 ml volumes of each reagent are used The urine extract is put up in a series of doubling dilutions from neat to 1 in 64 The normal controls are set up and the urine extract is tested for anticomplementary activity at strengths of neat, 1 in 2 and 1 in 4 In our experience, so far, any anti-complementary effects were diluted out at urine extract dilutions of 1 in 2 so that it is permissible to argue that any fixation of complement in the test series beyond this point is due to the presence of HCG At the end of each complement fixation test all the tubes are centrifuged and the end-point is taken as the final tube showing a button of erythrocytes

The complement fixation test already described here is being applied to rhesus-negative mothers in the third trimester of pregnancy, when it is hoped to confirm Scott's findings[8] that the larger than normal placenta of severely affected rhesus infants secretes an excessively high level of HCG Thus a high titre of HCG obtained in the complement fixation test and reported to the obstetrician at 32 weeks might well aid him in the decision to induce labour at the earliest possible moment

A full report on HCG titres found in the urines of rhesus-negative mothers with antibodies will be presented at a later date

It has proved impracticable to use the complement fixation test described here on pregnancy serum There are several possible explanations why this is so (1) HCG may be altered or destroyed by heating the serum at 56° C to inactivate complement, (2) the amount of circulating HCG may be too small to detect by ordinary means and is only concentrated by excretion in the urine, (3) HCG may be altered or destroyed by serum enzymes if there is a delay in separating off the serum

The HCG antiserum already described was subjected to immunodiffusion on cellulose acetate strips and by the plate method of Elek[9] and Ouchterlony[10] Precipitation lines were not obtained by either of these methods Immunoelectrophoretic investigations are being made

It is interesting to compare the results of the complement fixation test with biological pregnancy tests In a few instances (Table 1) it has been possible to compare results with the Hogben test performed on suitable urine dilutions The compari-

Table 1 COMPARISON OF COMPLEMENT FIXATION TITRES (CFT) WITH HOGBEN TITRES IN NORMAL RHESUS POSITIVE PREGNANCY URINES

Urine Ref No	Duration of normal pregnancy in weeks	HCG I U per ml extract CFT	HCG I U per ml extract Hogben*
1659	32	240	280
930	14	480	250–560
1412	4	30	28
1220	48	120	112
1952	N I	60	28
1974	N I	30	28
1978	N I	60	28
926	N I	30	28

N I , no information to hand as yet

* Minimal reacting dose = 28 I U per ml equivalent to 70 I U HCG per 2 5 ml for a 70-gm *Xenopus laevis*

son is somewhat crude and no correlation of figures has been attempted owing to the difficulties of performing quantitative bio-assays on large groups of animals

Conclusions Provided two *Xenopus*, known to be fully sensitive, receive 2 5 ml of each dilution of extract, the Hogben biological reaction compares well with the CFT

Addendum We thank Dr B M Hobson of the Usher Institute, Edinburgh, for the method of preparation of human chorionic gonadotrophin described as follows

Urine is obtained from the ante-natal clinic and pooled to give a final volume of 10 litres or more The urine is then filtered through a Buchner funnel fitted with two or three thicknesses of Whatman No 40 filter paper, which has been wetted by first dipping it in the urine Filtration is under suction (RB_4 via trap flask), and may have to be repeated if the first filtrate is not clear

The filtrate is measured and to each litre of filtrate is added 5 ml of glacial acetic acid while mixing thoroughly The *p*H of the filtrate is adjusted to 4 2–4 4 with hydrochloric acid or sodium hydroxide using bromophenol blue as indicator Test samples are returned to the filtrate 50 c c of a saturated solution of benzoic acid in acetone are now added to each litre of filtrate It is important at this stage to stir thoroughly so as to avoid surface precipitation The resulting mixture is allowed to stand overnight in the refrigerator

The following day, the supernatant fluid is decanted into a clean vessel The benzoic acid deposit is transferred into a Buchner funnel fitted with No 40 filter papers and the supernatant fluid is sucked through it twice, rinsing out the remnants of benzoic acid into the funnel Suction is continued until the powder is dry and cracking out, then the filter is removed and the filtrate saved for repeat extraction

The resulting powder is scraped off on to clean paper and chopped finely with a knife It is next transferred to a glass cylinder and suspended in an excess of acetone The deposit is allowed to form overnight in the refrigerator

Next day the supernatant fluid is decanted into a stock benzoic acid solution bottle The powder is transferred to a clean container and is stored in excess of acetone at 0°–4° C until required

The product may be purified by washing 4 or 5 times with acetone until no benzoic acid remains A clean glass slide dipped in the solution will detect traces of benzoic acid remaining, by forming an opaque film on the glass surfaces Washing is conveniently done by centrifugation

The purified powder should be dried in a desiccator to drive off the acetone It is then ground finely in a Griffith's tube and stored in a desiccator at room temperature until required The final preparation should be white in colour and is said to retain its potency for up to three years

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AN ELECTRON SPIN RESONANCE STUDY OF THE ALKALINE DEGRADATION OF 2-METHYL-1,4-NAPHTHOQUINONE

By DR THOMAS C. HOLLOCHER, jun.*
The Adolphus Busch III Laboratory of Molecular Biology, Washington University, St Louis
AND
DR MORTON M WEBER
Department of Microbiology, Saint Louis University School of Medicine, St Louis, Missouri

THE transport of electrons from the level of reduced diphosphopyridine nucleotide (DPNH) through the cytochrome pathway in particles of *Mycobacterium phlei* involves the participation of vitamin K_1 or a naturally occurring naphthoquinone[1] Using the electron spin resonance technique[2,3], a naphthosemiquinone has been identified as a reactant in these oxidation-reduction systems[4] With this DPNH oxidase and the soluble DPNH-2-methyl-1,4-naphthoquinone reductase systems of *M phlei*, we find that the concentrations of naphthoquinone free radicals are strongly dependent on *p*H (ref 5) In examining the causes of these *p*H effects, some of which are not related to enzyme activity, we observed that 2-methyl-1,4-naphthoquinone undergoes a rapid degradation in alkaline aqueous solutions by a reaction which leads to the appearance of stable free radicals in extremely high yields These observations and a postulated reaction mechanism are reported in this article The reaction imposes difficulties in the study of biological redox reactions of 2-methyl-1,4-naphthoquinone even at moderately high *p*H values[5]

The degradation was carried out in the following manner Highly purified 2-methyl-1,4-naphthoquinone in ethanol or as the solid is added to an aqueous or aqueous ethanol solution of 0 12 *M* potassium hydroxide in an amount sufficient to make

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* Present address Graduate Department of Biochemistry, Brandeis University, Waltham, Mass

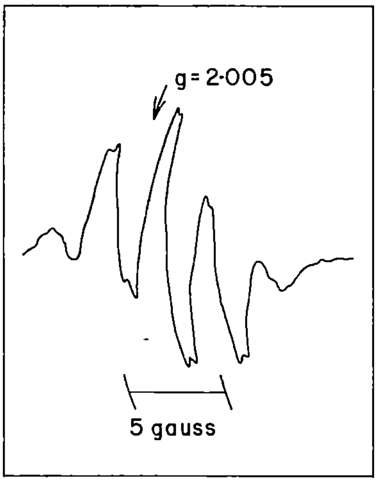

Fig. 1. Electron spin resonance spectrum obtained during the alkaline degradation of 2-methyl-1,4-naphthoquinone. 2-Methyl-1,4-naphthoquinone concentration is 2.5 m*M* at a *p*H of 12.5, modulation amplitude is 0.1 gauss, sweep time is about 1 min., and the time constant is 0.1 sec. Spectrum was taken about 3 min after mixing. The g-value mark and field scales were established using potassium peroxylamine disulphonate in sodium carbonate solution. The magnetic field increases from left to right in the figure

the final concentration of naphthoquinone from 2.5 to 10 m*M*. The cuvette containing the mixture is placed in the electron spin resonance spectrometer immediately after mixing. The first observation is made within 2 min., and observations are continued for as long as necessary. All spectra are taken at room temperature using a frequency of 9 kmc./s and a magnetic field modulation frequency of 1 mc./s.

Electron spin resonance signals, as shown in Fig. 1, appear immediately, intensify rapidly, and reach a maximum within about 10 min after mixing. Once the maximum value is reached, the signal intensity decreases very slowly under anaerobic conditions with a half-time of several hours. The addition of oxygen to the solution leads to a rapid disappearance of the signal.

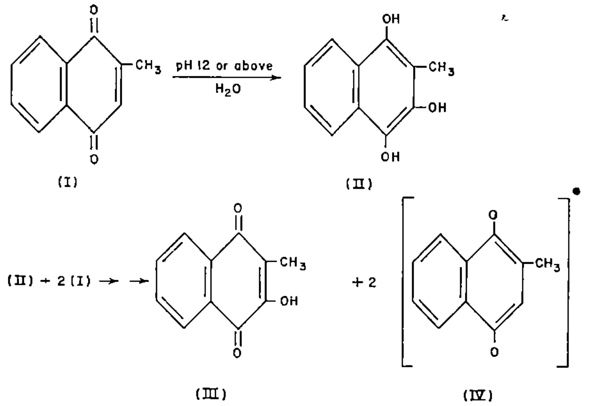

Fig. 2. Proposed mechanism for the formation of a free radical during the alkaline degradation of 2-methyl-1,4-naphthoquinone

The signal centres at $g = 2.004 \pm 0.001$ and consists of five hyperfine lines with intensity ratios of approximately 1 : 4 : 6 : 4 : 1 and with separations of 2.5 ± 0.2 gauss. Each of these five lines is further split into five lines with separations of 0.6 ± 0.1 gauss. Because of appreciable magnetic field inhomogeneity, these latter lines are not well resolved in Fig. 1. The characteristics of these electron spin resonance signals and their hyperfine splitting separations are identical with those described by Wertz and Vivo[6] and by Adams *et al.*[7] for the 2-methyl-1,4-naphthosemiquinone radical formed by chemical reduction.

Estimates of free radical concentrations were made by comparison with manganous ion standards[8]. These estimates were made with a precision of approximately 30 per cent and indicate that maximally 0.5–0.8 on a mole basis of the original 2-methyl-1,4-naphthoquinone is converted to the semiquinone radical.

These findings suggest that in the absence of an added reducing agent, 2-methyl-1,4-naphthoquinone or a derivative thereof must serve as its own one-electron reductant to provide a stable semiquinone

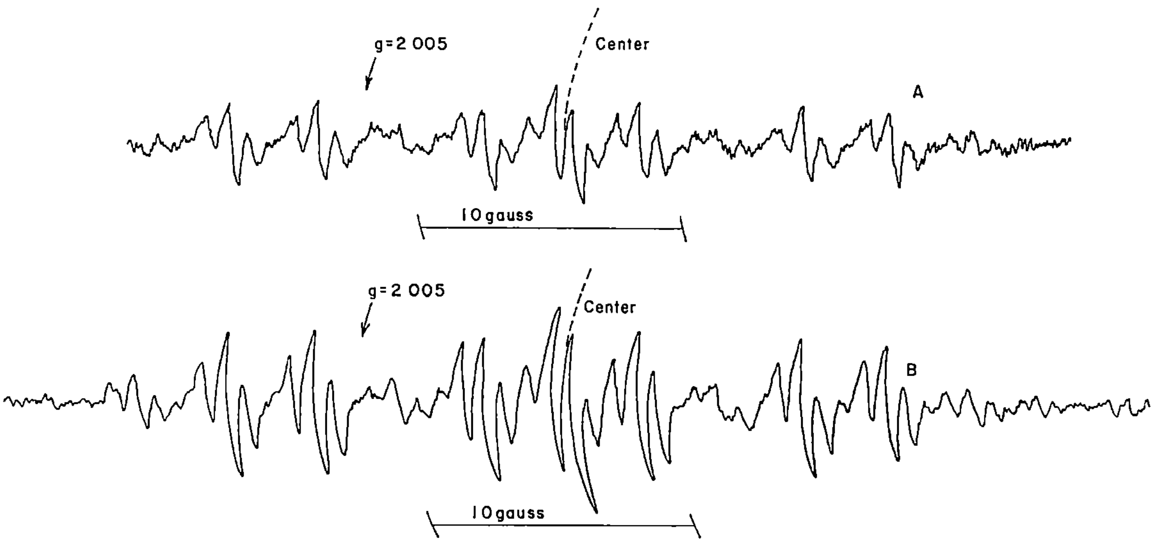

Fig. 3. *A*, high-resolution spectrum obtained with the products of the alkaline degradation of 2-methyl-1,4-naphthoquinone at *p*H 14, following reduction by $Na_2S_2O_4$ and subsequent partial reoxidation by oxygen. Original concentration of 2-methyl-1,4-naphthoquinone is 10 m*M*, modulation amplitude is 0.03 gauss, sweep time is 15 min., and the time constant is 1 sec. *B*, high-resolution spectrum obtained with 2-methyl-3-hydroxy-1,4-naphthoquinone at *p*H 14, following reduction by $Na_2S_2O_4$ and subsequent partial re-oxidation by oxygen. 2-Methyl-3-hydroxy-1,4-naphthoquinone concentration is 10 m*M*, instrument conditions are the same as in Fig. 3*A*. The g-value marks were obtained as in Fig. 1 and the field scales were established using calcium xanthone in tetrahydrofuran. The magnetic field increases from left to right in both Figs. *A* and *B*

in high yield A reasonable mechanism for the reaction is illustrated in Fig 2 2-Methyl-1,4-naphthoquinone (I) undergoes a 1,4 addition of water in alkaline solution to yield 2-methyl-3-hydroxy-1,4-naphthoquinol (II) The lower oxidation-reduction potential of (II) (ref 9) then permits the transfer of electrons to (I) with the formation of 2-methyl-3-hydroxy-1,4-naphthoquinone (III) and 2-methyl-1 4-naphthosemiquinone (IV) The semiquinone of (III) is apparently very reactive, and is not observed by the electron spin resonance technique during the reaction The radical observed (IV) decays only very slowly, presumably through redox dismutation, to yield (I) and 2-methyl-1,4-naphthoquinol or through dimerization The theoretical maximum yield of (IV) is 0 67 of (I) on a mole basis and is in agreement with the range of yields found

Oxygen rapidly oxidizes (IV) to (I) and results in the conversion of (I) to (III) in high yields That (III) is the final product of the alkaline degradation of (I) is confirmed by the identity of the high-resolution electron spin resonance spectra shown in Fig 3*A* and *B* Fig 3*A* represents the electron spin resonance spectra obtained when the product or products of the reaction, following oxygenation, are reduced with sodium hydrosulphite or sodium borohydride, in alkaline solution, and then partially reoxidized by oxygen. The results in Fig 3*B* are obtained when authentic (III) is similarly reduced and partially reoxidized These spectra are identical except with respect to amplitude and reflect differences in concentration only

In addition, the final product of the alkaline degradation of 2-methyl-1,4-naphthoquinone after extensive oxygenation is red-brown in colour in alkaline solutions, exhibits characteristic spectral changes as a function of *p*H, and is exceedingly stable in alkali These properties are consistent with our belief that the product is a hydroxylated naphthoquinone, specifically 2-methyl-3-hydroxy-1,4-naphthoquinone

This work was supported by grants (to B Commoner) from the Office of Naval Research and grant *C*–3983 from the National Cancer Institute, Public Health Service and grant *E*–3046 (to M M Weber) from the National Institutes of Health, Public Health Service We thank Dr Barry Commoner, who made this investigation possible We also thank Dr S I Weissman of the Department of Chemistry at Washington University for his co-operation and assistance in obtaining the high-resolution electron spin resonance spectra

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RELATIONSHIP BETWEEN TEMPERATURE AND PLASMA *p*H AND CARBON DIOXIDE TENSION IN THE TURTLE

By Prof EUGENE D. ROBIN

Department of Medicine, University of Pittsburgh Medical School, Pittsburgh and Mount Desert Marine Biological Laboratories, Salisbury Cove, Maine

THIS work was prompted by the following chance observation While examining acid-base metabolism in the turtle, *Pseudemys scripta elegans*, a series of measurements of plasma *p*H and carbon dioxide tension was performed on a group of turtles in Pittsburgh, Pennsylvania As shown in Table 1, control values of *p*H averaged 7 49 and of carbon dioxide tension averaged 41 mm mercury However, measurements of these parameters in another group of turtles of the same species performed in Salisbury Cove, Maine, showed an average *p*H of 7 72 and an average carbon dioxide tension of 20 mm mercury The only obvious difference between the two groups was the ambient temperature of each group Turtles in Pittsburgh were maintained indoors at a temperature between 26° and 28° C Turtles in Salisbury Cove were stored out of doors at an average temperature of 16°–18° C It seemed of interest to investigate the relationship between temperature and plasma carbon dioxide tension and *p*H

Table 1 A COMPARISON OF PLASMA *p*H AND CARBON DIOXIDE TENSION IN PITTSBURGH AND IN SALISBURY COVE IN THE TURTLE

	No of measurements	Mean plasma *p*H units	Mean plasma CO_2 tension mm Hg
Pseudemys scripta elegans (Pittsburgh)	5	7 49	41
Pseudemys scripta elegans (Salisbury Cove)	34	7 72	20

A group of 11 turtles (species, *Pseudemys scripta elegans*) weighing 0 5–1 0 kgm was studied Each turtle was exposed to temperatures of approximately 10°, 24° and 37° C The length of exposure at each temperature was approximately 24 hr The order of exposure was varied among the turtles so that all three possible orders of exposure were represented At the end of each 24-hr period, heparinized blood for analysis was obtained anaerobically by means of direct intracardiac puncture through a hole drilled in the plastron Between cardiac punctures, the hole in the plastron was filled with bone wax *p*H measurements were performed anaerobically by means of a standard glass electrode using an Instrumentation Laboratory *p*H meter These measurements were performed at each ambient temperature (presumably this represented the internal temperature of the turtle as well[1]) that the animal was exposed to In some animals whole blood total carbon dioxide contents were measured in a Van Slyke apparatus by the technique of Van Slyke and Neill[2] and carbon dioxide tensions calculated by means of the Henderson–Hasselbalch form of the mass action law from the plasma *p*H, the total carbon dioxide content of whole blood, the hæmatocrit, the temperature-corrected *p*Ka′ (ref 3) and a temperature-corrected carbon dioxide[4]. In other animals, carbon dioxide tensions were measured directly at the given ambient temperature by means of a Severinghaus electrode[5]. In these animals, plasma bicarbonate concentration was calculated by the Henderson–Hasselbalch statement of the mass action law using a temperature-corrected pKa' (ref. 4) and temperature-corrected carbon dioxide[5]. Comparisons between directly determined carbon dioxide tensions and calculated carbon dioxide tensions were performed on 5 bloods at the three temperature-ranges and were not significantly different.

The *in vitro* influence of temperature on turtle plasma *p*H, carbon dioxide tension and bicarbonate concentration was studied as follows. Blood was obtained from seven animals by intracardiac puncture in the usual fashion. Plasma *p*H's and carbon dioxide tensions were determined successively at 37°, 24° and 10° by direct measurement. Plasma bicarbonate concentrations were calculated as described here.

The *in vivo* data concerning the effect of temperature on carbon dioxide tension, *p*H and bicarbonate concentration are summarized in Tables 2, 3 and 4. It can be seen that, invariably, an increase of ambient temperature results in an increased plasma carbon dioxide tension. There is, on the average, an approximately three-fold increase in plasma carbon dioxide tension between 10° and 37° C. Corresponding changes occur in plasma *p*H so that plasma hydrogen ion concentration increases with exposure to higher temperatures. However, the fall in *p*H does not occur invariably in every animal. The changes of *p*H at 10° C and at 37° C and at 24° C and at 37° C are statistically significant at a $P < 0.001$ level. Those between 10° and 24° are significant at a level of $0.1 > P > 0.05$. The failure of *p*H to change to a greater extent between 10° and 24° in some animals probably reflects more effective renal compensation.

The mean changes in bicarbonate concentration are in the expected direction with the highest average plasma bicarbonate concentration being found in the animals at 37° C and the lowest in the animals at 10° C. There is considerable individual variation in this relationship again presumably reflecting different degrees of renal compensation.

Table 2. EFFECT OF DIFFERENT TEMPERATURES ON PLASMA CARBON DIOXIDE TENSION OF THE TURTLE

Turtle	CO₂ tension (mm. Hg) 10° C	24° C	37° C
1	17 (*a*)	29 (*b*)	64 (*c*)
2	13 (*a*)	26 (*b*)	—
3	13 (*a*)	24 (*b*)	59 (*c*)
4	22 (*b*)	40 (*c*)	62 (*a*)
5	27 (*b*)	52 (*c*)	61 (*a*)
6	27 (*b*)	31 (*c*)	45 (*a*)
7	14 (*c*)	19 (*a*)	79 (*b*)
8	15 (*c*)	25 (*a*)	44 (*b*)
9	20 (*c*)	21 (*b*)	53 (*a*)
10	13 (*c*)	21 (*b*)	47 (*a*)
11	12 (*c*)	20 (*b*)	42 (*a*)
Mean	18.6±6	28.0±10	56.0±12

$P < 0.001$ for all values at the three temperatures.
a, First; *b*, second; *c*, third temperature animal exposed to.

Table 3. EFFECT OF DIFFERENT TEMPERATURES ON PLASMA *p*H OF THE TURTLE

Turtle	*p*H (units) 10° C	24° C	37° C
1	7.72 (*a*)	7.66 (*b*)	7.35 (*c*)
2	7.76 (*a*)	7.66 (*b*)	—
3	7.70 (*a*)	7.66 (*b*)	7.42 (*c*)
4	7.60 (*b*)	7.50 (*c*)	7.44 (*a*)
5	7.58 (*b*)	7.45 (*c*)	7.43 (*a*)
6	7.55 (*b*)	7.60 (*c*)	7.50 (*a*)
7	7.60 (*c*)	7.65 (*a*)	7.25 (*b*)
8	7.71 (*c*)	7.69 (*a*)	7.50 (*b*)
9	7.66 (*c*)	7.68 (*b*)	7.43 (*a*)
10	7.67 (*c*)	—	7.57 (*a*)
11	7.68 (*c*)	7.68 (*b*)	7.48 (*a*)
Mean	7.66±0.02	7.63±0.09	7.44±0.09

$0.1 > P > 0.05$ for values obtained at 10° and 24°; $P < 0.001$ for values obtained at 10° and 37°; $P < 0.001$ for values obtained at 24° and 37°. *a*, *b* and *c* indicate order of exposure as in Table 2.

Table 4. EFFECT OF DIFFERENT TEMPERATURES ON PLASMA BICARBONATE CONCENTRATION OF THE TURTLE

Turtle	Bicarbonate concentration (m.moles/l.) 10° C	24° C	37° C
1	35.4 (*a*)	40.6 (*b*)	37.9 (*c*)
2	37.2 (*a*)	32.1 (*b*)	—
3	26.9 (*a*)	32.2 (*b*)	40.7 (*c*)
4	31.6 (*b*)	33.9 (*c*)	39.0 (*a*)
5	37.0 (*b*)	39.1 (*c*)	37.2 (*a*)
6	35.4 (*b*)	33.8 (*c*)	34.0 (*a*)
7	19.5 (*c*)	26.1 (*a*)	29.1 (*b*)
8	30.5 (*c*)	37.7 (*a*)	32.9 (*b*)
9	36.4 (*c*)	31.0 (*b*)	35.8 (*a*)
10	24.3 (*c*)	—	43.5 (*a*)
11	23.2 (*c*)	31.6 (*b*)	30.2 (*a*)
Mean	30.0±6.4	33.8±6.0	36.0±4.6

$0.1 > P > 0.05$ for values obtained at 10° and 24°; $P < 0.05$ for values obtained at 10° and 37°; $P < 0.2$ for values obtained at 24° and 37°. *a*, *b* and *c* indicate order of exposure as in Table 2.

The *in vitro* data are summarized in Table 5. An increase of temperature invariably results in an increase of carbon dioxide tension and a fall in *p*H. The mean values of bicarbonate concentration are essentially the same at all temperatures. The individual calculated bicarbonate concentrations at 10° tend to show relatively poor agreement with the values calculated at 24° and 37°. This poor agreement is probably related to the relative inaccuracy of the assumed α carbon dioxide at this temperature and on the fact that small inaccuracies of *p*H and plasma carbon dioxide tension will produce large differences in calculated bicarbonate concentrations.

Table 5. EFFECT OF TEMPERATURE CHANGES ON THE *p*H, CARBON DIOXIDE TENSION AND BICARBONATE CONCENTRATION OF TURTLE BLOOD *in vitro*

Blood No.	10° C	24° C	37° C
	CO₂ tension (mm. Hg)		
1	14	30	59
2	13	29	53
3	11	31	57
4	17	28	56
5	19	32	64
6	14	30	57
7	12	31	55
Mean	14.3±2.8 $P < 0.001$	30.1±1.4 $P < 0.001$	57.2±3.5
	*p*H (units)		
1	7.80	7.61	7.41
2	7.78	7.58	7.37
3	7.95	7.74	7.55
4	7.76	7.58	7.40
5	7.81	7.61	7.41
6	7.91	7.74	7.54
7	7.81	7.62	7.41
Mean	7.83±0.07 $P < 0.001$	7.64±0.07 $P < 0.001$	7.44±0.07
	HCO_3^- (m.moles/l.)		
1	36.2	35.8	38.2
2	32.0	33.0	32.0
3	42.0	52.5	51.9
4	40.0	32.6	35.2
5	51.3	40.0	39.4
6	47.5	50.5	52.5
7	32.4	40.8	36.0
Mean	40.2±7.2	40.7±8.0	40.7±8.1
	Not significant	Not significant	

The relation between temperature and acid-base metabolism is of considerable theoretical interest. The magnitude of temperature change in this study is probably not significantly different from temperatures that the turtle is subjected to during the course of ordinary existence since, during the winter, ambient temperature may be as low as 10° C and during basking in the Sun may probably come fairly close to 37°.

The obvious mechanism for the changes is the effect of temperature on the solubility of carbon dioxide gas at various temperatures, and the increase of H^+ activity that occurs with increasing temperature. An increase in temperature results in an increase of partial pressure of most gases in aqueous solution.

That this is indeed the basis of the observed changes is indicated by the results obtained, *in vitro*, in isolated turtle blood, which show that an increase of temperature is invariably associated with a rise in carbon dioxide tension and a fall in *p*H In the isolated closed physical-chemical system (the blood manipulated outside of the turtle), the direction and magnitude of the changes are strikingly consistent and predictable In the more open and complicated physical-chemical system (the blood inside of the manipulated turtle), there is more variability because of the large number of additional mechanisms besides temperature which produce changes in carbon dioxide tension and *p*H But the fundamental importance of body temperature in establishing a given level of carbon dioxide tension and *p*H in both systems seems clear As a matter of fact, the effect of temperature on gas solubility applies broadly to all biologically important gases and must therefore be a factor in regulating the value of carbon dioxide tensions in all biological systems However, its operation in homotherms is difficult to demonstrate for two reasons (1) Changes in temperature in such animals are small in magnitude (2) Homotherms regulate carbon dioxide tension and *p*H by varying external ventilation in such a way that a constant carbon dioxide tension is maintained Such control is usually neurally regulated

Since the turtle does not maintain a constant *p* carbon dioxide when temperature of the body is varied, it seems likely that the regulation of ventilation in the turtle does not depend on the carbon dioxide tension or *p*H of extracellular fluid Little is known concerning the regulation of ventilation in reptiles, but the turtle does not overtly hyperventilate when placed in an atmosphere enriched with carbon dioxide (unpublished observations), and appears to breathe erratically rather than regularly under basal conditions, and can spend considerable time under water without manifest external ventilation

In a number of mammals, the central regulation of temperature and of ventilation appear to be closely related[6] If the relationship between temperature and carbon dioxide tension applies not only to the turtle but generally to poikilothermic animals, it may well be that the regulation of carbon dioxide tension and *p*H within narrow limits is, from the evolutionary point of view, a temperature-dependent function

It seems clear that the maxim that 'life can only exist within a relatively small *p*H range' is not applicable to the turtle and may only be true of homothermal animals

This work was supported by research grant *H*–5059 from the National Heart Institute, U S National Institutes of Health, and by an institutional grant from the American Cancer Society

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RELATIVE IMPORTANCE OF DRY- AND WET-BULB TEMPERATURES IN CAUSING HEAT STRESS IN CATTLE

By Dr. W BIANCA

Department of Physiology, Hannah Dairy Research Institute, Ayr

THE two principal climatic factors which determine the stress imposed by a hot indoor environment are the dry-bulb and wet-bulb temperatures In man, wet-bulb temperature has been found to be far more important than dry-bulb temperature Indeed, wet-bulb temperature alone has experimentally been shown to be a useful indicator of heat stress[1] Where dry- and wet-bulb temperatures have been used in combination, wet-bulb temperature has always been regarded as being 5–10 times more important than dry-bulb temperature Recently, Provins, Hellon, Bell and Hiorns[2] obtained a clearly defined curve when plotting tolerance times of subjects resting in hot environments against the weighted scale dry-bulb × 0 15 + wet-bulb × 0 85

In cattle it is generally recognized that a high wet-bulb temperature causes more stress than a high dry-bulb temperature, but the relative importance of the two has not been defined An attempt has therefore been made to find what weighting factors for dry- and wet-bulb temperatures would be appropriate in cattle

Four Ayrshire bull calves, 9 months old, were simultaneously exposed in a climatic room to nine different environments (Table 1) The environments numbered 1–5 ranged from 30° to 50° C dry-bulb temperature at a constant dew-point temperature of 17 5° C The environments numbered 6–9 were at constant dry-bulb temperature of 40° C , that is, at approximately body temperature, with the dew-point temperature ranging from 8 3° to 30 6° C The corresponding wet-bulb temperatures are given in Table 1 Each animal was exposed three times to each of these environments for 5 hr at a time The resulting strain was expressed in terms of the rectal temperature recorded at the end of the 5-hr period and the mean values obtained were plotted against the weighted scale dry-bulb × 0 15 + wet-bulb × 0 85

Table 1 DRY-BULB (DB), WET-BULB (WB) AND COMBINED TEMPERATURES OF THE NINE EXPERIMENTAL ENVIRONMENTS

Environment No	DB °C	WB °C	DB×0 15+ WB×0 85 °C	DB×0 35+ WB×0 65 °C
1	30 0	21 5	22 8	24 5
2	35 0	23 0	24 8	27 2
3	40 0	24 4	26 7	29 9
4	45 0	25 7	28 6	32 5
5	50 0	26 9	30 4	35 0
6	40 0	20 4	23 3	27 3
7	40 0	25 1	27 3	30 3
8	40 0	29 1	30 7	32 9
9	40 0	32 5	33 6	35 1

It is evident from Fig 1 that the two sets of conditions, namely the environments 1–5 (which will be referred to as *A*) and the environments 6–9 (which will be referred to as *B*) gave divergent results There was good agreement between the rectal temperature responses to the environments 3 and 7, which were at the same dry-bulb temperature and at only slightly different wet-bulb temperatures The average rectal temperature attained after 5 hr in these two environments was 39.56° C and corresponded to 27° C on the weighted scale. However, below 27° C the curve for the responses to the *A* environments was below that for the responses to the *B* environments, above 27° C the situation was reversed. The two divergencies were almost symmetrical.

This finding indicates that the dry-bulb temperature had a much greater effect on the animals than was commensurate with a weighting factor of 15 per cent. In other words, a weighting of 15 per cent dry-bulb, 85 per cent wet-bulb temperature underrated the effect of dry-bulb temperature and overrated the effect of wet-bulb temperature.

Other dry-bulb–wet-bulb temperature combinations were therefore investigated, and it was found that with a weighting of dry-bulb × 0.35 + wet-bulb × 0.65 the rectal temperature responses to the environments *A* and *B* corresponded most closely (Fig. 2).

This means that whereas in man the effect of the wet-bulb temperature is almost six times as large as that of the dry-bulb temperature, in cattle it is only about twice as large. This difference could reflect species differences in the capacity for evaporation. According to Brody[3], on exposure to an atmosphere of 40.5° C, man can dissipate by evaporation of moisture about 190 per cent of his metabolic heat production, whereas European type cattle can dissipate only 105 per cent. Furthermore, in man evaporation occurs almost entirely from the skin, but in cattle it occurs from the respiratory tract as well as from the skin. Of these two modes of evaporation respiratory evaporation is less affected by a high air humidity than skin evaporation, because the air in the respiratory tract is normally warmer and therefore has a higher saturation vapour pressure than the air in contact with the skin. Indeed, moisture can be evaporated from the respiratory tract even in a saturated atmosphere, provided the temperature of the air surrounding the animal does not exceed the temperature of the air in the respiratory tract.

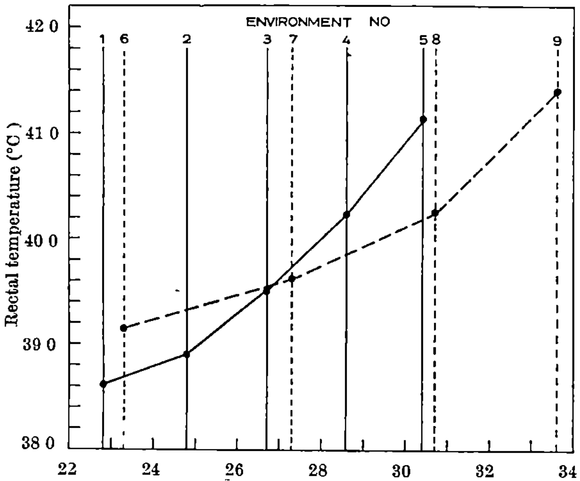

Fig. 1. Rectal temperatures of the calves after exposure to heat for 5 hr plotted against the weighted scale dry-bulb (DB) × 0.15 + wet-bulb (WB) × 0.85. Each point represents the mean of 12 readings.

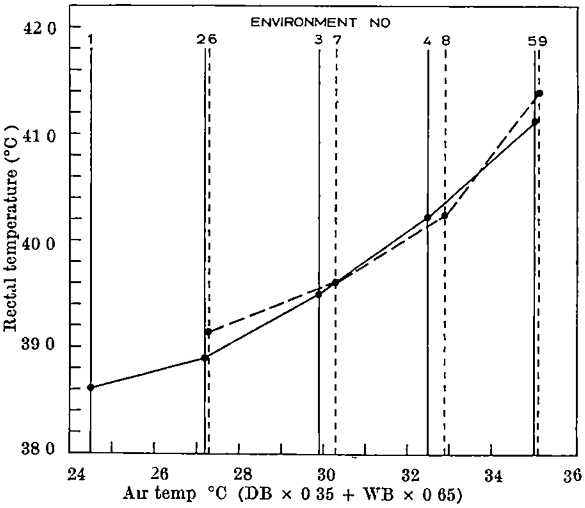

Fig. 2. Rectal temperatures of the calves after exposure to heat for 5 hr plotted against the weighted scale dry-bulb (DB) × 0.35 + wet bulb (WB) × 0.65. Each point represents the mean of 12 readings.

The weighting factors for dry- and wet-bulb temperatures arrived at in this work could become of practical value for predicting the heat strain imposed on cattle by various combinations of dry-bulb–wet-bulb temperatures. It would, however, be necessary to collect more data for the establishment of fiducial limits.

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TWO OPPOSITE BEHAVIOURAL EFFECTS OF THE ELECTRICAL STIMULATION OF BRAIN STEM STRUCTURES IN FREELY MOVING MONKEYS

By Prof. JUHN A. WADA and JOHN WRINCH
Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver

RAPID repetitive stimulation of the reticular core of the brain stem in freely moving animals has been reported to result in certain changes of behavioural state as well as electrocortical arousal. Such behavioural changes may range from awakening from natural sleep through the development of alert attitudes, to cowering or even frank flight[1]. Besides such global behavioural arousal, optimal stimulation may also produce a marked improvement in visual perception and motor reaction in tachistoscopic discrimination[2]. Entirely opposite effects, such as impaired performance of a learned task[3] or arrest of existent behaviour[4], have also been reported, and there is some evidence that 'a soporific effect on behaviour' may be obtained by electrical stimulation of certain points in the same functional system[5-7].

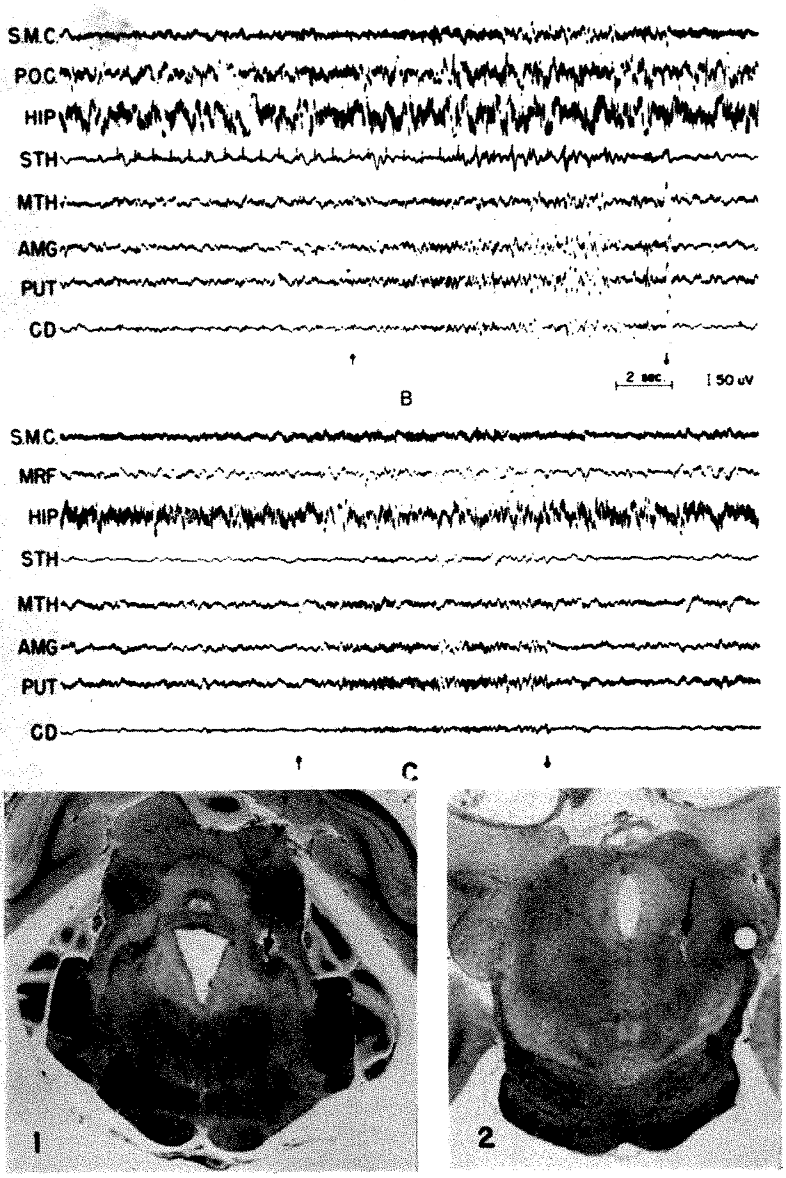

Fig. 1. Monkey "Goro". *A*, Behavioural sleep induced by reticular stimulation 4 V., 0·1 msec., 1·5 p.p.s. *B*, Spontaneous sleep. ↑ Closure of eyes after assuming sleeping position. ↓ Opening of eyes. *S.M.C.*, Sensori-motor cortex; *P.O.C.*, parieto-occipital cortex; *MRF*, mesencephalic reticular formation; *HIP*, hippocampus; *STH*, subthalamus; *MTH*, mid-line thalamic structure; *AMG*, amygdala; *PUT*, putamen; *CD*, caudate. *C*, Sites of stimulation. (1) "Goro". Electrode tip in the superior cerebellar peduncle. (2) "Saburo". Electrode tip in the lateral reticular formation

The frequency and nature of the electrical stimulation, as well as the environmental situation, seems to be important in obtaining the latter effect. Recently, we have been able to induce two distinctly opposite behavioural patterns by stimulating the same locus in the brain stem structures of macaque monkeys[8]. This communication is based on more than 100 observations on two monkeys during the last 10-month period. Each animal had multiple electrodes ('Teflon' coated bipolar platinum iridium wires separated 0·5 mm. at the tip) permanently implanted and was kept continuously in the observation box[8,9]. The behaviour was monitored through a one-way mirror system and the electrical activity was followed on a type 111*D* Grass electroencephalograph (EEG) and a DuMont type 332 oscilloscope. The EEG and behaviour of the animal were continuously and simultaneously cinematographed for later analysis. Electrical stimulation was delivered through the isolation units with two Grass type *S*4 stimulators using 1–200 p.p.s. at 2–8 V. and a 0·1 msec. square wave pulse duration. Electrode tips yielding the effects reported here were located in the lateral reticular formation (left) of one monkey and near the superior cerebellar peduncle (left) of the other (Fig. 1*C*).

(*a*) *Heightened Behaviour.* Behavioural arousal, ranging from alerting to a state of panic, followed repetitive high-frequency stimulation of the electrode sites shown in Fig. 1*C*.

The animal may be aroused from a natural drowsy state by 2 V., 0·1 msec., 200 p.p.s. If the same threshold stimulus was applied while the animal was awake, the only effect, and one consistently found, was that the animal raised its left hand and placed it on the edge of the collar plate. There was a few seconds latency for this action under the 2-V. stimulus, but this latency was practically eliminated when the voltage was raised to 2·5. Such stimulation by higher voltages was followed by a lifting of not only the left hand but also the right as well. When the left hand was occupied, as, for example, in holding a food pellet, the animal lifted its empty right hand. Since both animals were predominantly left-handed, it was easy to reproduce this last effect by offering them a piece of food immediately prior to the onset of brain stimulation. If the threshold brain stimulus was applied while the animal was engaged in eating from his left hand, his right hand was promptly raised and placed on the plastic collar, while the left hand remained unmoved or was lowered to the floor. Exactly the same behavioural patterns were repeatedly noted when the animal was alerted by an accidental noise. These findings seem to indicate that the lifting of the hand is not an involuntarily evoked motion but is an integral part of a voluntarily organized behavioural response in an alarming situation. As the voltage of the stimulation was further increased to 3·0, this 'prepared' behaviour was disorganized and replaced by sudden standing, rotation, aimless searching behaviour and other unmistakable patterns of panic. It seems possible that the stimulation of these electrode sites mobilized some neuronal system involved in the organization of patterned behaviour. This was evidenced by an appearance or disorganization of such behaviour depending on the intensity of stimulus applied.

(*b*) *Behavioural repose.* Low-frequency suprathreshold stimulation (6 V., 0·1 msec., 1 p.p.s.) of the same electrode sites where heightened behaviour was produced by high-frequency repetitive stimulation produced a slight twitching of the ipsilateral face and jaw following each stimulation. If the voltage was raised to 7, the jerking became so violent as to produce a noise of teeth cracking. When such a supramaximal stimulation was continued for more than 30 sec., the animal frequently began yawning. At this stage the animal kept its mouth wide open and thus minimized an annoying effect of the stimulation. Although the animal appeared to relax, sleep or a drowsy state never developed under these conditions (even if the stimulation was continued for more than 15 min.). A prolonged low-frequency, subthreshold stimulation of the same area, on the other hand, made the animal yawn, relax, assume sleeping position, close its eyes and finally go to sleep. The optimal stimulating conditions for evoking this reaction were found to be 4–5 V., 0·1 msec., 1·5–2 p.p.s. The onset of a relaxed behaviour progressing through drowsiness to sleep was accompanied by an electrographic pattern typical of similar states occurring spontaneously (Fig. 1*A*, *B*). Thus, the 30 c.p.s. fast activity in the depths as well as the surface usually observed at the onset was followed by rhythmic 3–5 c.p.s. slow wave bursts in the depths, the appearance of 10–12 c.p.s. high-voltage spindle bursts in the anterior cortices and the disappearance of lambda waves in the posterior cortex. Although this behaviour and electrographic state was repeatedly produced by stimulation of the brain, it could only be obtained if the environment was quiet. If a distracting stimulus was present, the animal did not sleep or relax. Furthermore, even after the animal closed its eyes and assumed a sleeping position, it could easily be aroused despite continued stimulus. The animal could also be awakened by simply changing to high-frequency repetitive stimulation after the animal had fallen asleep under low-frequency repetitive stimulation.

Sleep episodes commonly lasted 10–30 sec. in animals under low-frequency repetitive stimulation and, although repeated episodes could be induced, it has not been possible to make the animal sleep continuously. This finding suggests that the induced state of repose described here is probably due to facilitation of the mechanism of the onset but not the maintenance of sleep. It is possible that such brain stimulation, although unable by itself to affect overt behaviour, may, by its monotonous, repetitive nature, activate a process of inhibition similar to that of Pavlovian sleep. That such a process may involve more rostrally situated deep structures is suggested by the initial appearance of 30 c.p.s. fast activity in the prosencephalic structure. This assumption is in keeping with a recent disclosure that the caudate nucleus and its 'loop' serves as an integral part of inhibitory mechanism[10].

This work was aided by Federal Mental Health grant No. 609–5–129, the Provincial Mental Health Services of British Columbia, and the U.S. National Institute of Neurological Diseases and Blindness research grant No. *B*–2812. We thank Mr. Finn Bauck for assistance.

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MENTAL STRESS AND ACUTE DENTAL CARIES

By Dr. PHILIP R. N. SUTTON

Dental School, University of Melbourne

AN indication that a period of severe mental stress may be followed by the development of acute dental caries, in adults, is suggested by results obtained recently.

This observation links with those of Findlay[1] on psychosomatic gingival conditions, associated with the progestational and menstrual phases of the cycle in young females, and with those of Robertson[2] on psychosomatic stress and pregnancy sickness.

Acute dental crown caries is a severe condition which causes rapid destruction of the hardest of all the body tissues—the teeth. The signs of this form of caries are considerably different from those seen in chronic caries, and include the presence of a white halo around the cavity and soft, yellowish-white carious dentine. Bodecker[3], in his classification of types of caries, said that acute caries is active "during childhood and up. Fifteen to twenty-five years". He stated that the clinical data in that classification had been approved by L. R. Cahn, H. Holliday, H. J. Leonard, E. C. McBeath, A. T. Rowe, L. M. Waugh and D. E. Ziskin. Thoma and Goldman[4] made a similar statement.

Attention was attracted when, within a four-day period, acute caries was observed in six patients who were more than forty years of age. Five of them, judging from their remarks, had recently been through a period of severe worry, and the sixth complained of severe overwork. Following this observation, during the examination of all patients who were twenty-five years of age or more, an endeavour was made to find out whether, during the preceding year, the patient had been ill, worried or overworked, and all cases of acute caries were recorded. None of the subjects disclosed any major changes in their dietary patterns or in their oral hygiene procedures.

Data were recorded for each of the 661 non-edentulous patients, of twenty-five years of age or more, who were examined, on one or more occasions, during the period July 5, 1957–December 31, 1960. Acute caries was observed in 169 subjects, and 175 subjects admitted having recently undergone severe mental stress. The circumstances given by them as the causes of their stress were as follows (the frequency with which each was reported is shown in parentheses): (1) Severe illness of (*a*) husband or wife (16), (*b*) child (14), (*c*) parent or other close relative (28). (2) Worry regarding own health (36); the descriptions given were—'nerves' (7), 'nervous breakdown' (2), hypertension (5), suspected cancer (3), migraine, arthritis, hyperthyroidism, failure of eyesight, recent operation (each two cases) and one case of each of nine other conditions. (3) Severe business or financial worries (29). (4) Changes in, or unsatisfactory, employment (15). (5) Housing or building worries (10). (6) Other reasons (35). (7) Stress admitted but cause not disclosed (23). (8) Twenty-seven patients complained of mental stress as a result of having been very overworked for at least a three-month period.

Forty-eight subjects reported two causes of stress and five disclosed three causes. 17 per cent of the women, but only 7 per cent of the men, did not disclose the cause of their stress, and, as would be expected, the proportion of men having business and financial worries (26 per cent) exceeded that found in these women (16 per cent); and whereas 54 per cent of the women who had recently undergone stress attributed it to illness (of others or of themselves), only 35 per cent of the men who had been under stress admitted worry of this nature.

In ten subjects acute caries was observed during examinations conducted several months apart. In each case the patient reported a prolonged period, or a resurgence, of stress. The onset of the period of worry or overwork preceded the detection of acute caries by periods varying from eleven to only two months. In most cases the stress was said to have been of several months duration; acute caries was not seen to follow periods of stress which lasted only a week or two.

In order to consider variable factors which may influence this comparison, the data were divided into sixty-four categories. Each of the two main groups of subjects—those showing and those not showing acute caries—was separated into two divisions according to sex, and each of these into four age groups (25–34, 35–44, 45–54 and 55 years and more), and each of these groups into four parts according to the number of natural teeth present in the mouth of each subject (1–14, 15–20, 21–26, 27–32).

In each age-group the incidence of acute caries was similar in both sexes (differences not statistically significant). In view of the opinion of Bodecker[8] that acute caries "affects principally children and young adults up to about the age of 25 years" it would be expected that the incidence of that condition would be highest in the youngest age-group. However, the highest incidence, 33 per cent, was in the 45–54 years age group. It was 27 per cent in the 25–34 years group and 20 per cent in both the 35–44 and the 55+ years groups. The incidence in the 45–54 years age group was greater (just significant at the 1 per cent level) than that of the combined data of the other three groups and significantly greater (at the 0·1 per cent level) than that seen in the 35–44 years and the 55+ years groups.

The mean number of natural teeth present in the mouths of those with acute caries and those without that condition were, respectively, in each age-group 25–34 years 24·9, 23·0 (difference just significant at the 1 per cent level); 35–44 years 23·3, 20·7 (difference significant at the 1 per cent level); 45–54 years 18·9, 19·7 and 55 years and over 14·5, 16·1—neither of the last-mentioned two differences being significant.

The unexpected result was obtained, in each age-group, that there was only a slight suggestion (not significant) that the incidence of acute caries was related to the number of teeth present. Therefore, the slight differences which have just been mentioned, in the two youngest age-groups, between the number of teeth present in those with and those without acute caries, are unlikely to affect the comparison.

The results obtained are shown in Table 1.

Table 1. INCIDENCE OF ACUTE DENTAL CARIES IN SUBJECTS AGED TWENTY-FIVE YEARS AND MORE WHICH WERE EXAMINED DURING THE PERIOD JULY 5, 1957–DECEMBER 31, 1960, SHOWING THOSE FROM WHOM A HISTORY OF RECENT MENTAL STRESS WAS ELICITED

The four figures shown in parentheses give the totals that would be expected if there were no relationship between acute caries and mental stress

Recent stress		Elicited							Not elicited			Totals
Acute caries		Illness of others		Illness of self		Other worry or overwork		Totals			Totals	
	Sex	M	F	M	F	M	F	M+F	M	F	M+F	M+F
	Age in years											
Seen	25–34	5	11		8	10	21	55	1	1	2	57
	35–44	2	7		5	14	14	42		1	1	43
	45–54	4	17	7	7	8	13	56	1	1	2	58
	55+	1	1		5	2	1	10		1	1	11
	Totals	12	36	7	25	34	49	163 (45)	2	4	6 (124)	169
Not seen	25–34		1				2	3	52	98	150	153
	35–44		1			1	1	3	77	94	171	174
	45–54	1			1	2	1	5	45	70	115	120
	55+						1	1	22	22	44	45
	Totals	1	2		1	3	5	12 (130)	196	284	480 (362)	492
Totals		13	38	7	26	37	54	175	198	288	486	661

Not merely when the data shown in Table 1 are considered as a whole, but also when those of each of the four age-groups in each sex are assessed separately, it is obvious that there is a strong suggestion that there is an association between mental stress and acute caries. However, these results are open to criticism on several grounds: (*a*) Although all patients, twenty-five years of age and more, presenting for examination were included in this investigation, the sample cannot be said to be a random one from the general population. (*b*) Despite the fact that the signs of typical lesions of acute and chronic caries are considerably different, there is no unmistakable line of demarcation between the two types. (*c*) There is considerable difficulty in assessing the presence of mental stress and reliance had to be placed on the statements made by the subjects. However, of those who admitted stress, almost half (81) said that this was due to, or accompanied by, illness that was receiving medical attention. The fact that the majority of these subjects have demonstrated some degree of confidence in the examiner, almost half of them having returned for treatment for ten years or more and an additional 28 per cent for more than four years, may be considered to reduce the likelihood of undue reticence on their part.

In those with acute caries, 96 per cent admitted recent severe mental stress, but in only 2 per cent of those considered to be free from acute caries was the presence of recent stress elicited. The former percentage is unlikely to be too high, for there would appear to be no reason for pretending that stress had been experienced, and all except 23 of the 163 subjects with acute caries and stress disclosed the cause of their stress. It is more likely that some of those without acute caries may have recently experienced stress but did not admit this fact. However, in the very improbable event that these subjects—

if there were any—numbered as many as 400 (that is, that 412 of those without acute caries had experienced stress), there would still be a strong suggestion that mental stress was associated with acute caries in the subjects studied. Nevertheless, because of the difficulties and shortcomings that have been noted, it cannot be said that the results in Table 1 establish a causal relationship between mental stress and acute caries in adults.

Further investigations will be undertaken, for it is considered to be highly probable that these two factors are associated, and, at least in some adults, that severe mental stress predisposes to the development of acute dental caries.

I thank Sir Arthur Amies and Prof. James Aitchison for their suggestions, Mr. P. D. Finch for advice on statistical matters, and the University of Melbourne Research Fund.

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DIFFERENCES IN THE DEPOSITION AND PERSISTENCE OF PESTICIDES ON THE UPPER AND LOWER SURFACES OF LEAVES

By Dr. D. P. PIELOU, K. WILLIAMS and F. E. BRINTON

Entomology Laboratory, Canada Department of Agriculture, Summerland, B.C.

IN work on the deposition, persistence and effectiveness of pesticides on foliage in the field it is desirable to be able to determine the relative distribution of the chemicals on the upper and lower surfaces of leaves. The method devised by Rayner[1] allows the quantitative determination of certain fungicides on one side of a single leaf but, in the process, the deposit on the other side is destroyed.

An apparatus has now been devised by one of us (F. E. B.) which allows the separate and independent removal of certain pesticide deposits from the two surfaces of the same leaf (Fig. 1). Four compression springs (*CS*) hold together two square brass base plates (BP_1, BP_2). One of these plates is larger than the other. Each base plate has welded to it, and is penetrated by, a brass tube, 4 cm. in internal diameter, which acts as a solvent vessel (*SV*). These vessels can be closed at their outer ends by threaded brass caps (*C*) with polythene liners. In order to force apart the two halves of the apparatus, the larger base plate is placed on the support shoulder (*SS*) of a cradle. A lever device, with a forked arm (*FA*) which straddles the cap of the lower vessel, is then actuated so that the lower unit is forced down. A leaf (*L*) is inserted between the lips of the two vessels and the spring pressure is restored. The springs have been previously so adjusted that leaves will be in solvent-tight juxtaposition with the lips of the vessels but with pressure not so great as to perforate the foliage. A known amount of extraction solvent is placed in the upper vessel, which is then tightly capped. The apparatus is inverted and solvent is placed in the other vessel. The apparatus is then removed from the cradle and placed horizontally on a shaking machine.

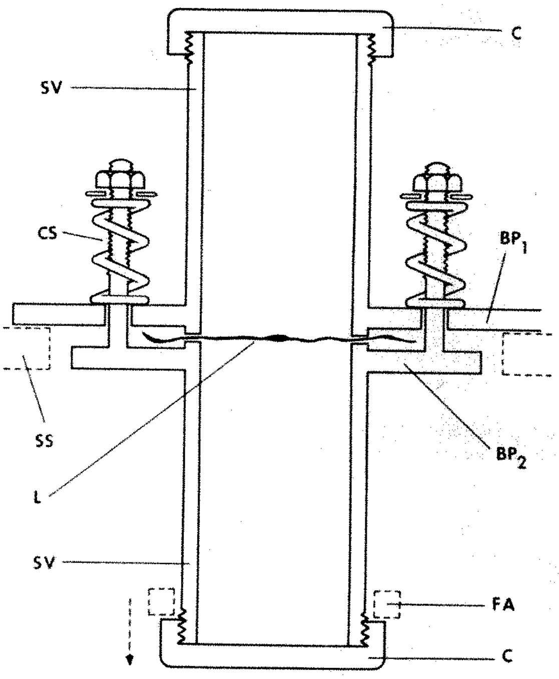

Fig. 1

Using 'Sevin' (1-naphthyl *N*-methylcarbamate) as an insecticide, and chloroform as an extraction solvent, agitation for 30 sec. was sufficient for recoveries of known deposits, from cherry and apple leaves, not significantly below those obtained by standard methods. In this period there was no translocation through the leaf by solvent action. This point was demonstrated by applying 'Sevin' to one surface of a leaf, placing chloroform in the vessel in contact with the opposite surface, and analysing for the presence of the insecticide in the solvent at intervals during agitation. With periods of up to 5 min. no transfer was detected. The method is speedy; extraction of 'Sevin' from both surfaces of 100 individual leaves can be carried out in 8 hr. A more detailed account of the construction and use of this apparatus and its effectiveness with other insecticides and other solvents will be published elsewhere[2].

Extensive use of this apparatus has shown that insecticides tend to be deposited more heavily on the lower surfaces of apple and cherry leaves than on the upper surface; and that weathering processes afterwards tend to accentuate this difference in distribution. For example, 'Sevin', 50 per cent wettable powder, was applied to cherry trees at the rate of 1 lb. per 100 imperial gallons until there was copious run-off from the leaves. Immediately after drying, 100 leaves were analysed; 75 of these had a higher deposit on the lower surface than on the upper. The mean deposit on the upper surface was 1·65 μgm./cm.^{2}; on the lower it was 2·38 μgm. or 1·44 times greater. Dwarf apple trees treated in the same way showed 130 leaves out of 144 as having heavier deposits on their undersides; the mean lower surface deposit was 1·84 times that of the upper. Essentially, similar results (ratio of lower to upper deposits, 1·75) were obtained with concentrate 'air-blast' spraying in which run-off of spray fluids does not occur. All these differences are statistically highly significant.

These findings cannot be explained in terms of the lower position of the operator with respect to the bulk of the tree because the small dwarf apple trees were sprayed as much from above as from below. It is highly likely, therefore, that the increased deposit is the outcome of some difference in structure between the two leaf surfaces. The difference may be related to the ultra-structure of wax on leaves that is revealed by electron microscopy of carbon replicas[3]. It is of interest to note the relative distribution on the two surfaces (but not the absolute amounts) was not significantly altered by the addition of 'Plyac', a film-forming surface-active agent of the polyethylene type (Allied Chemical Corporation, General Chemicals Division, New York, 6).

There was a clear indication that this difference was further enhanced with time. Deposit decline was somewhat more rapid on the upper surface even in the absence of rain. Thus, after 16 days the residual deposits on the lower surfaces of cherry leaves were 6·63 times those on the upper; the absolute values of both had, of course, fallen greatly from the initial values. It has been shown previously[4] that if rain, or water from overhead irrigation sprinklers, is present, deposits of 'Sevin' decline rapidly. This loss now appears to be primarily due to a reduction of deposit on the upper surfaces. Dwarf apple trees were irrigated, by overhead sprinklers, for 5 hr. with the equivalent of 2·1 in. of rain. Immediately before irrigation analysis showed that deposits on the upper and lower surfaces averaged 1·47 and 2·75 μgm./cm.^{2}, respectively; afterwards the values were 0·05 and 1·62 μgm./cm.^{2}, respectively.

The biological importance of these facts is obvious in the control of pests and diseases which predominate on one of the surfaces of a leaf, or for insects and mites that move freely from one side of a leaf to the other and show behaviour responses to insecticide deposits. Efforts are now being made to find adjuvants for pesticide formulations that will minimize the initial uneven distribution of deposits and reduce the rapid weathering from the upper surfaces of leaves.

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OBSERVATION OF SEX DIFFERENTIATION IN *SORGHUM* BY USE OF INDUCED MALE-STERILE MUTANTS

By Dr. Z. BARABÁS

Agricultural Research Institute, Hungarian Academy of Sciences, Martonvásár

MALE sterility has been reported in many species of plants which is derived, almost without exception, from spontaneous mutations or crosses[1,2]. Those deriving from crosses may in the final issue likewise originate from spontaneous mutations. Induced male-sterile (*ms*) mutations have been so far reported, to my knowledge, only in the tomato[3], in which, however, the frequency of spontaneous *ms* mutants is also rather high[4]. Theoretically, the artificial *ms* induction is important from a genetic point of view of evolution and practically in the utilization of heterosis effects in plant breeding. In connexion with these problems, as a part of a larger programme[5], the following experiments were conducted.

During the past 30 years three genetically analysed cases of male sterility in *Sorghum* have been described[6–8]. During seven years 35,000–40,000 plants were examined yearly in our nursery, but completely pollen-sterile spontaneous individuals were never found. From these results it may be concluded that spontaneous mutants are very infrequent. The monogenic *ms* Day *SA* 380 (No. 1) and the cytoplasmic Combine Kafir *SA* 6062–1–8–4 (No. 2) described by

Table 1. COMPARISON BETWEEN THREE NEW MALE-STERILE MUTANTS WITH THE ORIGINAL VARIETY

	Rancher (variety)	Male-sterile (*ms* 1645)	Microanther (*mth*, 1916)	Polygynœceous (*pg*, 2164)
Mutagen	—	Roentgen	colchicine	inbreeding
Dose and generation	—	20,000 r, X_5	0·1–0·5 per cent, C_4	—, I_7
Anthers/flower	3	3	3	0*
Anther weight (μgm.)	0·791	0·103	0·046	0·017*
Pollen sterility (per cent)	fertile	100·0†	100·0	100·0*
Gynæceum No./flower	1	1	1	4–13
Stigma No./flower	2	2	2	8·20‡
Gynæceum weight (μgm.)	0·869	0·197	0·778	2·900
Gynæceum : anther (weight ratio)	1:1	2:1	17:1	170 >:1
Seed per cent of the unbagged florets	80·6	76·6	1·5	26·7
Seed per cent of the bagged florets	75·5	0·0	0·0	0·0
ms × fertile/Rancher/ F_1 fertility	fertile	sectorial sterility§	fertile	fertile
F_2 fertile:*ms*	fertile	sectorial sterility§	3:1	3:1

* Anthers sometimes with fertile pollen occurs in 'intersexes'.
† On short days 1–2 per cent stainable pollen.
‡ 0–3 stigmas per ovary.
§ Sterile flowers in sectors on the panicle.

Stephens *et al.*[7,8], were the first *ms* forms of practical value. Mr. Stephens and Mr. Quinby kindly sent me three lines in 1955.

Homogeneous inbred strains of sorghum (*Sorghum vulgare* Pers. syn. *Sorghum* sect. *Sorghum* var. Rancher), which as a test object is endowed with a number of good qualities[9], were treated with doses of 10,000–35,000 r. X-rays in air-dried seed condition, and with 0·2–0·5 per cent aqueous solution of 0·1 per cent colchicine respectively injected in the scutellar node at the seedling stage. Colchicine was suggested, as gene-mutagen, by the results obtained by Franzke and Ross[10].

From the fertile Rancher strain three *ms* forms were obtained (Figs. 1–4, Table 1).

Male-sterile (*ms* 1645) Roentgen mutant attains about 60 per cent the height of the Rancher, its flowers are smaller, but its fertile sibs are of the same size. Its vitality is good, and seed setting complete.

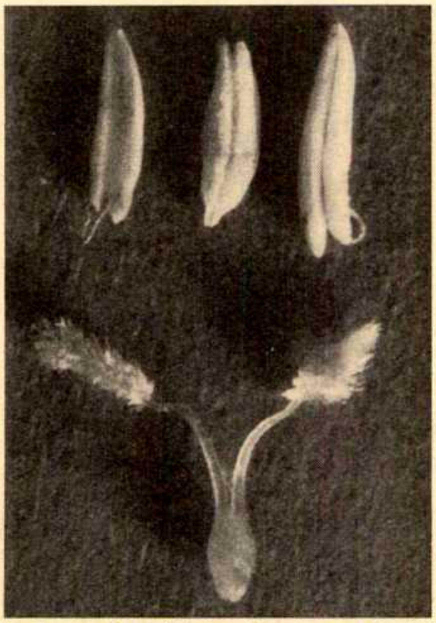


Fig. 1. Rancher, original variety, stamen and pistil

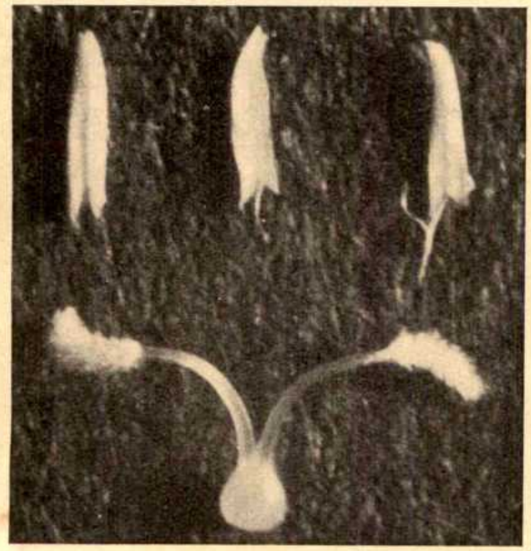


Fig. 2. Male-sterile mutant (*ms* 1645)

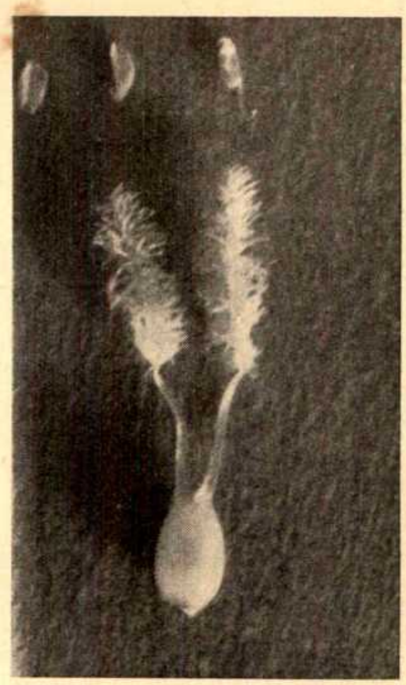


Fig. 3. Microanther mutant (*mth* 1916)

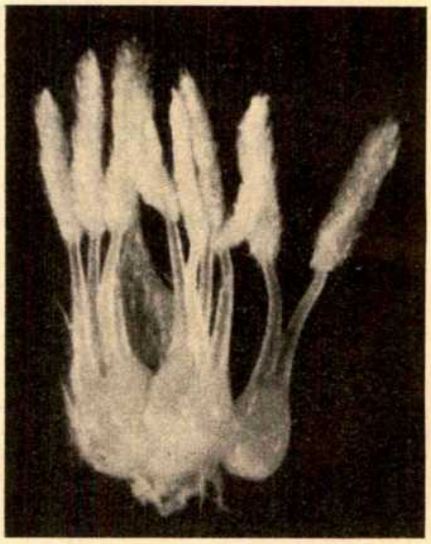


Fig. 4. Polygynæceous mutant (*pg* 2164)

Under short-day conditions it produces 1–2 per cent stainable pollen, but seed setting cannot be obtained from it. The constitution of the flower is similar to No. 2, but genetically different from it. This is shown also by the formation of F_2 and by the fact that F_1 is completely fertile when crossed with the counterpart of No. 2.

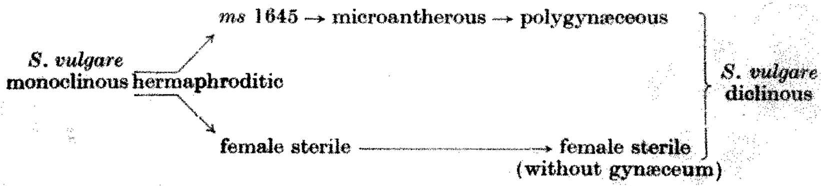
The microanther (*mth* 1916) colchicine mutant is a new form. No pollen is produced and its female sterility is also very high. The mutant is more viable, and it has more lateral shoots and panicles than the Rancher.

The polygynæceous (*pg* 2164) form has not appeared in *Sorghum*. Parallel mutants have been described in tobacco, barley, etc.[11]. In *pg* mutant up to four seeds for each flower occur. Multiplication is an important problem, since 10–12 pistils of the *pg* cannot be formed directly from three stamens. Contrary to Fig. 4 on some plants the partial transformation similarly occurs in a large number, making it difficult to ascertain the exact number of pistils. In some individuals, though stamens are often converted into an ovary, anthers may also occur, to a maximum of 6 per cent of the flowers, sometimes with fertile pollen ('intersex').

Frequently, the reduction of male sex is accompanied by the strengthening of the female sex. This is well expressed by the weight ratio obtained by measuring 35 sex organs, which were in fresh opened flowers, but did not yet fulfil their function. For example, in the fertile counterpart of No. 2 the gynæceum: anther ($G : A$) weight μgm. sex organ is 0·556 : 0·371; in the *ms* No. 2 it is 0·756 : 0·097, which means that pistil weight is higher in the *ms*. The $G : A$ ratio is about 1·5 : 1 and 8 : 1 respectively. The *ms* forms in Table 1 may be arranged on the basis of sex relations as follows: fertile Rancher → *ms* 1645 → *mth* → *pg*. The anther weight decreases roughly along exponential curve, while $G : A$ weight ratios are about $1 : 1 \rightarrow 2 : 1 \rightarrow 17 : 1 \rightarrow 170 > : 1$. Weight increase of the gynæceum is relative at first, then absolute. In the *pg* mutant the weight relation is about 170 > : 1 in the 'intersex', while increase to ∞ : 1 in the antherless mutant. The $G : A$ ratio establishes therefore the degree of sex reversal, and, to some extent, the sex index.

These steps, which are important from the morphological and evolutionary points of view, have a simple genetic background. The *ms* 1645 is not yet properly elucidated, but the male sterility may be simple recessive, as, for example, in case of No. 1. When back-crossed with the original form both the *mth* and *pg* show a segregation ratio of about 3 : 1 in the F_2. According to preliminary mitosis examinations of the types described, there is no evidence of division disturbances or chromosomal aberrations. Further investigations are needed, however.

An interesting completion of the *ms* series is the work of Casady *et al.*[12], reporting a female sterile form obtained by crosses also from hermaphroditic *Sorghum*. The female sterility is inherited by means of two gene-pairs. The female sex is present only in rudimentary form, but flowers without female organs have also occurred as a spontaneous mutation, but its maintenance was impossible for technical reasons[9]. On the basis of the foregoing, the sex differentiation develops in a similar way as was assumed in natural evolution by Correns (in ref. 13); a similar observation was made by Jones[14] in maize as spontaneous forms. In *Sorghum*, however, by means of these mutants, at least from the morphological point of view, intermediary steps could be examined likewise:


ms 1645 → microantherous → polygynæceous
S. vulgare monoclinous hermaphroditic
female sterile → female sterile (without gynæceum)
S. vulgare diclinous

The *pg* mutants, particularly the forms producing anthers too, are approaching the intersex postulated by Correns. A diclinous species was thus produced by way of mutations from a hermaphroditic (monoclinous) plant species. Some steps of sex differentiation occurring also in the natural evolution could be induced, therefore, artificially and observed *in statu nascendi*. Further investigations of these mutants will lead to a greater knowledge of sex inheritance in *Sorghum* and other hermaphroditic plants.

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CRYSTAL STRUCTURE OF NaCd₂

By STEN SAMSON

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena

PHASE-diagram studies of the sodium–cadmium system have established the existence of a compound with the approximate composition $NaCd_2$; the several references can be found in Hansen[1]. Pauling[2,3] reported that $NaCd_2$ has a cubic structure with the edge of the smallest unit cube slightly more than 30 Å., and that this unit contains about 384 sodium atoms and 768 cadmium atoms. The space group was found to be O_h^7-$Fd3m$ (Pauling, private communication).

A structure determination of $NaCd_2$ has now been made by the stochastic method. The structure is in accord with an almost complete three-dimensional set of single-crystal X-ray data obtained with copper $K\alpha$ radiation.

Octahedral crystals of $NaCd_2$ were prepared by allowing sodium–cadmium alloys containing excess sodium to cool slowly. The excess of sodium was dissolved by treatment with absolute alcohol, leaving the crystals of the intermetallic compound. This is the method also used by Kurnakow and Kusnetzow[4]. Chemical analyses for both sodium and cadmium in five single-phase samples prepared as above gave compositions varying between $NaCd_{1.99}$ and $NaCd_{2.12}$; the analytical data reported by Kurnakow and Kusnetzow[4] correspond to $NaCd_{2.17}$. The densities measured on several single-crystal samples varied between $\rho = 5{\cdot}38$ gm./cm.^{3} and $\rho = 5{\cdot}63$ gm./cm.^{3}. Although this phase is probably of variable composition as indicated by these data the name '$NaCd_2$' will be retained.

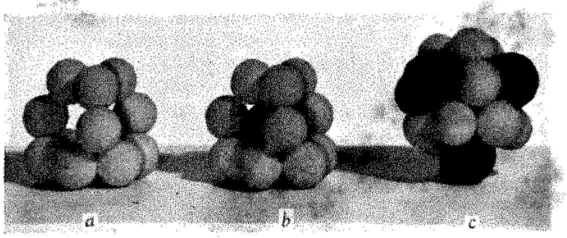

Fig. 1. *a*, *b*, The truncated tetrahedron; *c*, the Friauf polyhedron

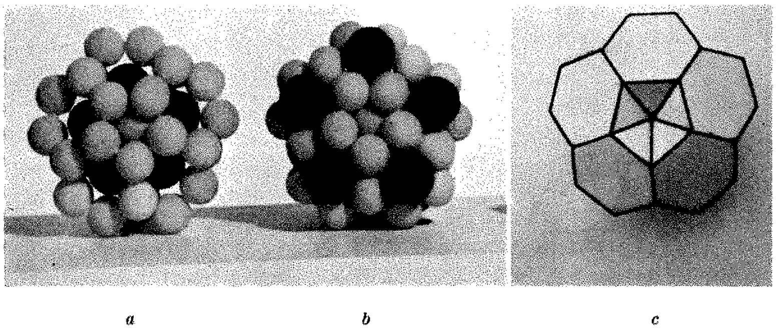

Fig. 2. *a*, Five truncated tetrahedra about a five-fold axis of symmetry; *b*, the F_5 polyhedron; *c*, a formal representation of the F_5 polyhedron. The atoms out from the centres of the hexagons are not indicated for the sake of perspicuity

The space group O_h^7-$Fd3m$ was confirmed, and the cube edge as measured for one crystal was found to be $a_0 = 30{\cdot}56$ Å.

On the basis of the experimental results given here, the unit cell contains between 1,120 and 1,190 atoms.

A structure to be tested by comparison of observed and calculated X-ray data was formulated with the assumption that many of the large atoms, the sodium atoms, have sixteen ligates, twelve cadmium atoms which are at the vertices of a truncated tetrahedron bounded by four hexagons and four triangles (Fig. 1*a*, *b*) and four sodium atoms which are located out from the centres of the hexagons of the truncated tetrahedron. The resulting polyhedron (Fig. 1*c*) in earlier papers[5,6], referred to as the Friauf polyhedron, is frequently observed in complex metallic structures containing atoms with metallic radii differing by about 15 per cent or more from one another. It was first observed in the Friauf phases $MgCu_2$ and $MgZn_2$ (refs. 7 and 8). Since the dihedral angles of a tetrahedron correspond to nearly one-fifth of a complete rotation, five Friauf polyhedra can be arranged about an approximate five-fold axis of symmetry in such a way that the five truncated tetrahedra have one edge in common on the five-fold axis, and each truncated tetrahedron shares two of its four hexagons with the two contiguous truncated tetrahedra (Fig. 2*a*). Such an aggregate of five Friauf polyhedra, which will be referred to as the F_5 polyhedron, represents the 47-atom complex shown in Fig. 2*b*. A formal representation of the F_5 polyhedron is given in Fig. 2*c*, in which the atoms out from the centres of the hexagons are not indicated for the sake of perspicuity. Six F_5 polyhedra are arranged about the vertices of an octahedron (Fig. 3). This octahedron is of T_d symmetry and hence only the (110) mirror planes are present. Each two F_5 polyhedra at the ends of the body diagonal of the octahedron are rotated 90° with respect to one another. The six F_5 polyhedra produce in their turn four Friauf polyhedra located at the vertices of a regular tetrahedron. These Friauf polyhedra are the dark ones shown in Fig. 3. The resulting aggregate consists of 34 Friauf polyhedra (6 × 5 + 4) and represents a 234-atom complex if the atoms out from the centres of the hexagons not indicated in Fig. 3 are included. Eight such 234-atom complexes can be fitted in the cubic unit in such a way that each complex shares atoms with four others (Fig. 4) such as to reduce the average number of atoms per complex to 144. The eight complexes thus contain 1,152 atoms. Eight more atoms have to be added, each one of them at the centre of a new Friauf polyhedron (F') formed by a set of six F_5 polyhedra. With the addition of thirty-two more atoms out from the centres of the triangles of eight such Friauf polyhedra (8 F'), the unit cell contains 1,192 atoms; and there are 280 Friauf polyhedra each about a central atom with ligancy 16 ($L16$). A closer examination reveals additionally 96 polyhedra of $L15$, 64 of $L14$, 128 of $L13$, and 624 polyhedra of $L12$ of which 528 are approximately regular icosahedra.

If each sodium atom is assumed to have ligancy 14, 15 or 16 and each cadmium atom ligancy 12, 13 or 14, and if of atoms with ligancy 14 there are as many sodium as there are cadmium, the unit-cell content corresponds to $Na_{408}Cd_{784}$ or $NaCd_{1{\cdot}92}$, and $\rho_{calc} = 5{\cdot}67$ gm./cm.^{3}.

With the atoms placed to fit this assumption, a fair agreement between observed and calculated structure factors was obtained. Several least-squares refinements resulted in an agreement index $R = 0{\cdot}15$ for the zero-layer reflexions, $R = 0{\cdot}21$ for the first 500 reflexions (*hk*0 through *hk*3), and $R = 0{\cdot}23$ for the almost complete three-dimensional set of reflexions; here $R = \Sigma|F_0 - F_c|/\Sigma|F_0|$.

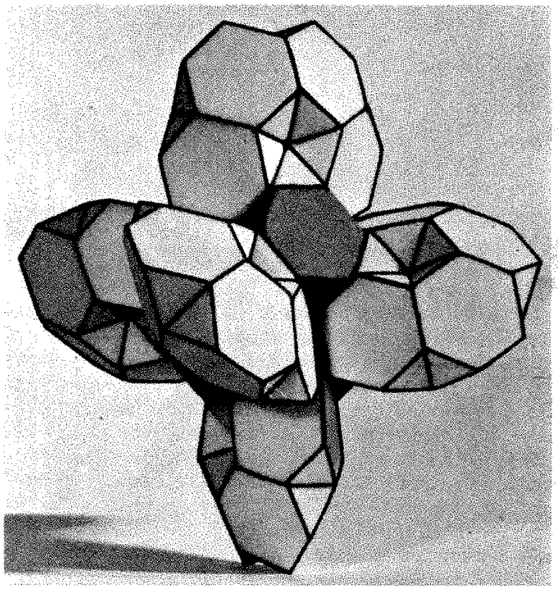

Fig. 3. The 234-atom complex formed by six F_5 polyhedra which are arranged around the vertices of an octahedron of T_d symmetry

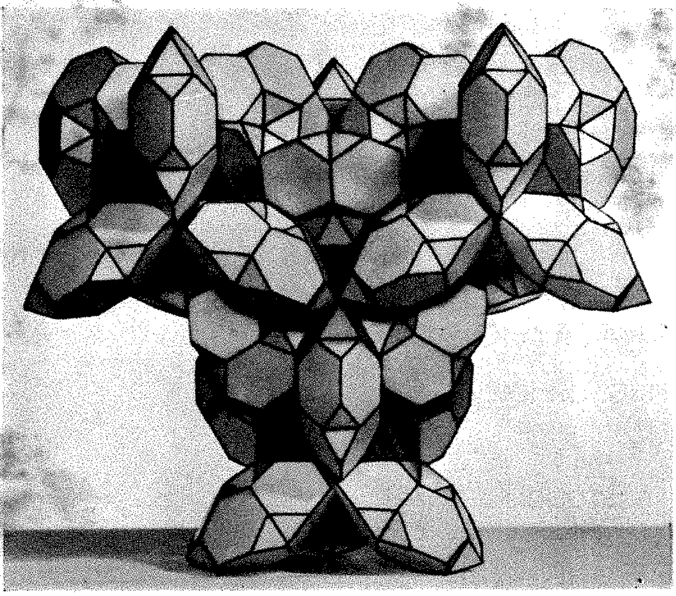

Fig. 4. Four 234-atom complexes arranged about one such complex. The four complexes are at the vertices of a regular tetrahedron

A Fourier section through the (011) plane was calculated with the use of all available data. Three peaks at assumed cadmium positions were found to correspond to approximately 0·5–0·75 of the reflecting power of cadmium, one sodium peak was higher than expected, and many of the other sodium peaks seemed somewhat lower than would correspond to complete occupancy. Least-squares calculations were then carried out with the inclusion of additional parameters that determine the approximate occupancy of the four positions just referred to, but the remaining sodium positions were taken to be completely occupied. Of the 1,480 reflexions used, 635 were too weak to be observed. At the present stage of the refinement 520 of the 'less than' reflexions are calculated lower than the minimum observable value of F, and 115 are still calculated somewhat too high. The agreement index, as defined here, is $R = 0{\cdot}17$ for all reflexions not including the 520 'less than' reflexions, and $R = 0{\cdot}13$ for the zero-layer reflexions, which are of considerably higher average intensity than those of the other layers.

The shortest and longest bond distances observed with the parameters obtained are Na–Na = 3·25–3·80 Å., Na–Cd = 3·12–3·80 Å. and Cd–Cd = 2·70–3·60 Å. The atomic positions, together with the as yet approximate positional parameters, are listed in Table 1. The least-squares calculations indicate that the disordered positions are occupied as follows: (1) 35 per cent Cd or 16 per cent Cd + 84 per cent Na in position $32e_4$, (2) 50 per cent Cd in position $32e_5$, (3) 70 per cent Cd or 62 per cent Cd + 38 per cent Na in position $96g_5$, and (4) 80 per cent Cd in position $96g_7$.

The crystals reacted with oxygen or moisture and gradually decomposed during X-ray photography. Therefore, several crystals had to be used to obtain the present results. The crystals were regular octahedra of diameter 0·20–0·27 mm., and absorption-correction factors[9] for spherical crystals of $\mu R \sim 7$ to 10 were applied to the measured intensities. Some systematic errors may accordingly have been introduced owing to inaccurate absorption corrections. It is also possible that some sodium was leached out of the crystals by the treatment with alcohol. It therefore did not seem justified to continue the refinement with the present data, although it became evident that the agreement factors could be considerably improved.

The work on $NaCd_2$ is now being continued with improved techniques for sample preparation and for the collection of intensity results. A detailed report on the structure and on the techniques used in determining the atomic positions will be published later.

A compound with structure apparently similar to that of $NaCd_2$ is βMg-Al (approximately Mg_2Al_3), which Perlitz[10,11] found by single-crystal X-ray studies to be cubic, space group O_h^7-$Fd3m$, with approximately 1,166 atoms per unit cube of edge $a_0 = 28{\cdot}22$ Å. Laves and Moeller[12] said that βMg-Al is isomorphous with Cu_4Cd_3; but the atomic positions were not known.

Preliminary work on these two phases has been carried out here in parallel with the above investigation. Crystals of βMg-Al were, unfortunately, always twinned, but the diffraction patterns are strikingly similar to those of $NaCd_2$. A single crystal of Cu_4Cd_3 has been isolated, and an almost complete set of intensity data has been obtained. The diffraction patterns of this phase differ drastically from those of $NaCd_2$ and βMg-Al. The probable space groups for Cu_4Cd_3 were found to be T_d^2, O^3 and O_h^5, and the cube edge to be $a_0 = 25{\cdot}83$ Å. This cubic unit contains approximately 1,116 atoms, according to the present experimental data.

The work on these two phases is also being continued. A detailed report will be published later.

I thank Prof. Linus Pauling for his interest and help. I also thank Prof. J. H. Sturdivant for useful discussions. Miss Brigitta Schönning carried out most of the preparative work and the X-ray photography as well as intensity measurements. Mr. Robert Ross contributed by checking and processing the intensity data.

Table 1

Position			Kind of atom	Ligancy	Co-ordination polyhedron
$8b$	$\frac{1}{2}\frac{1}{2}\frac{1}{2}$, etc		Na	16	Friauf polyhedron
$16c$	$\frac{1}{8}\frac{1}{8}\frac{1}{8}$, etc.		Cd	12	Icosahedron
$32e_1$	xxx, etc.	$x_{e_1} = 0{\cdot}216$	Cd	12	Icosahedron
$32e_2$	,,	$x_{e_2} = 0{\cdot}318$	Na	16	Friauf polyhedron
$32e_3$	,,	$x_{e_3} = 0{\cdot}378$	Cd	14	Irregular
$32e_4$	,,	$x_{e_4} = 0{\cdot}439$	Na*	14	Irregular
$32e_5$	,,	$x_{e_5} = 0{\cdot}600$	Cd*	13	Irregular
$48f$	$x00$, etc.	$x_f = 0{\cdot}144$	Na	16	Friauf polyhedron
$96h$	$\frac{1}{8}, x, \frac{1}{4}\text{-}x$, etc.	$x_h = -0{\cdot}012$	Cd	12	Icosahedron
$96g_1$	xxz, etc.	$x_{g_1} = 0{\cdot}146$ $z_{g_1} = 0{\cdot}218$	Cd	12	Icosahedron
$96g_2$	,,	$x_{g_2} = 0{\cdot}064$ $z_{g_2} = 0{\cdot}203$	Na	16	Friauf polyhedron
$96g_3$	,,	$x_{g_3} = 0{\cdot}217$ $z_{g_3} = 0{\cdot}488$	Cd	12	Icosahedron
$96g_4$	,,	$x_{g_4} = 0{\cdot}044$ $z_{g_4} = 0{\cdot}316$	Na	16	Friauf polyhedron
$96g_5$	,,	$x_{g_5} = 0{\cdot}218$ $z_{g_5} = 0{\cdot}650$	Cd*	12	Irregular
$96g_6$	,,	$x_{g_6} = 0{\cdot}134$ $z_{g_6} = 0{\cdot}487$	Na	15	Irregular
$96g_7$	,,	$x_{g_7} = 0{\cdot}179$ $z_{g_7} = 0{\cdot}569$	Cd*	13	Irregular
$192i$	xyz, etc.	$x_i = 0{\cdot}145$ $y_i = 0{\cdot}213$ $z_i = 0{\cdot}421$	Cd	12	Icosahedron

* At least predominantly occupied by atom indicated. See text regarding possible disorder.

This work was carried out under Contract No. *Nonr*–220(33) between the California Institute of Technology and the Office of Naval Research.

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OBSERVATION OF TRAPPED ELECTRONS IN POLYMETHACRYLIC ACID

By M. G. ORMEROD
Physics Branch
AND
L. G. STOODLEY
Radar and Telecommunications Branch, Royal Military College of Science, Shrivenham, Swindon

ELECTRON spin resonance is a useful tool in the study of the effect of additives on the radiation chemistry of solids. In particular, it has been used for elucidating the mechanisms of radiation protection in the solid state[1]. Another application is to use additives with special properties (for example, electron traps, radical scavengers) in order to help investigate the basic radiation chemical processes. In this article we report experiments of this type on polymethacrylic acid.

Our electron spin resonance work on polymethacrylic acid and its esters has led us to believe that the unpaired spins which are observed at 77° K. after irradiation at that temperature are trapped electrons[2]. In order to test this idea we decided to investigate the effect of some electron acceptors on the system, the chosen additives being pyromellitic dianhydride (PMDA) and tetracyanoethylene (TCNE).

The mixtures of 95 per cent polymethacrylic acid and 5 per cent additive were prepared by dissolving both compounds in ethanol and then driving off the solvent. The samples were sealed under vacuum in glass tubes and were irradiated at 77° K. with γ-rays from a 2 kilocurie cobalt-60 source (dose = 10^6 rads; dose-rate = 6×10^5 rads hr.$^{-1}$). They were examined in a conventional high-frequency (400 kc./s.) magnetic field modulation *X*-band spectrometer, all spectra being recorded as the first derivative of the absorption intensity as a function of variation in magnetic field. Unpaired spin concentrations were determined by numerical integration of the derivative curve and by comparison with a standard sample of α,α'-diphenyl β-picryl hydrazyl.

The electron spin resonance spectra obtained at 70° K. are shown in Fig. 1 and it can be seen that the additives had a marked effect on the nature of the radiation-induced spins. The spin concentration was not affected, the number of unpaired spins formed per 100 eV. of absorbed radiation (that is, *G*-value) being 1·5 in each case. The colour of the irradiated solid differed according to the additive, the colours being given in the caption.

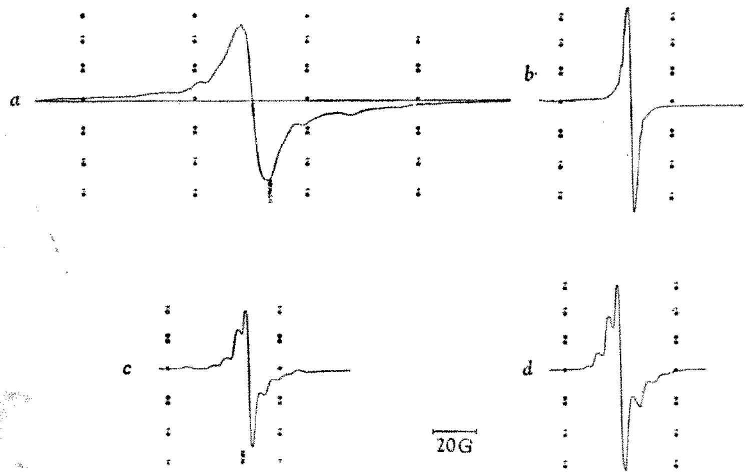


Fig. 1. Electron spin resonance spectra from some compounds recorded at 77° K. *a*, Polymethacrylic acid (brown) irradiated at 77° K.; *b*, polymethacrylic (red) plus 5 per cent PDMA irradiated at 77° K.; *c*, polymethacrylic acid (yellow) plus 5 per cent TCNE irradiated at 77° K.; *d*, the TCNE negative ion (yellow) in tetrahydrofuran. The colours in brackets refer to the colour of the solid at the time of recording the spectrum. The spectra have not been recorded at the same sensitivity

For the purpose of comparison we prepared the TCNE radical anion by reduction with sodium iodide in tetrahydrofuran solution. At room temperature this gave the known nine-line spectrum[3]. When cooled to 77° K. the resultant solid solution gave the spectrum of Fig. 1*d*, and although the anisotropic components of the hyperfine splittings are no longer averaged out, sufficient detail remains in the spectrum to show that it is identical with that obtained from the irradiated mixture of polymethacrylic acid and TCNE (Fig. 1*c*). Furthermore, both materials had the same yellow colour, showing that in the irradiated mixture we were observing the TCNE negative ion formed by the trapping of an electron which had been ejected by radiation from the polymethacrylic acid. In the pure polymer the electron is presumably trapped by the polymer itself. Similarly in the case of added

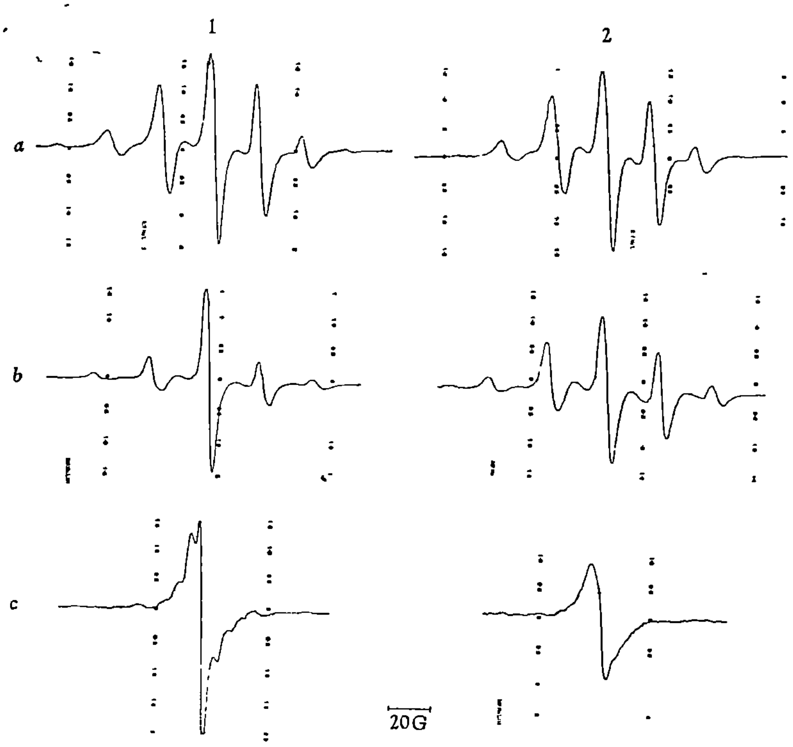
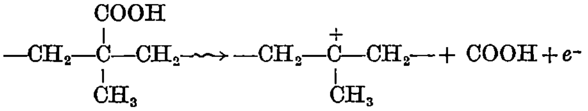


Fig 2 Electron spin resonance spectra recorded at room temperature from some compounds irradiated at 77° K (1) Immediately on warming to room temperature, (2) 24 hr later *a*, Polymethacrylic acid, *b*, polymethacrylic acid plus 5 per cent PDMA, *c*, polymethacrylic acid plus 5 per cent TCNE

PMDA it is presumably the PMDA negative ion which is observed although the lack of detail in the spectrum (Fig 1*b*) would render unprofitable any attempt at positive identification

There was no evidence of the presence of a positive ion which should also have been formed This could be explained by a reaction of the type



$$-CH_2-\overset{COOH}{\underset{CH_3}{C}}-CH_2- \rightsquigarrow -CH_2-\overset{+}{\underset{CH_3}{C}}-CH_2- + COOH + e^-$$

COOH breaking down to give gases and gaseous radicals which diffuse through the polymer and react with one another The ion $-CH_2-\overset{+}{\underset{CH_3}{C}}-CH_2-$ does not have an unpaired electron and will not be observed by electron spin resonance

On warming to room temperature the colours of the samples faded—rapidly in the pure polymer but more slowly in the case of the other two materials At the same time the electron spin resonance spectrum changes (Fig 2) These simultaneous changes in electron spin resonance spectrum and colour indicate that it is the trapped electrons which colour the materials The TCNE negative ion decays with a half-life of about half an hour to leave an unidentifiable residue (Fig 2*c*) while the PMDA ion decays to leave the stable spectrum that is obtained from the pure polymer at room temperature, showing that this ion is reacting with the polymer

Previously in those polymers in which the radiation-induced unpaired spins have been positively identified it has been found that electrically neutral free radicals were present, for example, in polyethylene[4,5], polytetrafluorethylene[4], and polypropylene[6] These free radicals are the result of either a radiation-induced excitation or an ionization The ions that are formed are comparatively short-lived and react to give neutral free radicals before electron spin resonance spectra can be recorded. On the other hand, the final radiation damage in polymethacrylic acid is the result of the reactions of comparatively stable ions and excitation is only playing a minor part These stable ions, which are also observed in the esters of polymethacrylic and polyacrylic acids[2], are presumably associated with the —COOR groups

We thank Prof A Charlesby for his advice

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PLUTONIUM IN MAN AND HIS ENVIRONMENT

By PHILIP W. KREY, DONALD BOGEN and EUGENE FRENCH
Isotopes, Inc , Westwood, New Jersey

THE two plausible modes of entry of fall-out plutonium into man are ingestion and inhalation In studying ingestion, large discrimination factors were observed in going through the ecological cycle from soils to man[1] With such discrimination, it was concluded that incorporation via ingestion was quite unlikely[2]

Although inhalation as a mode of entry of fall-out plutonium into man was also considered to be insignificant[2], animal studies by Stannard[3] and Bair[4] indicated the accumulation of plutonium in the pulmonary lymph nodes on a per gram of tissue basis after prolonged inhalation exposure This is analogous to reports by medical authorities that radioactive colloidal gold concentrates in the lymph nodes when injected into patients for therapeutic or diagnostic purposes[5] Stannard concluded that the pulmonary lymph nodes may limit inhalation exposure under many conditions, and that calculation of maximum permissible doses on this basis may be required In investigations on man, Langham has demonstrated the accumulation of plutonium in the pulmonary lymph nodes of a plutonium operator who was exposed to a low-level chronic plutonium air-borne concentration for about six years In this investigation, the question of the critical organ following chronic inhalation exposure was raised again[6]

A second fact which prompted further investigation of inhalation as the mode of entry of fall-out plutonium into man was the estimates of the ground-level air concentrations of plutonium Baus[7] has reported that the average strontium-90 concentration for ground-level air in Washington, D C, during mid-1957 was about 1 5 DPM per 100 standard cubic metres This corresponds to about 0 2 per cent of the maximum permissible concentration in air of soluble plutonium-239 or -240 for continuous exposure of the general population with bone as the critical organ (A reduction to 1/10 of the maximum permissible concentration in air for occupational workers has been applied for the general population[9])

Eight air filter samples which were collected in Washington, D C, during the spring of 1959 were analysed in this laboratory for plutonium These samples represented a 24-hr collection period and were made available by Dr L B Lockhart, jun, of the U S Naval Research Laboratory The analyses showed an average ground-level air concentration of plutonium of 0 14 DPM/100 m^3 or about 1 per cent of maximum permissible concentration in air for the general population on the same basis as above This agrees with the British estimate of 0 2 DPM/100 m^3 for this same period[10] Based on these estimates and measurements, a cursory investigation of plutonium in human and animal tissue was undertaken

The α-spectra of the plutonium separated from several samples were determined using a thin cæsium iodide (thallium) crystal as a detector similar to the system described by Martinez and Senftle[11] and a 100-channel pulse height analyser The spectra of these samples showed pure plutonium-239 and/or -240 with no other α-contamination

Since the plutonium activities in the samples which were analysed were generally low, extreme caution was exercised to eliminate possible contamination of the sample during the analytical processing Reagent blanks were processed concurrently with samples to monitor the contamination-level if any existed In addition, lung tissue autopsied prior to 1945, made available through the courtesy of Colonel J M Blumberg, deputy director, Armed Forces Institute of Pathology, Washington 25, D C, was processed to represent ideal tissue blanks In all cases, the blank samples reflected no detectable α activity

Human tissue analyses The results of the human tissue analyses are given in Table 1 The errors represent one standard deviation of the counting error All samples were obtained in the New York metropolitan area The sample solution obtained from a 1959 lung sample was split and each aliquot analysed separately to reflect the reproducibility of the radiochemical procedure Analyses of other duplicate samples and standards in which the counting errors were minimal gave an average precision of better than ±5 per cent The composite organs in group I consisted of combined samples from six cadavers whose age at time of death was at least 70 years

The *National Bureau of Standards Handbook*[9] proposes a maximum annual dose of 15 rems for most individual organs of the body and 5 rems for the gonads or the whole body for occupational workers By applying a 1/10 reduction of this dose for the general population, the plutonium concentration in most individual organs can be converted to per cent of maximum permissible dose to that organ by multiplying the DPM plutonium/gm of tissue by a factor which converts the α-energy per disintegration a minute to rems per annum and dividing by the maximum permissible dose in rems per annum, that is

$$\frac{\text{DPM Pu}}{\text{gm of tissue}}\ \frac{\left(\frac{5\ 15\ \text{MeV}}{\text{Disint}} \times \frac{1\ 6 \times 10^{-6}\ \text{ergs}}{\text{MeV}} \times \frac{5\ 25 \times 10^{5}\ \text{min}}{\text{yr}} \times \frac{\text{rad}}{10^{2}\ \text{ergs /gm}} \times 1 \times 10^{1}\ \text{RBE}\right) \times 10^{2}}{1\ 5\ \text{rems/yr}} = \begin{array}{l}\text{per cent}\\ \text{of MPD}\\ \text{to organ}\end{array} \qquad (1)$$

$$\frac{\text{DPM Pu}}{\text{gm of tissue}} \times 28\ 8 = \text{per cent of MPD to organ} \qquad (2)$$

Since the maximum permissible dose (MPD) to the gonads is 1/3 that of most individual organs

$$\frac{\text{DPM Pu}}{\text{gm of gonads}} \times 86\ 4 = \text{per cent of MPD to gonads} \qquad (3)$$

Equations (2) and (3) were used to calculate the percentage of MPD, column 6 in Table 1

The most striking result obtained from the analyses of the human tissue is the high percentage of the maximum permissible dose delivered to the lungs, pulmonary lymph nodes and gonads by the plutonium deposited in these organs This is particularly emphasized by comparison with the average percentage of MPD delivered by strontium-90 to the adult skele-

Table 1 PLUTONIUM ANALYSES OF HUMAN TISSUE

Sample	Year of death	Sample weight (gm)	DPM±*S D*	DPM/gm of tissue± *S D* (10^{-3})	Per cent MPD ±*S D*
Lung-1	1953	950	0 409±0 030	0 430±0 037	0 012±0 001
Lung-2	1954	1,000	0 760±0 062	0 760±0 062	0 022±0 002
Lung-3	1958	398	4 75±0 44	11 9±1 1	0 34±0 03
Tracheo-hilar lymph nodes-3	1958	3 4	0 22±0 11	65±32	1 9±0 9
Lung-4*A*	1959	450	0 565±0 007	1 26±0 17	0 036±0 005
4*B*	1959	450	0 611±0 007	1 36±0 17	0 039±0 005
Composite Organs—Group 1					
Lung	1959	2,340	4 06±0 39	1 74±0 17	0 050±0 005
Pulmonary lymph nodes	1959	53 6	0 57±0 21	11±4	0 32±0 11
Spleen	1959	889	1 02±0 19	1 15±0 21	0 033±0 006
Kidney	1959	949	0 94±0 19	0 99±0 22	0 028±0 006
Gonad	1959	96	0 76±0 18	7 9±1 9	0 68±0 16

Samples collected from 1953 to 1958 were made available by Dr Arthur Schulert, Lamont Geological Observatory, Palisades, New York
Samples recovered in 1959 were supplied by Dr Roth, head Pathologist of Bergen Pines Hospital, Oradell, New Jersey

Table 2 Plutonium Analyses of Animal Tissue

Sample	Time of collection	Sample weight (gm)	DPM ± *S D*	DPM/gm ± *S D* (10^{-3})	Per cent MPC ± *S D*
Steer No 1 organs	Spring 1959				
Spleen		453	41 7 ± 1 6	92 1 ± 3 5	0 84 ± 0 03
Liver		482	3 11 ± 0 37	6 45 ± 0 77	0 059 ± 0 007
Heart		609	2 01 ± 0 31	3 30 ± 0 51	0 033 ± 0 004
Steer lung	Spring 1959	381	0 71 ± 0 16	1 9 ± 0 4	0 017 ± 0 004
Stewing beef samples	Autumn 1958 to Spring 1959				
1		516	35 5 ± 1 6	68 8 ± 3 1	0 62 ± 0 03
2		462	46 4 ± 2 2	100 ± 5	0 91 ± 0 04
3		540	16 0 ± 0 7	27 8 ± 1 3	0 25 ± 0 01
(*a*) Tissue		432	1 29 ± 0 24	2 99 ± 0 56	
(*b*) Fluid		32 6	13 7 ± 0 7	420 ± 21	
4		270	1 42 ± 0 33	5 26 ± 1 22	0 048 ± 0 011
(*a*)		213	0 64 ± 0 12	3 0 ± 0 6	
(*b*)		18 4	0 78 ± 0 31	42 ± 17	
Pork liver (average of 5 samples)	Autumn 1958 to Spring 1959	390	1 75 ± 0 73	4 48 ± 1 87	0 041 ± 0 017

ton Kulp[12] has reported that the average strontium-90 concentration in adult bone in 1959 for Western culture in the northern hemisphere was about 0 30 μμc /gm calcium or about 0 15 per cent of MPD The percentage of MPD for plutonium in lungs, pulmonary lymph nodes and gonads ranges from 1/3 to about ten times this percentage of strontium-90 MPD to the skeleton The gonad burden raises the question of genetic effects in addition to possible somatic effects

The distribution of total plutonium in the human organs measured supports the theory that inhalation is the significant mode of entry of fall-out plutonium into man In the composite organs of group 1, only one lung from each cadaver was analysed so that the activity (4 1 DPM) for the total lung sample should be doubled when comparing the plutonium content of the various organs It is clear that the total activity of plutonium in the lungs is about ten times greater than any other organ measured Further, the high activity per gram in the pulmonary lymph nodes (a factor of about 6 greater than the corresponding lungs) correlates with theories predicting that the plutonium deposited in the lungs will concentrate in the lymph system This suggests that the pulmonary lymph nodes may be the critical organ so far as plutonium inhalation is concerned It is interesting to note that the values of plutonium found in the lungs autopsied in the spring of 1959 agree with a predicted value of 1 DPM/gm calculated from the Harriman lung model of pulmonary deposition and the measured air concentrations[13]

Animal tissue analyses The results of the animal tissue analyses are given in Table 2 The samples comprising the steer No 1 organs were recovered from the same animal by making arrangements with a local slaughterhouse All other samples in Table 2 were purchased in supermarkets in the northern New Jersey area during the autumn of 1958 and the spring of 1959 In the stewing beef samples, numbers 3 and 4, the fluid was drained from the meat and each fraction analysed separately

The plutonium activities per gram of beef spleen, stewing beef and particularly the fluid extract from the stewing beef samples are relatively high Stewing beef samples are extracted from the shoulder of the animal in the vicinity of the thoracic duct Since these samples are all associated with the lymph system, there is an apparent correlation between high plutonium content and this system

The one lung of a steer analysed contained about the same quantity of plutonium per gram as did human lung Unfortunately, this lung was not recovered from the same animal as the No 1 group of samples Therefore, no definitive correlation between total plutonium burden and organ can be made to delineate the mode of entry of the plutonium into the animal

The general level of plutonium found in the other tissue in Table 2 reflects its somewhat ubiquitous nature The per cent of MPC for soluble plutonium with bone as the critical organ for ingestion of these meat products by the general population is also included in Table 2 These values were calculated by comparing the activity per gram of meat with the permissible activity per millilitre of drinking water Although the percentage approach 1 per cent in the spleen and stewing beef samples, the general levels are of no real significance so far as ingestion hazards of the total diet are concerned This has also been demonstrated by the plutonium analysis of several total diet samples which were collected by Consumers Union in their examination of strontium-90 during 1959 [14] The highest concentration of plutonium found in these samples (0 095 DPM/kgm) represents only 10^{-3} per cent of MPC for continuous ingestion by the general population

These data, particularly the results from the human tissue analyses, have shown that fall-out plutonium has entered the biosphere and man, and that inhalation is the main mode of entry into man In addition it appears that the pulmonary lymph nodes should be considered in the assignment of the critical organ so far as inhalation of plutonium is concerned The presence of plutonium in man as a result of nuclear weapons testing offers an opportunity to study the metabolism and translocation of this element within the body although the activity-levels encountered are low

The work was supported by the Defense Atomic Support Agency, Contract *DA*–29–044–*XZ*–609

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INTEGRATED SURFACE EFFECTS OF ATMOSPHERIC POLLUTION

By E. N. LAWRENCE

Meteorological Office, Bracknell, Berkshire

SOME recent work[1] on the measurement of atmospheric sulphur dioxide by the commonly used lead dioxide (cylindrical) candle method[2] (with the louvred box at a height of 2 m. above ground) suggests that this or similar equipment may have a number of applications to atmospheric pollution problems in general. Results show that, under certain conditions, with a fairly sheltered site, the accumulation of sulphate on the cylinder (measured by P, the monthly mean daily number of mgm. of SO_3 collected by 100 cm.^{2} of 'batch A' lead dioxide) is related to V (the monthly mean daily concentration, vol. of SO_2 per 100 million vol. of air), by the formula

$$P = V(0\cdot 08u + 0\cdot 11) \qquad (1)$$

where u is the mean wind speed in m.p.h. When u is suitably large, P is proportional to u, for a given value of V, that is, $\Sigma P \propto \Sigma V u$, further evidence[1] from exposed moorland sites confirms this. These results are approximately what one might expect in an environment where temperature, humidity and rainfall have only second or lower order effects[2] on the total or 'integrated' surface effects of atmospheric pollution.

The dependence of the lead dioxide gauge on wind speed is confirmed by laboratory wind-tunnel data for a cylinder (with axis perpendicular to the wind direction). Powell and Griffiths[3] found that the rate of evaporation (E) therefrom is given by

$$E = 1\cdot 83 \times 10^{-6} u^{0\cdot 61}(p_w - p_a) \qquad (2)$$

where p_w and p_a are the vapour pressures of the saturated surface and the ambient air respectively, for a range of u from 0·5 to 3·0 m./sec. The basic data used to obtain equation (1) give P as an approximate power law of u with index of 0·5–0·6; the range of wind speed (monthly means) varied from 0·5 to 2·0 m.p.h. but, as stated above, the evidence[1] suggests that this index is nearer to unity for higher wind speeds (of the order of 5–10 m.p.h. at the height of 2 m.).

This lead dioxide equipment may well be of use in problems concerning the assimilation or absorption of a gaseous pollutant by a horizontal ground surface, although the laws governing the physical processes involved in the latter are different from those of a cylinder exposed in a polluted air stream. Following Sheppard[4], by replacing the eddy diffusivity by a term consisting of the sum of the molecular diffusivity in air of the gaseous pollutant (δ) plus $ku_* z$ where k is von Karman's constant, u_* the friction velocity and z the height above ground, but using concentrations instead of humidity mixing ratios, and considering the rate of deposition (D) instead of evaporation, we have

$$D = \frac{ku_*(\chi - \chi_0)}{\log ku_* z/\delta} \qquad z \gg \delta/ku_* \qquad (3)$$

where χ is the concentration at height z and χ_0 is the surface concentration. Allowing for atmospheric stability, by using a generalized form of k, we may write

$$D = \frac{ku_*(\chi - \chi_0)\, f(Ri)}{[\log u_* + \log kz\, f(Ri)/\delta]} \qquad z \gg \frac{\delta}{ku_*\, f(Ri)} \qquad (4)$$

where $f(Ri)$ is a function of the Richardson number (Ri). The appropriate form for $f(Ri)$ has been discussed by many authors[5–13]. The variety of forms quoted are all equivalent to

$$f(Ri) = 1 - \sigma Ri \qquad (5)$$

when Ri is small. Based on a number of studies, Blackwell[13] suggests that $\sigma = 7$ for $Ri > 0$ and $\sigma = 15$ for $Ri < 0$. For conditions near the ground, Ri tends to be small and $f(Ri)$ tends to unity, that is, under these conditions, there is a tendency for a neutral atmosphere; values of Ri near the ground are shown, for example, in the diagrams given by Priestley[8] and Pasquill[14]. Deposition, in practice, requires eddy exchange, which implies low Ri. Thus, over a long period (for example, a month), with σ of the order of 10 and Ri on average within 0·01 of zero, an error in the amount of deposition of within 10 per cent may well be achieved, on the assumption that $f(Ri)$ is unity. Further, if χ_0 is zero or small compared with χ and if z is such that the constant in the square brackets is substantially greater than $\log u_*$ (see, for example, Sutton[15]), equation (4) approximates to

$$D = \text{constant} \times (u_* \chi) \qquad (6)$$

Thus it would not be surprising if a practical empirical relation existed between the accumulation of sulphate by the standard lead dioxide cylinder and that by a horizontal ground surface (of given roughness), provided due allowance is made for any accumulation of sulphur dioxide, etc., on the latter, due to washout by rain (see, for example, Chamberlain[16]) or to scavenging processes in fog[17].

Results obtained by the lead dioxide gauge, or by any similar equipment, refer only to the point of measurement, where irregularities in topography give rise to variation in both wind and pollution concentration (but particularly the former) over very short distances. Measurements obtained, for example, on the perimeter of a forest-stand are not applicable to the well-sheltered interior. A close network of pollution gauges (preferably supported by run-of-wind gauges) is necessary for assessing the distribution of the total or integrated effect of wind-driven pollution. With only distant sources and the consequent more homogeneously polluted atmosphere, it is probable that wind is the dominant factor controlling the relative total effect between exposed sites (at least within a confined area and for a specific type of pollution) and that 'run-of-wind' data would provide such relative measures. Though maximum (surface) concentrations of pollution tend to occur with light winds under stable conditions, maximum 'integrated' effects may result from more turbulent conditions, and it may be useful to consider the degree of turbulence as well as the run-of-wind, in the interpolation of relative values

The magnitude of total surface accumulation of a pollutant by a suitable sensitive surface, such as the standard lead dioxide candle surface, may well be of greater practical application than that of the concentration of atmospheric pollution In polluted areas, it is certainly of paramount importance, for example, in problems relating to the use of ventilation in horticultural techniques or to pollution damage with non-streamlined building designs and to similar exposure problems in medicine, animal and crop husbandry and forestry—that is, in domains where atmospheric pollution effects are dependent on both atmospheric concentration and wind speed

The use of standard (artificial) surfaces as distinct from natural surfaces is not new to scientific recording techniques The painted surface of the Duvdevani dew-gauge[18], or the polystyrene block of the surface wetness recorder[19] for the estimation of leaf or plant surface wetness are examples In laboratory wind-tunnel work, various types of surface have been used to show surface flow direction or to distinguish between laminar and turbulent flow in boundary air-flow patterns These techniques (for example, refs 20–23) involve physical and chemical changes in the surface used, and depend on the visual observation of the degree of evaporation or sublimation, or, as in the 'contamination' techniques, the deposition of dust, etc , or the intensity of a chemically formed stain Surfaces are 'scoured' or 'swept', and reveal the degree of air-interchange, air-renewal or 'run-of-wind' close to the surface, near the boundary of zero wind velocity and the régime of molecular diffusion

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ACID LEACHING OF THORIUM CARBIDE

By YASUICHI SASAKI, FUJIO ICHIKAWA, HISASHI IMAI and SHINOBU URUNO

Japan Atomic Energy Research Institute, Ibaraki-ken

RECENT developments of nuclear research has stimulated the study of new fuel materials such as carbides of uranium, plutonium and thorium These carbides have high melting-points and can be used in the reactor which is operated at high temperatures Furthermore, they show greater thermal conductivity than that of oxides

In the semi-homogeneous breeder reactor development programme, which has been planned in this Institute, the use of thorium carbide as a fertile material is considered

There are many problems in handling this material as a blanket fuel For example, protection from hydrolytic decomposition by moist atmosphere, physical and chemical actions in reactor circumstances and method of re-processing are the most essential

So far, Hyde *et al* [1] have carried out nitric acid leaching of compact carbide fuel composed of thorium and uranium

The existence of two compounds, ThC and ThC_2, is known, but experimental investigations were confined to the latter, as it was difficult to get the monocarbide in a single phase Compacted ThC_2 was prepared by the following method Powder of natural graphite and thoria was mixed, in the ratio of 1 part of thorium to 5 parts of carbon (by weight) The mixture of 1 gm was shaped into a compact 0 63 cm in diameter and 0 63 cm high under about 7,000 kgm /cm ^{2} in a briquetting machine The compact was converted into ThC_2 by heating at about 2,000° C for 3 hr in a vacuum Tammann furnace It was proved by X-ray diffraction analysis that the products were composed of ThC_2 and traces of graphite, and no ThO_2 or ThC was detected

The ThC_2 prepared was stored in a desiccator over phosphorus pentoxide, because ThC_2 reacts easily with atmospheric moisture to give gaseous hydrocarbons and $ThO_2(2H_2O)$

Acid Leaching of ThC_2 at Room Temperature

Acid leaching of the ThC_2 compact was examined using concentrations of acids, such as hydrochloric, sulphuric and nitric The degree of reaction was measured as the percentage of dissolved thorium to total thorium The method was as follows

A ThC_2 compact of about 1 gm was placed into 30 ml acid, then broken into pieces The solution was filtered after the reaction had proceeded overnight The residue was ignited in a platinum crucible at 900° C for 1 hr After graphite was oxidized into carbon dioxide, thorium was measured as thoria Meanwhile, the filtrate was dried and redissolved in 1 *N* hydrochloric acid, then thorium was precipitated as thorium oxalate by adding 10 ml of 10 per cent oxalic acid The precipitate was ignited after filtration, and the remaining thorium weighed as the dioxide

(i) *Hydrochloric acid leaching of ThC_2.* When the ThC_2 compact was put in a solution of hydrochloric acid, it disintegrated spontaneously and liberated gases with an acetylene-like odour. Almost all the ThC_2 was dissolved in this acid in a concentration-range of 0.5–12 *N* (Fig. 1).

(ii) *Sulphuric acid leaching of ThC_2.* In case of sulphuric acid leaching, ThC_2 dissolved with liberation of gases in the concentration range 0.5–1 *N*. But the solubility became gradually smaller with increasing concentration of this acid, and ThC_2 did not completely dissolve in the acid at a concentration above 10 *N* (Fig. 2). It is known that the solubility of $Th(SO_4)_2$ in sulphuric acid is lowered with increasing concentration of the acid. The leaching process of ThC_2 by sulphuric acid can be divided into two steps, namely, decomposition of ThC_2 and dissolving of $Th(SO_4)_2$ in sulphuric acid. The latter step is controlled by the solubility of $Th(SO_4)_2$ in the acid.

(iii) *Nitric acid leaching of ThC_2.* In the case of nitric acid leaching, an interesting result was obtained (Fig. 3). About 90 per cent of ThC_2 was decomposed in the concentration range 0.5–1 *N*, but the reaction grew less with increasing concentration of this acid.

In about 6 *N* nitric acid, the solubility of ThC_2 reached a minimum, then it gradually increased and recovered at higher concentration of the acid until up to a limit at about 50 per cent. Liberation of gaseous hydrocarbons was not observed at concentrations above 4 *N*, and charcoal-grey material appeared in colloidal form. It was observed that this material does not have a C—H bond as indicated by the results obtained from infra-red spectroscopic analysis and that the greater part of it precipitates in an aggregate form at about *p*H 1. When this material is heated in air, it burns out easily. The X-ray diffraction analysis proved that the insoluble material is ThC_2 and not thoria.

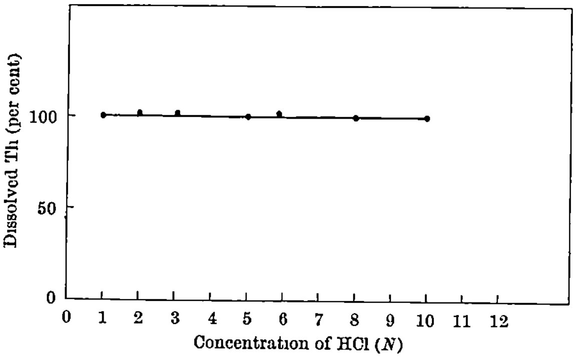

Fig. 1. Dissolving of ThC_2 in hydrochloric acid at room temperature (24 hr.)

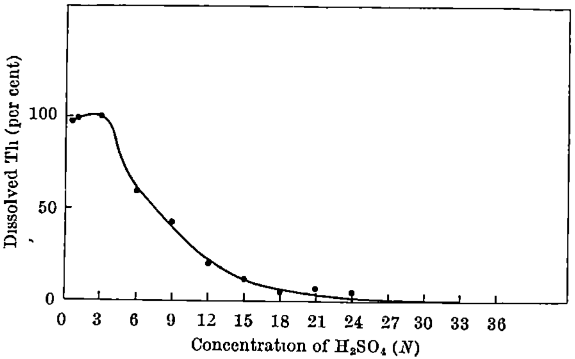

Fig. 2. Dissolving of ThC_2 in sulphuric acid at room temperature

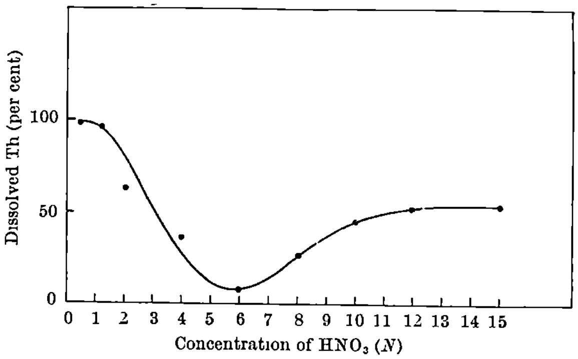

Fig. 3. Dissolving of ThC_2 in nitric acid at room temperature

From the experimental results described, this charcoal-grey material might be colloidal carbon rather than organic material. It is considered that ThC_2 is soluble in dilute nitric acid as well as in hydrochloric acid, while in the case of concentrated nitric acid, gaseous hydrocarbon does not generate and free carbon is isolated because of increased oxidation produced by increasing concentration of the acid. The isolated carbon becomes colloidal carbon and/or dispersed in solution.

A clear explanation cannot be advanced for the redissolving of ThC_2 in nitric acid at concentrations above 6 *N*, but it might be speculated tentatively that the dissolving reaction would be effected by the dissolving-rate of intermediate compounds of thorium which are formed on decomposition of ThC_2. As a tentative explanation for this phenomenon, the following reaction is postulated:

$$ThC_2 + 4HNO_3 = Th(NO_3)_4 + (CH_2)_n$$

$$ThC_2 + 4HNO_3 = ThO_2(2H_2O) + 4NO_2 + 2C$$

$$ThO_2(2H_2O) + 4HNO_3 = Th(NO_3)_4 + 4H_2O$$

Acid Leaching of ThC_2 at 80° C

Experiments were carried out also at 80° C. Leaching was continued for 7 hr. The experiments were conducted only at concentrations of 8 *N* and 12 *N* for nitric and sulphuric acids, respectively, because the results at room temperature showed the minimum solubility at these concentrations.

(i) *Sulphuric acid leaching of ThC_2 at 80° C.* For comparison with the results of leaching at the higher temperature, leaching at room temperature was also carried out under the same conditions. The solubility of ThC_2 at room temperature was 1.8 per cent in 12 *N* sulphuric acid, whereas at 80° C it was 6.1 per cent. The effect of temperature on the solubility of ThC_2 is small, and this means that the principal factor for sulphuric acid leaching is the solubility of $Th(SO_4)_4$ in sulphuric acid.

(ii) *Nitric acid leaching of ThC_2 at 80° C.* In the case of 8 *N* nitric acid, the solubility of ThC_2 at room temperature was 19 per cent and rose to 99 per cent at 80° C.

Table 1. EFFECT OF TEMPERATURE ON DISSOLVING OF ThC_2 IN 8 *N* NITRIC ACID AND 12 *N* SULPHURIC ACID

Acid	Leaching temp.	Leaching time (hr.)	Dissolved Th (per cent)
H_2SO_4	At room temp.	7	1.8
	At 80° C	7	6.1
HNO_3	At room temp.	7	19
	At 80° C	7	99

The effect of temperature on ThC_2 dissolution into 8 *N* nitric acid and 12 *N* of H_2SO_4 are shown (Table 1).

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LETTERS TO THE EDITOR

GEOPHYSICS

Space Density of Dust in the Stratosphere

THE Smithsonian meteoritic dust programme has included a number of aerosol collections made by various aircraft in different altitude ranges These include collections in the range 40,000–48,000 ft by a *B*-52, roughly 60,000 ft by a *U*-2, and 51,000–87,000 ft by an *F*-104*A* The purpose of these collections was to obtain samples of meteoritic material, both so that we could estimate the rate of accretion by the Earth of extraterrestrial matter and analyse it chemically

Our first step has been to investigate the space density of dust particles of all types as a function of height and of time The space density was calculated simply by dividing the total number of particles of a certain size-range by the volume encountered by the opening of the collector, taking into account the efficiency of the collector Wind-tunnel tests provide us with a set of values for the efficiency of the collector under various conditions of velocity and altitude

Table 1 MEAN SPACE DENSITIES

Aircraft	Height (ft)	Mean space densities (particles > 3μ diam /m ^{3})
B-52	40,000–48,000	4,000
U-2	60,000	2,800
F-104*A*	51,000–87,000	1,000

The true space densities for atmospheric particles are given in Table 1 The values for the lower altitude flights were computed from the counts of particles of all types, from which background counts as determined from unexposed (but flown) filters were subtracted The original counts were for particles with mean dimensions greater than 6μ These we transformed into counts for particles larger than 3μ by means of the known size distribution[1] To derive the space densities tabulated, we then divided the counts by the effective volume of air encountered The tabulated results clearly show a decrease of particle space density with altitude

Meteoritic spherules Small, spherical particles that strongly resembled, both in appearance[1] and chemical composition[2], meteoritic droplets such as are found surrounding falls of larger meteorites[3] were discovered on five *B*-52 filters Because of recent wind tunnel tests, the previous calculations of the rate of fall of such particles to the surface must be revised upward The correct space density of these particles is approximately $3/\text{m}^3$ at 45,000 ft If as Stokes's law would indicate, they fall at the rate of approximately 1 cm /sec from this altitude, the expected rate of fall on the Earth's surface is 0 3 spherule/cm ^{2}/day This is not significantly different from the actually observed rate of fall of such particles in the Arctic[4], which is approximately one spherule/cm ^{2}/day We believe that the essential agreement of these greatly different experiments is good evidence that our conclusions about the meteoritic origin of the particles are correct Furthermore, the present results indicate that approximately 2×10^8 kgm of this kind of meteoritic material fall to the Earth a year (using standard assumptions[2]) This is not inconsistent with our previous value of 5×10^8 kgm /yr (ref 4) and is in excellent agreement with the results of Crozier[5] and Thiel and Schmidt[6], who collected spherules from land and ice sediments, respectively

F W WRIGHT

Smithsonian Astrophysical Observatory
and Harvard College Observatory

P W HODGE

Smithsonian Astrophysical Observatory
and University of California at Berkeley

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Equatorial Micropulsations and Ionospheric Disturbance Currents

THE early evening maximum of occurrence of regular geomagnetic pulsations, *pc*, of period 20–30 sec , was first detected on the Earth current recordings obtained at Legon, Ghana, during the International Geophysical Year[1], but more recently, Cardus[2] has confirmed that the occurrence of evening *pc* is also a feature of micropulsational activity at other equatorial stations No satisfactory hypothesis has yet been proposed to explain why this type of geomagnetic activity is a day-time phenomenon at all mid-latitude stations, with a maximum of occurrence and amplitude at approximately mid-day, while, in the region of the magnetic equator, there is an evening maximum of both occurrence and amplitude, with lesser maxima at mid-day and early morning[3] Several attempts have, however, been made to relate the occurrence of *pc* pulsations with ionospheric disturbances, and interesting results have recently been obtained by Campbell and Matsushita[4], who suggest that the onset of micropulsation storms is associated with an increase in ionospheric disturbance, and by Hope[5], who puts forward the proposal that the movement pattern of the ionospheric disturbance currents produces maximum geomagnetic activity at different times of the day at high, middle and low latitudes

So far as the Legon micropulsation results are concerned, it has been considered for some time that the evening maximum of *pc* activity must be associated in some way with the other known equatorial disturbance phenomena, but previous attempts at correlation of *pc* occurrence at Legon with the ionospheric parameters from a neighbouring station have proved unfruitful However, following the ideas suggested by Campbell, Matsushita and Hope, the correlation between *pc* activity and the disturbance daily variation, S_D, has recently been determined and, in this case, the results are encouraging

Using the continuous recordings of the Earth current potential gradients at Legon between April 1958 and March 1959, the disturbance daily variation of the Earth currents has been determined, and in Fig 1 the resultant amplitudes of the S_D potential gradients for each hour are given The daily variations of (*a*) the

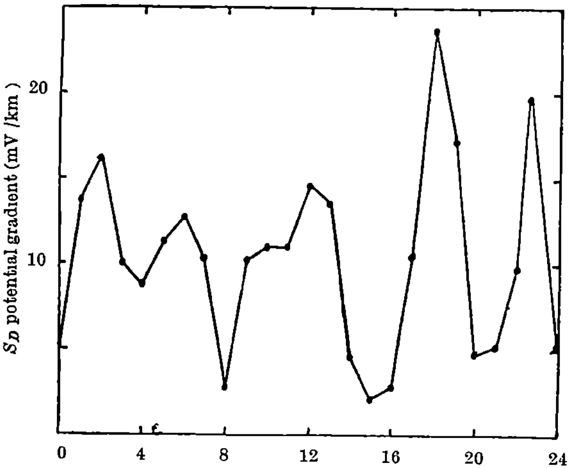

Fig 1 The annual mean S_D variation of Earth current potential gradients at Legon

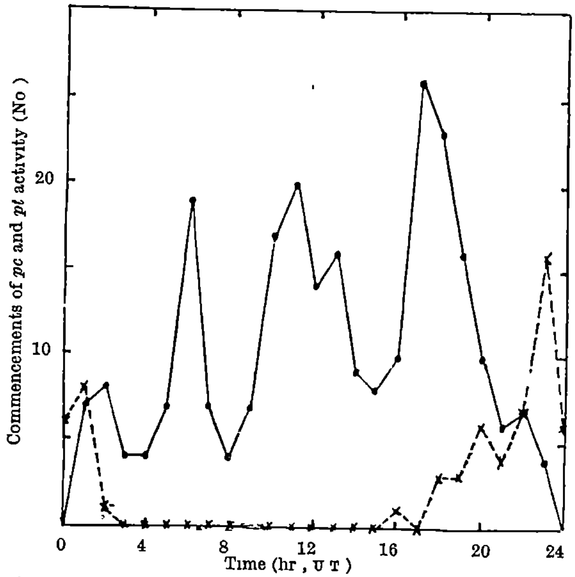

Fig 2 The daily variation of the number of commencements of *pc* and *pt* activity during one year at Legon ——, *pc* pulsations, – – – –, *pt* pulsations

frequency of occurrence of *pc* pulsations, of amplitude greater than some arbitrary fixed level, and (*b*) the number of commencements of such pulsational activity during each hour were also obtained from the same Earth current records Both variations (*a*) and (*b*) were found to agree reasonably well with the S_D variation, the agreement being particularly good for variation (*b*), which is given in Fig 2 Examination of Figs 1 and 2 suggests that there is a close correspondence between the probability of onset of *pc* pulsational activity and the magnitude of the disturbance daily variation of the Earth current potential gradient at the same station The correlation coefficient between these two variables has been determined for the time-interval 3–21 hr and found to be 0 6 As this value is highly significant, it may be concluded that the most probable times of onset of geomagnetic activity of type *pc* coincide with times of S_D maxima, that is, 6 hr, 12 hr and 18 hr On the other hand, as the correspondence for the night maxima of S_D is not so good, it is suggested that the night maxima are related to some other form of pulsational activity For example, *pt* pulsational activity is known to have a maximum of occurrence frequency at approximately 22 hr, the daily variation of the number of occurrences of *pt* at Legon during a 12-month period being represented by the dotted line in Fig 2 From a comparison of Figs 1 and 2 for the 6-hr period around midnight, it seems reasonable to conclude that the S_D peaks at 2 hr and 22 hr are associated with *pt* activity

In considering the S_D variation of Fig 1, it must be remembered that the Earth current values reflect the rate of change of the magnetic field variations and hence also the rate of change of the currents in the ionosphere Investigations of the S_D variation of the Earth currents at Legon thus indicate that the disturbance currents in the equatorial ionosphere experience their most rapid change at approximately 18 hr, and that at 2 hr, 6 hr, 12 hr and 22 hr, there are additional large changes in the ionospheric current strength It may also be noted that the S_D Earth current flow at Legon has been found to be more or less uni-directional, but with the mid-day and early evening current flowing from north to south and the night-time current flowing in the reverse direction[6]. There is thus a tendency for the north-to-south current flow to be associated predominantly with *pc* activity and the south to north flow with *pt* activity

The correlation between the commencement of *pc* activity and increase in ionospheric disturbance currents is thus well established at this equatorial station and agrees well with the auroral zone studies of Campbell and Matsushita and also with the comments made by Hope[7] on the Earth current S_D variation at Kakioka However, the estimation of the extent to which the movement of the S_D current may account for the mid-day maximum of *pc* activity at mid-latitude stations and the evening maximum at the equator requires an examination of similar data from a wide network of stations

Rosemary Hutton

Department of Physics,
University of Ghana

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PHYSICS

Core Kinetics of Fission Reactors

Our theoretical and experimental work on the core kinetics[1] of water-moderated fission reactors has been continued

The autocorrelation function of reactivity fluctuations in a reactor operating at low power has been computed from a record of the fluctuations (Fig 1) The experimental results support the simplified kinetic theory For a reactor in which delayed neutrons are neglected, this predicts a power spectral density $W(f)$ of the form

$$W(f) = \frac{A}{1 + \omega^2 \left(\frac{l}{\beta}\right)^2} \qquad (1)$$

and an autocorrelation function $\psi_{aa}(\tau)$ of the form

$$\bar{\psi}_{aa}(\tau) = \frac{A}{4\left(\frac{l}{\beta}\right)} e^{-\frac{\beta|\tau|}{l}} \qquad (2)$$

where A is a constant, l is the mean effective prompt neutron lifetime, β is the delayed neutron fraction, ω is the angular velocity and τ is the correlation time The departure of the function from the exponential form indicates the presence of higher frequency components in the core fluctuation The apparent periodicity is inherently linked with the nuclear events which take place within the core

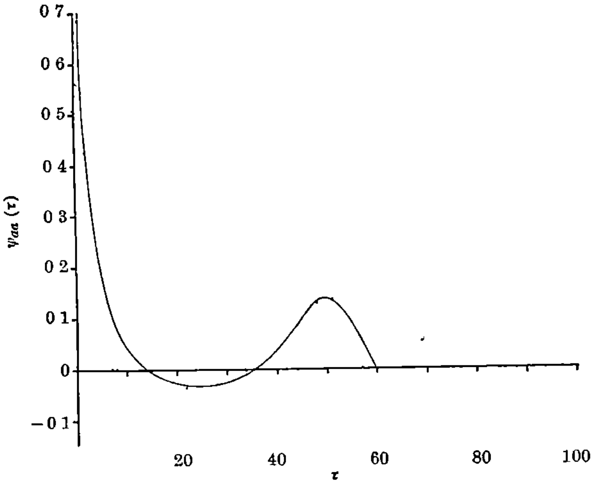

Fig 1 Autocorrelation function of reactor fluctuation (τ = sec 10^{-2})

In Fig 2 is shown the proposed experimental arrangement for the study of core kinetic fluctuations by means of cross-correlation Correlations may be measured between nuclear and non-nuclear parameters and system interactions may be studied without any major disturbance to normal reactor operating conditions Provided that the assumptions concerning system linearity are valid, the following integral relationships now hold for the reactor core being examined

$$\psi_b(t) = \int_0^\infty \psi_a(t-\tau)h(\tau)\mathrm{d}\tau \qquad (3)$$

$$\psi_b(p) = n(p)\psi_a(p) \qquad (4)$$

$$\bar{\psi}_{aa}(\tau) \approx W(f)\delta(\tau) \qquad (5)$$

$$\bar{\psi}_{ab}(\tau) = \int_0^\infty \bar{\psi}_{aa}(\lambda_M-\tau)h(\tau)\mathrm{d}\tau \qquad (6)$$

where, in this case, $W(f)$ is the power spectral density of ψ_a, $\delta(\tau)$ is a delta function, $\bar{\psi}_{ab}(\tau)$ is the cross-correlation function, $h(t)$ is the reactor impulse response, $n(p)$ is the reactor transfer function and p is the Laplace transform variable The driving function $\psi_a(t)$ is designed to introduce a pseudo-random fluctuation into the reactor core Its autocorrelation function is, approximately, a delta function

This investigation, together with measurements concerned with the propagation of periodic and pulsed signals in a reactor core and the variance of a reactor control channel, is being carried out in collaboration with the Research Reactor Group at Harwell[2]

The objective of these experiments is to develop new techniques for (1) The measurement of reactor system parameters (2) The characterization of nuclear reactor types by means of such functions as their 'impulse response' and the systematic monitoring of these characteristics for incipient instability.

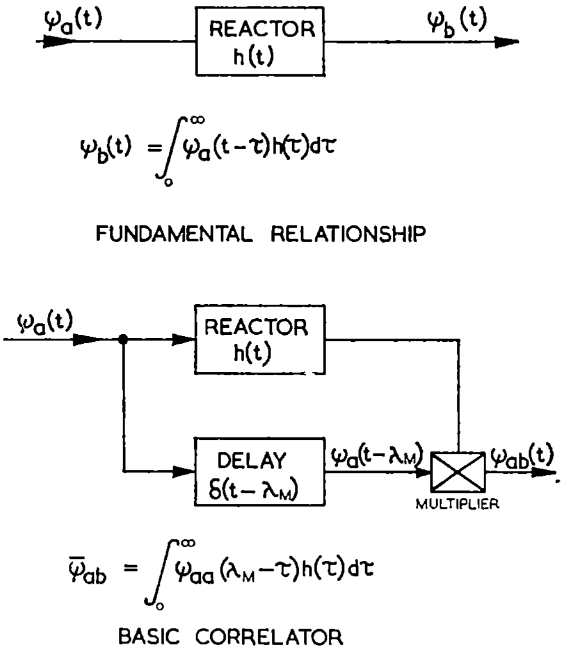

Fig 2 Schematic of instrumentation

L G KEMENY

Nuclear Engineering Laboratory,
Queen Mary College,
University of London, E 1

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Half-Lives of Carbon-10 and Neon-19

THE half-lives of carbon-10 and neon-19 are not well known Only two measurements of the half-life of carbon-10 have been reported, the first of 8 8 sec [1] was in error due to an impurity in the boron powder used; the second, due to Sherr *et al* [2], was 19 1 ± 0 8 sec In the case of neon-19, values ranging from 17 4 ± 0 2 sec [3] up to 20 3 ± 0 5 sec have been reported[4]

During the course of experiments on the reactions ^{10}B(p,n)^{10}C and ^{19}F(p,n)^{19}Ne it became possible to measure the carbon-10 and neon-19 half-lives for β^+ decay by using an *R I D L* 400-channel pulse height analyser (model 34–12) in its time analyser (multichannel scaler) mode of operation As a timing unit a 100-kc /sec crystal oscillator, having a stability better than 1 part in 10^5, was scaled down to provide a pulse which advanced the analyser address every 2 sec

For measuring the half-life of carbon-10 a 200–250 μgm /cm ^{2} self-supporting boron-10 target (we are indebted to Mr F A Howe and associates at the U K Atomic Weapons Research Establishment, who made this target) was irradiated for 60 sec with 0 1 μamp of 7 5 MeV protons from the Canberra tandem accelerator, and the decay of the induced activity followed for 290 sec , that is, about 15 half-lives The β^+ decay of carbon-10 goes 98 4 per cent to the first excited state of boron-10 (0 717 MeV excitation) and 1 65 ± 0 2 per cent to the second excited state[5] Since the decay of carbon-10 is uniquely identified by the 0 717 MeV line, it was measured using a 3 in × 3 in NaI(Tl) crystal spectrometer feeding a single channel analyser with the window set over the relevant γ-ray photopeak By following the decay of counts in the window it was possible to obtain single component decay curves on a constant, low background To check that other activities were not affecting the results, several determinations were made by following the decay of the coincidence counting-rate from a coincidence unit of 0 2 μsec resolving time Two 3 in × 3 in NaI(Tl) crystals, placed on opposite sides of, and $\frac{1}{2}$ in from, the target, were used, one feeding a single channel analyser with a window over the 0 511 MeV photopeak and the other a similar analyser with a window over the 0 717 MeV photopeak By following the decay for many half-lives it was shown that in both methods the backgrounds were constant, and accurate subtractions could be made (Fig 1)

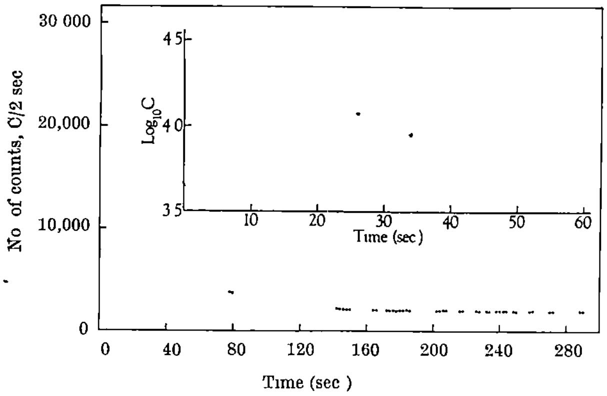

Fig 1 A typical decay curve for carbon-10, together with a logarithmic plot of the data after subtraction of the background This curve consists of the sum of a number of runs, in each of which counting loss and gain-change effects are negligible

A logarithmic plot of the results was made, and a straight line fitted to it by a statistically weighted least squares treatment, the slope of the straight line so obtained gave the half-life and its root mean square error directly Table 1 gives the results for the half-life of carbon-10 obtained for several independent determinations

Table 1

Half-life values for ^{10}C (sec)	Half-life values for ^{19}Ne (sec)
19 37 ± 0 12	17 40 ± 0 13
19 55 ± 0 12	17 48 ± 0 13
19 62 ± 0 12	17 29 ± 0 13
19 63 ± 0 15	17 32 ± 0 13
19 33 ± 0 12	17 64 ± 0 13
19 40 ± 0 12	

For measuring the half-life of neon-19 the fluorine-19 target was obtained by vacuum evaporation of manganous fluoride on to a 300 μgm /cm ^{2} gold foil, and was irradiated with 0 1 μamp of protons at approximately 4 8 MeV The decay of neon-19 goes 100 per cent to the ground-state of fluorine-19 (ref 4), and hence the decay was followed with the single channel analyser window covering the 0 511 MeV annihilation γ-ray photopeak For the check, the decay of coincidences between the annihilation quanta was followed This did not exclude from the measurement other positron activities present on the target However, the linearity of the logarithmic plots indicated that the effect of any such impurities was negligible The data were analysed as before, and the values obtained are given in Table 1

From these data the half-lives and their standard errors were determined as

$$\tau\,(^{10}\text{C}) = 19\ 48 \pm 0\ 05 \text{ sec}$$
$$\tau\,(^{19}\text{Ne}) = 17\ 43 \pm 0\ 06 \text{ sec}$$

L G Earwaker
J G Jenkin
E W Titterton

Research School of Physical Sciences,
Australian National University,
Canberra

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Temperature Coefficient of Electrical Resistance of High-Purity Rhodium

The temperature coefficient of electrical resistance between 0° C and 100° C , $\frac{(R_{100} - R_0)}{100\,R_0}$, is an accepted criterion of the purity of platinum Very high-purity platinum, such as is used in the manufacture of resistance thermometers and thermocouples, has a temperature coefficient approaching 0 003927, and this represents the purest platinum commercially available For rhodium, the other element used in standard thermocouples, the temperature coefficient of electrical resistance is not at present used for assessment of purity, but measurements made by us indicate that it might be a useful criterion for this metal also The highest value recorded in the literature[1] for the temperature coefficient of electrical resistance, between 0° C and 100° C , for high-purity rhodium is 0 00457

Recently, wires made from two samples of rhodium powder produced by the Mond Acton Refinery were found to have temperature coefficients considerably greater than that value, and the purity of these samples has been determined by mass-spectrographic analysis (determined by Dr T A Davies, Birmingham Research Laboratory, International Nickel Co (Mond), Ltd) and correlated with temperature coefficient One of the powder samples was of normal commercial grade, selected because spectrographic analysis indicated a low impurity content, the second sample, which had been specially prepared, was known, from neutron-activation analysis[2], to have a low iridium content

The resistivities were determined at the ice and steam points, on resistance thermometers wound from wires 0 0068 in in diameter, and having a resistance of about 1 5 ohms at 0° C The wire was fabricated from the rhodium powder by vacuum-sintering hydrostatically pressed powder compacts at 1,500° C , and rotary swaging at 1,100°–1,200° C to rod 0 125 in in diameter The rod was hot-drawn through tungsten-carbide wire dies to 0 015 in diameter, and then cold-drawn to the final size The values of the temperature coefficient of electrical resistance and the mass-spectrographic analyses of

Table 1 TEMPERATURE COEFFICIENT OF ELECTRICAL RESISTANCE AND MASS-SPECTROGRAPHIC ANALYSES OF HIGH-PURITY RHODIUM

	Commercial purity rhodium	Specially purified rhodium
$\frac{R_{100} - R_0}{100\ R_0}$	0 00461	0 00463
	Atomic (p p m)	
Pb	0 2	0 12
Au	0 2	0 6
Ir	30	3 5
W	0 15	<0 1
Te	9	0 25
Sb	0 4	0 2
Sn	2 5	0 4
Ag	0 5	1
Pd	40	4
Ru	45	5 5
Sr	2 5	2
Se	0 9	<0 06
As	2	0 25
Zn	6	7 5
Cu	50	12
Ni	20	12
Co	2	0 5
Fe	700*	100
Mn	5	1 5
Cr	40	10
Ti	8	8
Ca	1,000*	2,000*
K	30	30
S	25	22
P	20	18
Si	400	300
Al	250	140
Mg	12	6
Na	12	6
B	10	5
Pt	3†	1†

* Denotes evidence of segregation

† These values are a maximum since all or part of the content may have been due to surface contamination, arising from the handling of the samples with platinum-tipped tongs

The following elements were not detected in either sample

Element	Limit of detection (p p m atomic)
Bi, In, Tm, Ho, Tb, Pr, La, Y, Sc	0 03
Tl, Ce, Ba, Rb	0 04
Re, Ga	0 05
Eu, Zr	0 06
Os	0 075
Hf, Er, Cs, I, In, Nb, Ge	0 09
Hg, Yb, Cd	0 1
Dy, Sm, Nd	0 11
Gd	0 13
Mo	0 18
V	uncertain

Table 2

Iridium content of rhodium (p p m atomic)

	Neutron activation analysis determined on powder	Mass-spectrographic analysis determined on wire
Specially purified rhodium	2	3 5
Commercial purity rhodium	92	30

the wires are given in Table 1 The iridium contents of the samples determined by mass-spectrographic analysis and neutron-activation analysis, are compared in Table 2

The mass-spectrographic analyses do not give an absolute determination of the impurities present since standards were not available, but the relative levels of any element in the two samples are reliable

The lower impurity content of the specially purified rhodium compared with that of the commercially purified material is clearly shown It is believed that the lower contents of iridium, ruthenium and iron in particular contribute to the improvement of the temperature coefficient

High calcium, aluminium and silicon contents, as found in these samples, have been noticed also in high-purity platinum[3], they are possibly due to segregated particles of oxide, from which preferential sparking occurs during mass-spectrographic examination The iron contents of the rhodium wires are also comparable with those found in high-purity platinum, and it is considered that the iron is not due to any contamination during hot-working procedures used on the rhodium

E G PRICE
B TAYLOR

The International Nickel Co (Mond), Ltd ,
Development and Research Department,
Bashley Road,
London, N W 10

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GEOLOGY

Aragonitic Fossils in the Lower Carboniferous of Scotland

ALTHOUGH many invertebrates form skeletons partly or wholly of aragonite, this normally inverts to calcite within a geologically negligible period of time, a few tens of thousands of years at the most[1] Indeed, more than 50 per cent of the aragonite laid down by some of the marine invertebrates studied by Lowenstam[2] inverted to calcite within a year As the geothermal gradient lies completely within the stability field of calcite it has been maintained that aragonite within buried sediments could exist only in peculiar local conditions of high hydrostatic pressure and moderate temperature[3] Aragonite in rocks of a respectable age may therefore be presumed rare, and any record of such an occurrence is of interest both because of the problems posed by the preservation of this metastable mineral and the desirability of obtaining unaltered shells for palæo-ecological studies

Initially in this department finely preserved nacreous shell material of ammonites from the Cretaceous Speeton and Gault clays was found to consist wholly of aragonite This form of preservation, not uncommon in some Tertiary and younger Mesozoic rocks, was afterwards recognized in a small quantity of material from the Palæozoic, where it is very rare, and it is with this that the present communication is concerned

The fossils occur in three specimens of shale, in the collection of H M Geological Survey in Edinburgh, from the Cot Castle (or Basket) Shell-bed in the Lower Carboniferous, Upper Oil Shale Group, zone P_1 of Currie[4] *M* 2132^L and *M* 2135^L come from a depth of 263 ft in the No 1 bore, Baads Mine, and *E* 8010 from about 150 ft depth in the Fraser No 1 shaft, West Calder, West Lothian Fragments of the shells of several molluscs, of either nacreous or chalky appearance, were subjected to X-ray analysis using the following technique

Samples were obtained by breaking or scraping material from the shells into an agate mortar and grinding by hand with an agate pestle under acetone No inversion of calcite to aragonite occurs under these conditions Smear mounts on glass slides were prepared and the samples tested on a Phillips *PW* 1050 X-ray goniometer, using automatic chart recorder, filtered copper radiation, slits 1° and 0 1 mm , scan-rate 1° 2θ per min , range scanned 10°–50° 2θ Approximate *d*-spacings and relative intensities were obtained from these charts and compared with data cards in the A S T M powder data file

Aragonite and calcite were the only phases giving prominent reflexions

The nacreous shells of a goniatite (*Beyrichoceratoides*) and an orthocone nautiloid were found to consist wholly of aragonite while corresponding shells of microgranular chalky appearance consist of mixtures of variable amounts of aragonite and calcite The chalky shell of a zygopleurid gastropod was also found to consist of both minerals It appears, therefore, that breakdown of the nacreous shells has been accompanied by partial inversion to calcite, though possibly in the case of the gastropod the original shell consisted of a mixture of the two minerals, as in some modern forms It is worthy of note that in recent aragonitic shells the fine structure tends to break down before any inversion occurs[1]

According to Mr R B Wilson, preservation of nacreous or chalky shells is very exceptional in the Scottish Carboniferous Usually the shells have been dissolved away or replaced by calcite or pyrite It is not clear why aragonite should have been preserved in this instance It may be partly a consequence of the impermeable character of the shale matrix of the Cot Castle Shell-bed in particular and the Oil Shale Group in general, together with the absence of surface weathering The shale of the matrix seems, however, to lack exceptional features In thin section it is seen to be homogeneous and unlaminated, with scattered pyrite granules and rare fragments of detrital quartz An analysis by the Geochemical Department of the Grant Institute indicated some 26 per cent of calcium carbonate, 2 9 per cent of organic carbon and 2 5 per cent of sulphur The mineral composition of carbonate, which consists in part of small shell fragments, was not determined

This seems to be the oldest recorded occurrence of aragonite The only other certain Palæozoic occurrence known to us was reported by Stehli[5], who records aragonitic molluscs in Upper Carboniferous (Pennsylvanian) shales and asphalt of Oklahoma and Kentucky

We thank Mr R B Wilson for making available the fossils and providing useful information

A HALLAM
M J O'HARA

Grant Institute of Geology,
University of Edinburgh.

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Inclusions in Garnets

RECENT developments in structural mapping in the Dalradian rocks of Scotland have led to the re-interpretation of regional metamorphism and recognition of its phase-by-phase development[1-5] In this respect, textural studies have proved particularly useful in so far as relict textures are preserved as inclusions within porphyroblasts, thus permitting a recognition of fabrics[6], which in the rock as a whole, owing to subsequent recrystallization, have disappeared In the middle-grade zones of regional metamorphism of Dalradian rocks, garnet porphyroblasts are particularly abundant, and their inclusions can be grouped into the pre-porphyroblast, the syn-porphyroblast and the post-porphyroblast

The pre-porphyroblast (residual) inclusions can be divided into two types (*a*) dimensionally strongly orientated inclusions of mainly quartz, feldspar, calcite and ores which trend at an angle to the foliation outside the porphyroblast, (*b*) dimensionally weakly orientated inclusions of mainly quartz, feldspar and calcite showing triple junctions and having the trend which parallels or is continuous with the external fabric In hundreds of examples considered by us, the former inclusions have a grain-size much smaller than the grain-size of the fabric outside the porphyroblast, while the latter inclusions have a grain-size comparable to that of the minerals outside the porphyroblasts It is suggested that in the former case the garnet with its internal fabric belongs to an episode of metamorphism earlier than the deformation which has caused the rotation of the garnet with respect to the external fabric In the latter case, however, the garnet has grown under static conditions and belongs to essentially the post-tectonic episode of crystallization and recrystallization responsible for the partial obliteration of the dimensional preferred orientation of the external fabric Identical geometric criteria can be applied to other porphyroblasts, including feldspars, kyanite, staurolite and chloritoid The degree of preservation of residual inclusion may depend on complexities of growth and dissolution, but it must be emphasized that their existence is independent of the existence of the porphyroblast

The syn-porphyroblast (cogenetic) inclusions are those which have been generated either in the matrix surrounding a growing porphyroblast or at the growing surface of such a porphyroblast The former type includes quartz, feldspar ores, sphene, etc, while the latter are of graphite, ores and sphene The pattern of the former type of inclusions is conditioned partly by concurrent deformation or crystallization and partly by the velocity of growth of the porphyroblast For example, if a porphyroblast is growing at the same time as the rock is subjected to deformation, *S*- or *Z*-shaped trends of inclusions will be observed within the porphyroblast. Clearly the tightness of the loops of the *S* will depend on the velocity of the growth of the porphyroblast with respect to deformation The pattern of the inclusions generated at the growing surfaces of porphyroblasts will depend on the shape of the crystals concerned In larger garnets, in particular, such patterns can be used to trace changes of the growing faces The experimental work of Stober[7] may be relevant to the growth of such inclusions When, however, Galwey and Jones[8] claim that we have advanced the view[1] that, contrary to Stober, "the high rate of crystallization results in the development of inclusions", they are misinterpreting our ideas, since we were dealing with residual inclusions in the process of dissolution and not cogenetic inclusions in a stage of growth

The post-porphyroblast inclusions in garnets can be divided into two groups those which have no obvious systematic relationship to the garnets and merely occupy cracks in it and those which show systematic crystal orientation with respect to garnets and are in the main micas and feldspars, which in certain cases replace the garnets entirely These inclusions, which have no bearing on relations of garnet growth to deformation, will be described elsewhere by one of us (N R), and have been called the penetration inclusions

Since there are numerous examples of combinations of these effects, in so far as concentric zones of garnets can show different inclusions, it is not surprising that Galwey and Jones find a wide variety of inclusions which they list without explanation. The position is further complicated by the fact that the most common post-tectonic garnets in the Dalradian of Scotland overgrow various textural patterns in the rock, resulting in preservation of complex inclusion trends which may be difficult to interpret on geometric relationships alone. Consequently in tracing the relationships of inclusions to garnets in addition to purely qualitative microscopic examination, the nature of deformation and recrystallization was estimated by petrofabric examination of the quartz inclusions and their comparison with the quartz of the matrix. This method enabled us to prove that features of preferred optical orientation still preserved in certain pre-porphyroblast types of inclusions cannot be discovered in the surrounding matrix. This more than anything else convinced us that the hypothetical pore solutions *A* and *B*, dendritic crystallization, etc., invoked by Galwey and Jones, and for which there is no evidence, are not factors of importance. In any event, our interpretations are based not just on garnets, but on all other minerals as well. We agree with Galwey and Jones that very great care must be exercised in interpreting inclusion fabrics. Indeed, the temptation to resolve difficulties by recourse to conjectural chemical reactions is not easy to resist.

N. Rast

Department of Geology,
University of Liverpool.

B. A. Sturt

Department of Geology,
Bedford College, London.

A. L. Harris

c/o Department of Geology,
University College of Wales,
Aberystwyth.

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MINERALOGY

Measurement of the Surface Conductivity of Mineral Grains

During an investigation into the principles underlying the electrostatic separation of minerals in a corona-discharge, roll-type separator, it was found necessary to measure the surface conductivity of individual mineral grains.

The apparatus used consisted essentially of two stainless steel rollers, each 0·5 in. in diameter and 2 in. long[1], mounted in polytetrafluoroethylene end-plates on a metal base (Fig. 1). One of the rollers was set eccentrically on its bearing stub so that the distance between the rollers could be set at half the diameter of the grain being measured, the minimum grain size being in practice about 72 B.S. mesh (211μ). The roller assembly, with an antimicrophonic lead fitted to each roller, was set in an earthed brass box fitted with inlet and outlet tubes which enabled air at a controlled relative humidity to be passed through the box. Humidity control was effected by a closed-circuit system, consisting of diaphragm pump, sintered glass bubbler containing a saturated solution of electrolyte[2], glass-wool spray trap, stainless steel gauze ion trap, rotameter, and the electrically screened brass box containing the rollers. The minimum amount of insulating material was used in the construction of the apparatus because it was found that static charges tended to build up on the insulators, giving false readings.

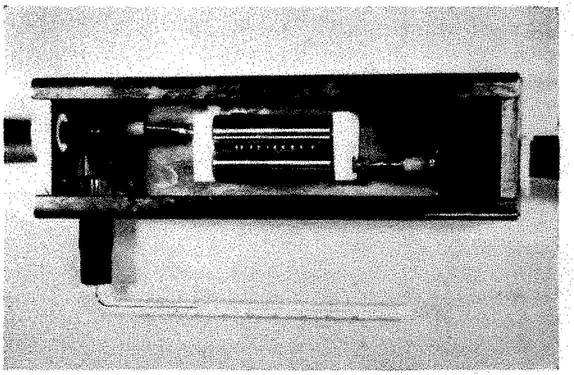

Fig. 1. Apparatus for measuring the surface conductivity of mineral grains

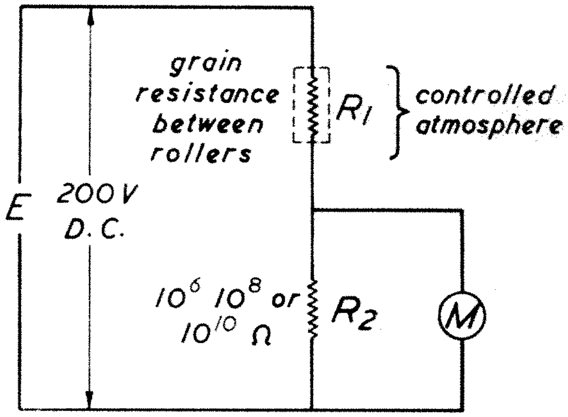

Fig. 2. Circuit for measuring surface resistance of mineral grains. *V*, Voltage; R_1, grains; R_2, resistance box; *M*, Vibron electrometer

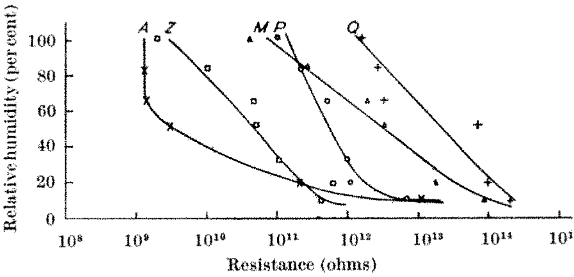

Fig. 3. Resistance of 10 grains as a function of relative humidity. *A*, Albite; *Z*, zircon; *M*, monazite; *P*, pyrochlore; *Q*, quartz

The equivalent electrical circuit used is shown in Fig. 2. The potential across the mineral grains was fixed at 200 V. d.c. (*E*), as this value gave reproducible results and also approximates to the potential difference existing across the grain when a charge has been acquired from the ion current in the corona discharge separator.

R_1 is the resistance of the mineral grains on the rollers, R_2 is a standard electrometer resistance box of 10^6, 10^8 or 10^{10} ohms, which in practice was always at least two orders of magnitude less than R_1. *M* is a vibrating condenser electrometer measuring the potential difference (V) across R_2. From the circuit constant and the measured value of V, $R_1 = ER_2/V$, since $R_1 \gg R_2$.

Mineral grains were closely sized, washed in distilled water, dried at 110° C., and conditioned at the selected relative humidity for at least 6 hr. Ten grains were placed between the rollers, the system was given at least 10 min. to settle, and the resistance was then measured. Ten measurements were normally made at each relative humidity value, namely, at 10, 20, 33, 52, 66, 84 and 100 per cent. A fresh set of grains was used for every measurement to obviate errors caused by ion transport or polarization in the mineral grain[3].

When plotted, the curves of log resistance against per cent relative humidity were linear or gently curved. The results were found to be reproducible to within half an order. This apparently low reproducibility is due to the fact that the property being measured is the resistance of an insulator, at best a somewhat dubious parameter. Typical results are shown in Fig. 3, each point on the graph representing the mean of ten readings. Our curves differ from those obtained by Kakovsky and Revnivtsev in that only in a few cases are they strictly linear, and that some minerals attain a low limiting value of resistance due to saturation of the mineral surface by a water film at high relative humidities.

L. Gilbert
G. R. Hobbs

Warren Spring Laboratory,
Department of Scientific
and Industrial Research,
Stevenage, Herts.

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Cell Dimensions and Interatomic Forces in Layer Lattice Silicates

The factors which control the sheet dimensions of the layer lattice silicates have been carefully re-examined recently[1], both from a structural point of view[2], and by the methods of multiple regression analysis[3]. This has led to a new, more explicit geometrical model[4] for the octahedral layers of these minerals, and thence to a detailed study of the balance of forces which must be involved. The results of the latter are outlined here.

Megaw, Kempster and Radoslovich[5] have attempted to understand the felspar structure, anorthite, by treating the network of bonds and bond-angles rather as a problem in statics, comparable with the design of bridge trusses. The same kind of approach is profitable for the layer lattice silicates, with the following general conclusions.

(1) The primary control of all three dimensions, *a*, *b* and *c*, is vested in the octahedral layer. In this layer the highly charged cations are only partly shielded electrostatically from each other across the shared octahedral edges. There is a strong mutual repulsion which is balanced by the limit of stretching of the cation–anion bonds, and the limit of compression towards each other of pairs of anions in these shortened shared edges (in accordance with Pauling's rules).

(2) Other factors exert control at a secondary level, or in extreme cases in an over-riding fashion. These factors include: (*a*) interlayer cation-surface oxygen bonds; (*b*) net surface charge (for example, cation exchange capacity effects); (*c*) net octahedral layer charge; (*d*) polarization of anions, particularly surface hydroxyls; (*e*) interlayer hydrogen bonding; (*f*) surface hydroxyl–hydroxyl bonding; (*g*) limitations of tetrahedral deformation, governed by bond-lengths and anion–anion compression. I believe that detailed examples of each may be found in various layer silicate structures already determined accurately.

(3) For several quite accurate structures the reported minor variations in bond-lengths—both within one structure, and between structures—appear to be consistent with these general concepts. The variations apparently may be 'explained' by treating these minerals as essentially ionic structures and then considering the effects on the bonds of small but known variations in effective electrostatic valency. Very similar arguments have been used by other authors in discussing several rather unrelated inorganic structures determined with notable precision recently.

(4) If these ideas are substantially correct then they: will allow much better prediction of trial structures of layer lattice silicates; should give convincing explanations for observed composition limits for the naturally occurring minerals[6]; cause considerable rethinking about the forces governing the morphology of kaolins and serpentines[7]; and have real bearing on the relative stability under weathering, and on the observed polymorphism, of the micas.

I thank Dr. K. Norrish, Division of Soils, and Mr. L. G. Veitch, Division of Mathematical Statistics, C.S.I.R.O., for their assistance.

E. W. Radoslovich

Division of Soils,
Commonwealth Scientific and
Industrial Research Organization,
Adelaide.

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ELECTRICAL ENGINEERING

Electrode Phenomena in Magnetohydrodynamic Power Generation

Recently[1] Maycock, Noé and Swift-Hook have described some results obtained in a magnetohydrodynamic power generation experiment involving the use of a rocket motor and a kerosene–oxygen fuel mixture seeded with potassium. Both carbon and water-cooled copper electrodes were used, with the unexpected result in the latter case that high conductivities could be obtained despite the low electrode surface temperature of about 400° C. A possible explanation was suggested in terms of a thin carbon layer found on the electrode surface at the end of the experiment. It was thought that this layer might provide thermionic emission of electrons into the gas stream and thus permit 'electronic' conductivity to apply to this situation.

Working with cold electrodes in air plasmas produced by an electrically driven shock tube[2], we have observed similar high values of conductivity, but in a situation in which no carbon is present.

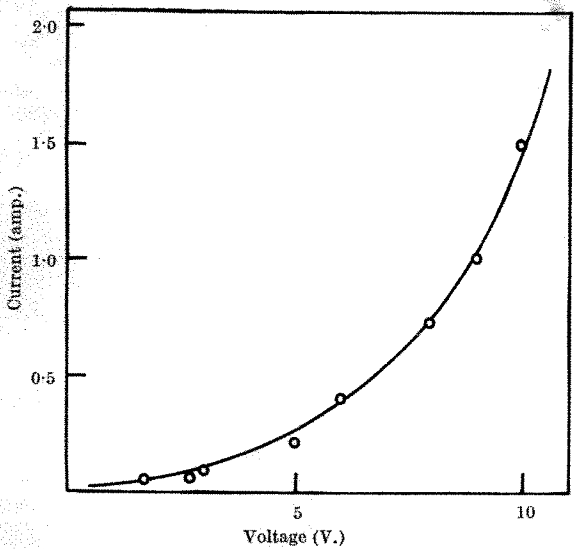

Fig. 1. Current-voltage characteristic for an air plasma produced in an electrically driven shock tube. Parallel disk probes, 0·5 cm. diam., 1·4 cm. apart. Initial air pressure = 1 mm. mercury; shock speed = Mach 12 (equilibrium temperature, 4,200° K. approx.)

Typical results for the current flowing between two parallel disk electrodes as a function of applied voltage are shown in Fig. 1. The resistance between the probes at high current densities (of the order of 50 amp./cm.[2]) is found to correspond to the 'electronic' conductivity of the air plasma, after allowing for fringing effects. However, at lower current densities the resistance is very much higher and indicates the presence of sheath voltage drops at the electrodes. Similar results have been obtained for various electrode materials (for example, nickel, brass, and gold).

The physical processes capable of providing continuity of 'electronic' current at the cathode at high current densities are believed to be complex and may involve several different phenomena. An analogous situation exists at the cathode of the cold-cathode arc, for which many hypotheses have been proposed[3]. That favoured by us for the present situation involves collision ionization in a thin layer of gas adjacent to the cathode when the field strength (and thus current density) is high enough. At still higher field strengths this region extends to fill the whole space between the electrodes, but does so without the sharp transition normally separating the 'dark' discharge region from a glow discharge and spark breakdown. Typical oscilloscope records of current are shown in Fig. 2, with multiple exposures superimposed to show the different effects. At low field strengths the current pulse lasts only for the duration of the shock-heated gas between the probes (approximately 10 μsec.); but at higher field strengths the plasma can remain highly conductive for periods long after the initial hot gas has passed the probes. The current finally extinguishes at different times from shot to shot presumably owing to variable diffusion and recombination processes.

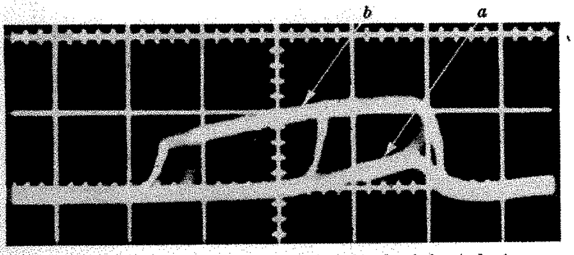

Fig. 2. Current flow between probes in shock-heated air as a function of time. (Time reads from right to left. Scales: 0·5 amp. per division; 10 μsec. per division.) Initial air pressure = 0·2 mm. mercury; shock speed = Mach 9 (equilibrium temperature = 3,000° K. approx.). *a*, Low field strengths; *b*, high field strengths, with breakdown

Thus the interesting possibility is raised of using not only cooled electrodes but also the enhanced conductivity of secondary ionization processes for magnetohydrodynamic power generation. Under high-temperature conditions the electric field strengths required to create breakdown are very small compared to normal cold gas situations. This proposition has been discussed by Westendorp, Bishop, Hurwitz, Goldman and Ben Daniel[4], who also present some interesting results for non-thermal ionization in helium plasmas seeded with cæsium. Similar breakdown in high-temperature gases at low field strengths is being observed in steady-state experiments being carried out here with argon between graphite electrodes. In the latter case high equilibrium conductivities are also being measured (approximately 10^8 times the theoretical argon conductivity for the same temperature and pressure), suggesting that impurities from the graphite electrodes (commercial grades) may in fact be seeding the gas. Further experiments with very pure graphite (reactor-grade *A*) are being carried out to establish whether this or some other mechanism is the explanation for the high conductivity.

We acknowledge the financial support for the energy-conversion project at the University of Sydney provided by the Electrical Research Board and the Australian Institute of Nuclear Science and Engineering, which is making possible this experimental programme.

D. W. George
H. K. Messerle
School of Electrical Engineering,
University of Sydney.

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CHEMICAL ENGINEERING

Detonation in Ammonium Nitrate

It has been appreciated for many years[1-5] that ammonium nitrate, even when free from combustible material, can detonate. This is not surprising, since the products of decomposition must be entirely gaseous and have substantially less energy of formation than the original substance. For example, if the nitrogen is not oxidized, the energy released at constant volume, with water in the vapour phase, is 380 cal./gm. or 8·7 kcal. per mole of gas. This is, of course, rather low compared with typical values for explosives, which lie in the range 20–50 kcal./mole gas. The temperatures and pressures reached in the detonation wave will therefore also be relatively low, and one might expect the substance to detonate with difficulty and only under good confinement. This is found to be so. For normal material at densities around 1 gm./cm.[3], cartridge diameters of many inches or heavy metal tubes are necessary[6,7], and even then the measured speeds are much lower than the maximum values calculated for products $N_2 + 2H_2O + \frac{1}{2}O_2$. This has led some authors[8] to conclude that an equilibrium involving oxides of nitrogen is more appropriate, and that the release of energy is of order 230 cal./gm.

It is well known that reduction in the loading density of an explosive makes propagation easier, and this can be explained in a general way by the increase in compressibility, so that, although the overall energy yield is no greater, more extreme conditions arise in the wave-front. Velocities greater than 2 km./s. have been reported[9] for an ammonium nitrate at 0·65–0·7 gm./cm.^{3} in steel tubes, particularly at elevated temperatures.

We have recently made some measurements on material the physical form of which results in an unusually low bulk density, 0·52 gm./cm.^{3}. This was loaded into light-weight cellulose acetate or cardboard tubes, and initiated by primers of Blasting Gelatine. Its behaviour was recorded by placing lead plates under the charge, and the detonation velocity measured by the Dautriche method at the larger cartridge diameters and with a streak camera at the smaller. The direct streak record was supplemented by a back-lit silhouette record because of the low level of light given out.

Steady propagation was recorded down to 2-in. diameter over at least a 2-ft. length of charge, and at $1\frac{1}{2}$-in. diameter over a 9-in. length. At 1-in. diameter the wave died out quickly. The velocities were as follows:

Charge diameter (in.)	$1\frac{1}{2}$	2	3	$5\frac{1}{2}$	$7\frac{3}{4}$
Detonation velocity (km./s.)	1·40	1·55	1·78, 1·95	2·05	2·23

When these velocities are plotted against reciprocal charge diameter, they are fitted quite well by a straight line, and extrapolation to infinite diameter indicates a limiting speed of 2·37 km./s. and a reaction time of around 7μs. Calculations by the method of ref. 6 with assumed products $N_2 + 2H_2O + \frac{1}{2}O_2$ predict the following maximum speeds:

Density (gm./cm.^{3})	0·5	0·6	0·7	0·8	0·9	1·0
Detonation velocity (km./s.)	2·36	2·56	2·76	2·98	3·22	3·48

Interpolation gives 2·40 km./s. at 0·52 gm./cm.^{3}. It seems, therefore, that this analysis describes correctly the behaviour of ammonium nitrate as a detonating explosive, at least at low charge density. Certainly, the observed speeds are too high to be reconciled with a release of energy of 230 cal./gm., which would imply a limiting velocity of only 1·87 km./s.

STEWART PATERSON
JEAN M. DAVIDSON

Imperial Chemical Industries, Ltd.,
Nobel Division,
Stevenston, Ayrshire.

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Bubbles in Fluidized Beds

GAS fluidized beds are characterized by 'bubbles' which rise through them, modifying the gas flow path and moving solid particles. Bubbles are largely responsible for solids mixing, for high bed to wall heat transfer-rates and for the pattern of gas/solids contacting, important in most chemical engineering applications of the technique.

We have recently taken X-ray ciné photographs of these bubbles. Glass spheres 0·5 mm. in diameter were fluidized by air in a 14-cm. diameter vessel. The bed was 24 cm. deep and the field of view was 12 cm. diameter centred about half-way up the bed. A pulsed

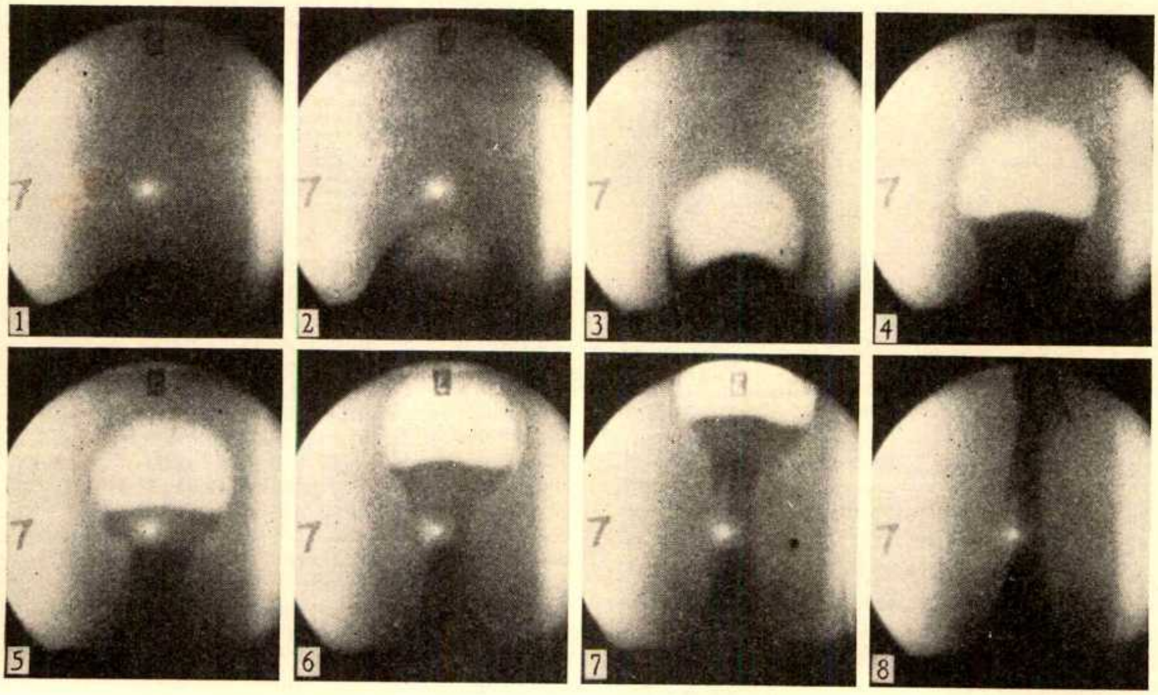

Fig. 1

X-ray source and an image intensifier were used and ciné photographs were taken at 25 frames per second with an exposure time of 1/100 sec.[1]. In one experiment, the lower half of the bed was made up of lead glass spheres and the upper half of soda glass with a horizontal interface between them originally. A bubble was filmed as it crossed this boundary, and the movement of a horizontal layer of particles was followed as well as the bubble itself.

The sequence of movement is shown in the series of photographs (Fig. 1). In the first, a bubble is lifting the darker lead glass spheres up and away from its path. Particles slip round the bubble and in the second picture it is just visible through a curtain of lead glass. The outline of the bubble is plainly visible in the third picture with the last of the lead glass draining from its lower edges. It is seen to be about 5 cm. in diameter. As it continues to rise, a spout of the identifiable lead glass is drawn up behind it and, in the fourth, fifth, sixth and seventh pictures, a wake containing the dark particles is seen to be travelling with the bubble. It finally leaves a vertical drift profile[2] surrounded by barb-like fragments shed from the wake. (The central light spot and light bands at the sides in all the photographs are optical effects and should be ignored.)

The bubble is empty and its shape corresponds closely to that of a sphere with the bottom cap (of height about 2/5 sphere diameter) removed. The wake region behind the bubble is seen to be defined approximately by this cap. The rate of rise of the bubble is in good agreement with Davies and Taylor's[3] value for gas bubbles in an inviscid liquid as proposed by Davidson and others[4]. The bubble is growing in diameter slightly as it rises. The final drift profile of the eighth picture has already been reported by Rowe and Partridge[5] and is a direct consequence of the streamline flow of particles around the bubble.

From other photographs we have seen that bubbles frequently split or coalesce so that measurements of bubble velocity that do not define the instantaneous bubble size and state may be misleading.

P. N. ROWE
B. A. PARTRIDGE
E. LYALL

Chemical Engineering Division,
Atomic Energy Research Establishment,
Harwell.

G. M. ARDRAN

Nuffield Institute for Medical Research,
University of Oxford.

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CHEMISTRY

Reaction of Hydrogen Peroxide and Nitric Oxide

RECENT work[1,2] on the nitrogen dioxide sensitized hydrogen–oxygen reaction has shown that at 360° C. the reaction:

$$HO_2 + NO \rightarrow OH + NO_2$$

is fast relative to other reactions of HO_2 in that system. On this evidence it was predicted[1] that at similar temperatures hydrogen peroxide and nitric oxide would react by a chain mechanism, since above 420° C. hydrogen peroxide decomposes homogeneously by a mechanism[3] involving the radicals OH and HO_2. This prediction has now been verified and a preliminary investigation of the reaction is described here.

The overall reaction was found to be:

$$H_2O_2 + NO \rightarrow H_2O + NO_2 \qquad (1)$$

and the initial rate of formation of NO_2 is given by equation (I).

$$\mathrm{d}P_{NO_2}/\mathrm{d}t = \frac{k.P_{H_2O_2}^{\,x}}{P_{N_2}} \qquad (I)$$

The value of x is dependent upon the nitrogen pressure and lies between 1·6 and 1·9. The initial rate is independent of both the nitric oxide pressure and the vessel diameter.

The reactions were examined in plane-ended cylindrical quartz vessels by continuous measurement of the nitrogen dioxide concentration and of the total pressure. The former was recorded using a double-beam photometer[1,4], and the latter with a bourdon gauge manometer[5]. 99·2 per cent w/w unstabilized hydrogen peroxide was used. The reactants were pre-mixed before admission to the reaction vessel, and an excess of nitrogen was present in all experiments.

It has been shown that NO_2 is rapidly formed from H_2O_2 and NO at temperatures from 297° to 473° C. Equation (I) summarized the results of a detailed investigation at 299° C. in which the NO pressure was varied from 1 to 25 mm., the H_2O_2 pressure from 1 to 14 mm. and the N_2 pressure from 30 to 300 mm. Neither a change of vessel diameter from 15 to 38 mm. nor a change of mixing time from 5 to 300 sec. affected the rate. The order with respect to H_2O_2 varied from 1·86 ± 0·06 at 300 mm. total pressure to 1·68 ± 0·10 at 100 mm. In many experiments a pressure decrease was observed in the early stages of the reaction. The rate of this decrease rarely exceeded 10 per cent of the simultaneous rate of NO_2 formation.

It was concluded that equation (I) was consistent with the stoichiometry of the reaction since more than 90 per cent of the initial NO present might be accounted for by the NO_2 formed with only a small concurrent change in pressure. However, the decrease in pressure does indicate the presence of a minor product during the initial reaction, accounting for up to 10 per cent of the total products.

The form of the rate equation (I) excludes the possibility of a homogeneous molecular reaction, and a heterogeneous path is unlikely in view of the lack of diameter dependence. The possibility of an initial rate-determining decomposition of H_2O_2 to oxygen, followed by the combination of NO and O_2, may be rejected since the rates of formation of NO_2 are much greater than those expected from the termolecular reaction. Thus it is concluded that a homogeneous radical reaction occurs. Of the possible schemes considered[1], only the following leads to the correct rate equation :

$$\begin{array}{rll}
H_2O_2 + NO \rightarrow & HNO + HO_2 & \\
 & HNO_2 + OH & k_2 \\
 & HNO_3 + H \rightarrow OH \text{ or } HO_2 & \\
OH + H_2O_2 \rightarrow & HO_2 + H_2O & k_3 \\
HO_2 + NO \rightarrow & OH + NO_2 & k_4 \\
OH + NO + M \rightarrow & HNO_2 + M & k_5
\end{array}$$

The application of the stationary state hypothesis to this scheme, assuming long chains, leads to the rate equation (II) :

$$\mathrm{d}P_{\mathrm{NO}_2}/\mathrm{d}t = \frac{k_2k_3.P_{\mathrm{H_2O_2}}{}^2}{\Sigma k_{5,M}.P_M} \qquad \text{(II)}$$

This is in good agreement with the experimental equation (I) apart from the order with respect to H_2O_2. The most probable causes of this discrepancy are either that the long-chain assumption is not valid, or that H_2O_2 is an efficient third body in reaction (5) relative to nitrogen. At present there is no evidence to suggest a choice between the alternative initiation reactions.

The reaction scheme does not account for the pressure decrease observed unless nitrous acid can accumulate at this temperature, which is improbable on thermodynamic grounds[6]. It is more likely that the decrease is due to the establishment of a stationary concentration of nitric acid, since this decomposes slowly at 300° C.[7], and could be formed in the system. Equation (I) does not apply to the variation of the rate within a single run. This effect has not been examined in detail, but it is probably due to secondary reactions caused by the products. In particular, it has been found that H_2O_2 and NO_2 react at both 70° and 299° C., and at the lower temperature this reaction, followed by changes in P_{NO_2} and total pressure, conforms to the equation:

$$2NO_2 + H_2O_2 \rightarrow 2HNO_3$$

It is intended to investigate further both the reaction with NO and with NO_2.

I wish to thank Dr. P. G. Ashmore for many helpful discussions during the course of this work, and Laporte Chemicals, Ltd., for a gift of hydrogen peroxide.

B. J. TYLER

Department of Physical Chemistry,
University of Cambridge.

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Bromination of Triphenyl Phosphine Borine

In most compounds containing hydrogen linked to boron, the hydrogen atoms have a more or less pronounced electronegative or hydridic character. This is evident in the mildness of the conditions required for their reaction with water or hydrogen chloride to give molecular hydrogen. In the phosphinoborine ring polymers, however, the BH_2 groups are exceptionally inert towards hydrolysis. Burg *et al.* have attributed this to a diminution in the hydridic character of the hydrogen atoms, caused by partial utilization of the B–H bonding electrons in forming π bonds between the boron and phosphorus atoms[1–3]. Triphenyl phosphine borine, $(C_6H_5)_3P.BH_3$, is another unusually inert boron hydride derivative. Since our preliminary report that it resisted hydrolysis[4], we have found that it is not affected by refluxing for 1 hr. with 6 *N* hydrochloric acid; nor, when dissolved in benzene, does it react with hydrogen chloride gas at room temperature. This stable (m.p. 188°) and easily prepared compound appeals to us as a material with which to investigate the chemical behaviour of deactivated hydrogen atoms attached to boron. We here report the first of a series of experiments by means of which we hope to characterize more fully the type of reactivity possessed by these hydrogen atoms.

Triphenyl phosphine borine was first made by passing diborane into a benzene solution of triphenyl phosphine. Using high-vacuum apparatus, bromine was distilled into a suspension of the triphenyl phosphine borine in dry carbon disulphide. Reaction occurred smoothly at −40° according to the equation:

$$(C_6H_5)_3P.BH_3 + 3Br_2 \rightarrow 3HBr + (C_6H_5)_3P.BBr_3$$

The second reaction product was identified by comparison with a specimen of the triphenyl phosphine–boron tribromide complex prepared by mixing the components in carbon disulphide solution. The two substances gave: (*a*) indistinguishable infra-red spectra (potassium bromide disk method); (*b*) agreeing melting points (reaction product 212°–214°, $(C_6H_5)_3P.BBr_3$ 215°–217°, mixed m.p. 214°); (*c*) the same hydrolysis products, when refluxed for an hour with carbon tetrachloride and water.

Table 1

Reactants		HBr formed *, †	From hydrolysis of involatile reaction product‡	
$(C_6H_5)_3P.BH_3$	Br_2		H_3BO_3	BHr †
0·855	2·56	2·54	0·85	2·57
0·873	2·62	2·59	0·87	2·64

All quantities in m.moles. Reaction in 1·5 ml. of carbon disulphide at −40°.

* Pumped off from reaction mixture at room temperature.

† Determined both gravimetrically as silver bromide and acidimetrically.

‡ The hydrolysis also gave rise to triphenyl phosphine oxide, which was not determined quantitatively. This presumably arose from atmospheric oxidation of the triphenyl phosphine first formed; it was also formed in experiments in which triphenyl phosphine or its boron tribromide adduct were refluxed under the same conditions.

Table 1 shows the practically quantitative stoichiometry of the reaction.

Taken in conjunction with the known lack of hydridic reactivity of the borine hydrogens in this compound, the reaction with bromine may well imply protonic ionization of the borine hydrogens, leading to their replacement by bromine in an S_E1 process. But more experiments are needed before alternative mechanisms, such as fission of the P–B bond by bromine and its subsequent re-formation, can be ruled out.

H. G. HEAL
I. O. MADDEN

Department of Chemistry,
Queen's University of Belfast.

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Reaction of Phenols with Peroxy Radicals

Although it has been known for a long time that phenols inhibit the autoxidation of many organic substances, the exact nature of the inhibition process is still not completely understood[1]. Most experimental evidence suggests that the reaction involves a straightforward abstraction of the phenolic hydrogen by the substrate peroxy radicals to give a hydroperoxide and a comparatively unreactive phenoxy radical:

$$ROO\cdot + \varphi OH \rightarrow ROOH + \varphi O\cdot \qquad (1)$$

However, several workers have shown that replacement of the phenolic hydrogen by deuterium has

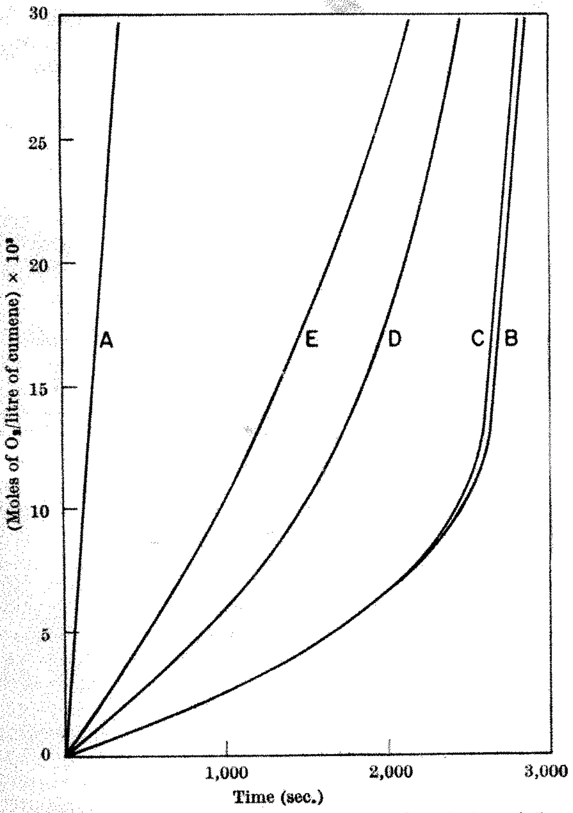

Fig. 1. Oxidation of cumene at 65° C. initiated by 0·327 mole/l. of AIBN and inhibited by 10^{-2} moles/l. of BMP. *A*, Uninhibited oxidation; *B*, BMP inhibited; *C*, BMP inhibited + water; *D*, deuterated BMP inhibited; *E*, deuterated BMP + deuterium oxide

either no effect, or else only a comparatively small effect (~ 0–60 per cent) on the rate of this reaction[1–3]. It has therefore been suggested that the rate-determining step involves the prior formation of a complex between the peroxy radical and the phenol followed by a rapid, non-rate determining, hydrogen abstraction[1,2]. Alternatively, small isotope effects could be secondary effects or could arise in abstraction from the activated complex[3]. We have now found that the apparent smallness or complete absence of the expected isotope effect is most probably due to the very rapid exchange that occurs between a deuterated phenol dissolved in an organic substrate and traces of moisture or the hydroperoxide products of oxidation. The oxidation of cumene under 760 mm. of oxygen initiated by α,α′-azo-*bis*-isobutyronitrile (AIBN) was studied in a conventional constant pressure apparatus. The oxidation was partially inhibited by 2,6-di-*tert*-butyl-4-methylphenol (BMP). In the presence of this inhibitor the initial rate is quite low, but it rises to the rate of the uninhibited reaction as soon as the inhibitor is consumed. This initial rate is proportional to the AIBN concentration and inversely proportional to the BMP concentration, and its reciprocal is proportional to the rate of reaction 1. We have found that exchange can be partially suppressed by using rather high concentration of deuterated BMP and that under these conditions a fairly large isotope effect is observed which increases as the inhibitor concentration is increased. For example, some preliminary experiments at 70° C. with an AIBN concentration of 0·34 mole/l. gave measured isotope effects of 1·6, 1·8 and 2·2 at BMP concentrations of 10^{-2}, 2×10^{-2} and 4×10^{-2} moles/l. respectively. Theoretically, the true isotope effect in the absence of exchange could be obtained by extrapolating to infinite inhibitor concentration. A simpler and, probably, much more accurate procedure is to add an excess of deuterium oxide to the reaction system (~ 1·0 c.c. D_2O to 5 c.c. cumene), thereby maintaining the BMP in its fully deuterated state throughout the early part of the reaction in which the rates are measured. Fig. 1 shows some results obtained in this way at 65° C. with an AIBN concentration of 0·327 mole/l. and a BMP concentration of 10^{-2} moles/l. In the presence of deuterium oxide the initial rate is ⩾ 4·2 times that obtained with the undeuterated inhibitor, whereas, in the absence of deuterium oxide, this factor was only 2·3. That is, from the average of three determinations, the true deuterium isotope effect for reaction 1 between cumyl peroxy radicals and BMP at 65° C. is ⩾ 4·2 ± 0·5. Our assumption that the major, and possibly only, part played by the deuterium oxide is to maintain the BMP in its fully deuterated state is confirmed by the fact that water has no appreciable effect on the undeuterated inhibitor (see Fig. 1) and that the effects of water and deuterium oxide on the uninhibited oxidation are very small. Experiments of a similar nature using styrene as the substrate gave an isotope effect of about 10·6 at 65° C.

In conclusion, the reaction between phenols and peroxy radicals does not show the unusual features previously reported. That is, the reaction of 2,6-di-*tert*-butyl-4-methylphenol with both tertiary and secondary peroxy radicals is a normal hydrogen abstraction process exhibiting an isotope effect of about the expected magnitude for a transition state involving appreciable stretching of the phenolic O–H bond.

K. U. INGOLD
J. A. HOWARD

Division of Applied Chemistry,
National Research Council,
Ottawa, Canada.

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BIOCHEMISTRY

Disk Electrophoresis of Basic Proteins and Peptides on Polyacrylamide Gels

DISK electrophoresis on small columns of polyacrylamide gels, a new method for the separation of serum proteins, has been developed by Ornstein and Davis[1,2].

We have modified their procedure to make possible the separation of basic proteins and peptides[3]. This technique permits excellent resolution of mixtures of proteins or peptides with samples as small as 50 μgm. and within as little as 20 min. The method achieves this by concentrating the components of dilute samples into very thin starting zones and by utilizing the frictional properties of the gel to aid separation by molecular sieving.

Disk electrophoresis is carried out in columns of polyacrylamide gel consisting of three sections: (1) a large-pore anticonvection gel containing the protein sample; (2) a large-pore spacer gel in which electrophoretic concentration takes place; and (3) a small-pore gel in which electrophoretic separation is accomplished. Buffer trays, graphite electrodes and

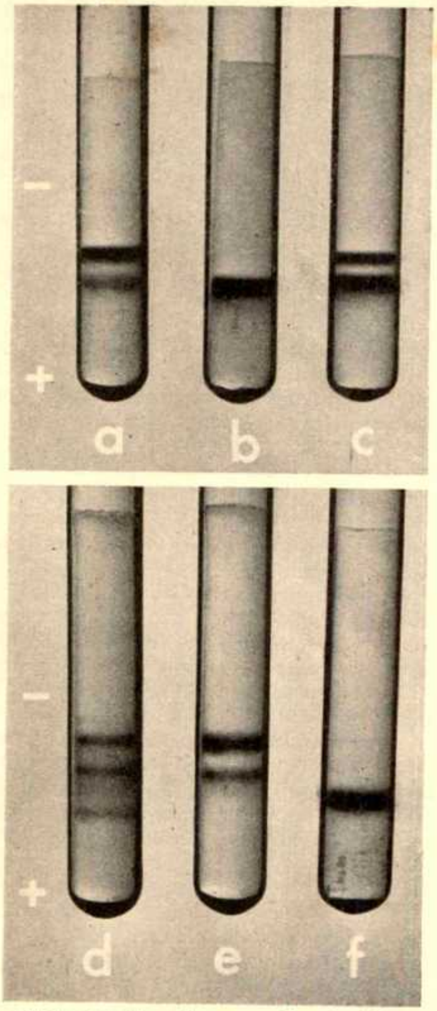

Fig. 1. Electrophoretic patterns. *a*, Trypsin (Worthington, crystallized three times); *b*, chymotrypsin (Worthington, crystallized three times); *c*, 1 : 1 mixture of trypsin and chymotrypsin. Porcine α-melanocyte-stimulating hormone during various stages of purification (*d*, *e*, *f*)

a power supply (300 V., 200 m.amp.) complete the arrangement.

Glass tubes (7 × 0·5 cm. internal diameter) are tightly closed at one end with flat-topped rubber stoppers. Standing upright on these stoppers, the tubes are each filled with 0·85 ml. of small-pore solution (see below for preparation) at room temperature. This solution is carefully overlaid with 0·1 ml. of distilled water and allowed to polymerize for 30 min. The water layer is removed, and the remainder of each tube rinsed once with large-pore solution, after which 0·15 ml. of this solution is added and carefully overlaid with 0·1 ml. of distilled water. A daylight fluorescent lamp (15 W.) is placed behind the tubes, about 3 in. away. The large-pore solution becomes opalescent and polymerizes within 15 min. The water layer is removed and large-pore solution (0·15 ml.), which is mixed with a sample of 50–200 μgm. of protein contained in 5–10 μl., is added. Whenever this amount of sample is contained in a larger volume, the large-pore gel must be applied in a concentration sufficiently higher to offset this dilution. The sample layer is photopolymerized for 15 min. The remaining space in each glass tube is filled with tray buffer (β-alanine acetate), the rubber caps are removed, and the tubes, sample gel uppermost, are attached to the anode buffer compartment, which is then filled with tray buffer. A hanging drop of buffer is attached to the bottom of each tube. The anode compartment, supported on a ring stand, is placed so that the tubes are immersed about ¼ in. into the buffer contained in the cathode compartment. An alternative method of sample application is to layer the sample solution (up to 0·15 ml.) on the spacer gel after the anode compartment is filled with buffer. To prevent mixing, the density of the sample solution is increased with sucrose. Electrophoresis is carried out by applying a current of 6–8 m.amp. per tube for 20 min.

When a current is applied, the β-alanine–potassium ion boundary, originally present at the buffer-gel interface, moves into the gel towards the cathode, sweeping up the protein components. The proteins are concentrated between the potassium and the β-alanine in a series of disks in order of electrophoretic mobility. The ratio of the concentrations of the proteins in these disks to that of the potassium ions is equal to the ratio of the respective mobilities per single charge[4]. When the stack of disks passes into the small-pore gel, the β-alanine–potassium ion boundary is accelerated in this region of lower *p*H and the protein disks are left behind. The disks are now in a linear voltage gradient, each in a very thin starting zone. The protein components separate according to charge and molecular size.

When electrophoresis is completed, the gels are immediately removed from the glass tubes by gently rimming them with a 22-gauge syringe needle through which a thin stream of water is allowed to pass. The gels are fixed and stained for 1 hr. in a solution of 1 per cent 'Amidoschwarz' in 7 per cent acetic acid. Background stain is removed electrophoretically by placing the gels into glass tubes (7 × 0·8 cm. internal diameter). One end of each tube is slightly tapered to hold a ¼-in. plug of small-pore gel (7·5 per cent acrylamide). The de-staining tubes and buffer reservoirs are filled with 7 per cent acetic acid. A current of 15 m.amp. per tube is applied, allowing the excess dye to flow towards the anode. All background stain can thus be removed in 30–40 min. The gels are stored in screw-cap vials containing 7 per cent acetic acid.

The following stock solutions are necessary to prepare the small-pore and large-pore solutions:

(*A*)	*N* Potassium hydroxide	48 ml.
	Acetic acid (glacial)	17·2 ml.
	Tetramethylethylenediamine	4·0 ml.
	Water to make	100 ml.
(*B*)	*N* Potassium hydroxide	48 ml.
	Acetic acid (glacial)	2·87 ml.
	Tetramethylethylenediamine	0·46 ml.
	Water to make	100 ml.
(*C*)	Acrylamide	60 gm.
	Methylene *bis*acrylamide	0·4 gm.
	Water to make	100 ml.
(*D*)	Acrylamide	10 gm.
	Methylene *bis*acrylamide	2·5 gm.
	Water to make	100 ml.
(*E*)	Riboflavin	4·0 mgm.
	Water to make	100 ml.

The tray buffer (*p*H 4·5) is prepared as follows: β-alanine, 31·2 gm.; acetic acid (glacial), 8·0 ml.; water to make 1 litre.

The following working solutions are prepared from the stock solutions:

Small-pore solution (*p*H 4·3)
1 part *A*
2 parts *C*
1 part water

This solution is mixed before use with an equal volume of a freshly prepared solution of ammonium persulphate (0 28 gm /100 ml) This will result in a solution containing 15 per cent acrylamide For samples with molecular weights greater than 30,000, a 7 5 per cent acrylamide concentration may be more advantageous To achieve this, stock solution (*C*) is prepared by using 30 gm acrylamide and 0 8 gm of methylene *bis*acrylamide

Large-pore solution (*p*H 6 8)
1 part *B*
2 parts *D*
1 part *E*
4 parts water

All stock solutions have a shelf life of several weeks when stored in brown glass bottles in the refrigerator

The buffer reservoirs can be easily made from polystyrene refrigerator dishes (4–5 in diameter), the anode compartment being prepared by drilling equally spaced holes ($\frac{3}{8}$ in diameter) near the periphery Rubber grommets ($\frac{1}{4}$ in inside diameter) are placed into these holes Each reservoir has a carbon electrode ($\frac{1}{4}$ × $2\frac{1}{2}$ in) fastened at the centre

Fig 1 depicts some of the results we obtained with this method The crystalline preparation of trypsin was shown to be heterogeneous, the minor component having the mobility of chymotrypsin as demonstrated by the electrophoretic pattern of a mixture of equal parts of trypsin and chymotrypsin The chymotrypsin preparation was also shown to contain a minor component A preparation of porcine α-melanocyte-stimulating hormone is shown during various stages of chromatographic purification The final product appears to be homogeneous We have also found inhomogeneity in the following preparations ribonuclease (Worthington, crystallized three times), one major and three minor components, protamine sulphate (Nutritional Biochemicals Corporation), one major and three minor components, globin (Nutritional Biochemicals Corporation), one major and four minor components, lysine vasopressin (N I H , Endocrinology Study Section), two major and four minor components

The method makes it possible to analyse eluate fractions from column chromatography since one person can analyse as many as 20–30 samples per day We have found the method to be highly reproducible, as well as rapid, simple, and most inexpensive to set up

R A Reisfeld
U J Lewis
D E Williams

Merck Sharp and Dohme Research Laboratories,
Rahway, New Jersey

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Polyribonucleotide-directed Protein Synthesis using an *E. coli* Cell-free System

Since the discovery by Nirenberg and Matthaei[1] that a synthetic polynucleotide, when added to an *E coli* cell-free system, led to the synthesis of a specific polypeptide, various groups have used similar methods to find code letters for the common amino-acids We should like to report certain observations, some of which are at variance with those already published

Nirenberg and Matthaei's original observation, that poly U coded specifically for phenylalanine, appeared to give a clear path for the solution of the genetic code, assuming a triplet nature for the code[2], one sequence for phenylalanine would be UUU Using, in general, polymers composed of uridylic acid residues, with small amounts of the other bases, C, A, G or I, Ochoa *et al* have produced code letters for nineteen amino-acids and have predicted the twentieth[3–5] Many of these triplets have been confirmed by Matthaei *et al*[6] Several code letter assignments depend on the incorporation of an amino-acid in small amounts, stimulated by a polymer the composition of which has frequently been left unanalysed, but is assumed to have the same overall composition as the reaction mixture from which it was made[3–5] That analysis is essential is shown by the composition of reaction mixture and product of several polymers we prepared (Table 1), though naturally results may differ somewhat for enzymes from different sources and with different incubation conditions

Table 1

	μM reactants	Analysis of product
Poly UC	40 UDP + 37 5 CDP	C/U = 1 9
Poly AC	40 ADP + 40 CDP	C/A = 1 7
Poly UG	25 UDP + 25 GDP	G/U = 32
Poly UCG	59 5 UDP + 10 5 CDP + 10 5 GDP	U/C/G = 2 1 1

Comparison of reactant concentrations and product composition using a polynucleotide phosphorylase (of specific activity 23) from *Azotobacter vinelandii* Incubation mixture contains per ml 100 *μM tris p*H 8 2, 10 *μM* magnesium chloride and 2 units of enzyme for poly UC and poly AC, or 10 units of enzyme for poly UG and poly UCG.

In assigning a triplet, the ratio of incorporation of the amino-acid to that of a standard (generally phenylalanine) is compared with the composition of the polymer This assumes that the polymer is random in sequence, that peptides of different compositions are detected with equal efficiency (usually insolubility in hot trichloroacetic acid), and that only one triplet is read for the standard (for example, UUU) The latter two assumptions appear to be unlikely

It seems possible, therefore, that in some cases the stimulations observed may be caused by triplets other than those to which the code letters have been assigned

Evidence for the degeneracy of the code has been provided by the stimulation of leucine by poly UC and poly UG[5,7], and by poly UA[5] Serine is reported to be represented by UUC and UGC[6], asparagine by UAA and UAC[5], and threonine by UAC[5] and originally by UCC[4]

We find that in a similar cell-free system the stimulation of amino-acid incorporation by poly U is not limited to phenylalanine, but that variable amounts of leucine (5–25 per cent in different experiments) are always incorporated An incorporation of about 3 per cent leucine has also been reported by Matthaei[6] Both the time course and the temperature dependence (tested at 25° C and 37° C) of incorporation are similar for these amino-acids The leucine incorporation is not due to an impurity in our carbon-14 L-leucine sample because a hundred-fold excess of carbon-12 L-leucine (of purity better than one part in a thousand) quenches the incorporation almost completely Five preparations of poly U, with S_{20} values lying between 4 3 and 1 7 (the last prepared by degrading a larger polymer with snake venom phosphodiesterase), have been tested, and no difference in the ability to incorporate leucine could be observed Hydrolysis of a poly U sample showed that the total impurities of C, A, G or I were less than 1 per cent moreover, no poly U sample incorporated serine or proline (expected of a poly UC), isoleucine (expected of a poly UA), or valine (expected of a UG or UI polymer) Two of the poly U samples had been prepared from independent sources of synthetic UDP We conclude that poly U, when placed in this cell-free system, leads to the incorporation of more than one amino-acid This effect indicates either that intact cells make this 'mistake' or that the *in vitro* system has been damaged somewhat, or that a systematic alteration of the polymer has taken place in the reaction mixture Whatever the reason, it suggests that small amounts of incorporation may be misleading, as may be the incorporation of leucine into those polymers which mainly consist of U's

All code letters produced so far have U's in them This seems unlikely since it would give an overwhelming abundance of U's in a hypothetical messenger[8], which would lead one to expect a high A/G base composition for DNA That it is unnecessary for triplets to contain U's is shown by a poly CA (S_{20} = 2 0, made from 4 CDP + 1 ADP, C/A = 5 6, with maximum impurities of 1 per cent U, 0 8 per cent G) This has been tested for 18 amino-acids (the usual 20 except tryptophan and cysteine) and incorporates mainly proline, and smaller amounts of threonine and histidine Another poly CA (S_{20} = 2 9, 1 CDP + 1 ADP, C/A = 1 7) incorporates mainly proline, some threonine and histidine together with a much smaller amount of glutamine Each incorporation has been checked by quenching with the corresponding C12 amino-acid Both these poly CA's function with about equal efficiency (as judged by total incorporation when our system is saturated with polymer), which is about one-third as efficient as a poly CU (C/U = 4 3)

Table 2

	Poly CA C/A = 5 6	Poly CA C/A = 1 7	No polymer
Proline	530	520	17
Threonine	215	350	32
Histidine	70	160	5
Glutamine	58	70	36

Incorporation with these two polymers in $\mu\mu$moles/mgm ribosomes is shown with figures for the incorporation in the absence of polymer Incubation mixture contains per ml 16 μM Mg $(CH_3COO)_2$, 60 μM *tris* *p*H 7 8, 85 μM KCl, 12 μM mercaptoethanol, 1 2 μM ATP, 0 25 μM GTP, 4 5 μM phosphoenolpyruvate, 80 μgm phosphoenolpyruvate kinase (from Boehringer), 1 2 mgm ribosomes, 3 2 mgm concentrated dialysed supernatant, 40 mμM of a carbon-14 L-amino acid and 0 3 μM of each of the other 19 common amino-acids, and 40 μgm of the polymer Samples were incubated at 37° C for 15 min Incorporation was measured by radioactive hot TCA precipitable material

It was originally reported[4] (though more recent work by the same authors has shown this to be unlikely) that a triplet for threonine was UCC With UC polymers of composition U/C = 2 8, 0 7 and 0 23, only slight threonine stimulation could be detected, being always less than 5 per cent of the total incorporation by the polymers This indicates that the UCC assignment is incorrect Matthaei *et al* have come to a similar conclusion It is of interest that these three poly UC's function with roughly equal efficiencies

We conclude that not all codons must contain U, and that the assignment of triplets on the basis of small amounts of incorporation can lead to mistakes

We thank Dr F H C Crick for many helpful suggestions, Dr J Ofengand for details of the protein synthesis system which he developed, and Dr A M Michelson for a sample of chemically synthesized UDP We acknowledge with thanks the award of a Salters' Scholarship (to M S Bretscher), and a grant from the U S National Institutes of Health, No C 45 80 (to Marianne Grunberg-Manago).

M S BRETSCHER
Medical Research Council,
Laboratory of Molecular Biology,
Hills Road, Cambridge

M GRUNBERG-MANAGO
Institut de Biologie Physico-Chimique,
13 Rue Pierre Curie,
Paris Ve

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Use of Indium Trichloride for the Separation of Native, Denatured and Single-stranded Deoxyribonucleic Acid

A PREVIOUS communication from me[1] described the use of indium trichloride for the separation of DNA and RNA This work has now been extended to the point where it has become possible to separate different molecular species of DNA in the presence of indium chloride and varying concentrations of sodium chloride

Calf thymus DNA isolated by the method of Kay *et al* [2] was dissolved in 0 14 *M* sodium chloride so that a 0 4 per cent solution resulted Aliquots of this solution were taken and subjected to one of the four following experimental conditions (1) no treatment, that is, native DNA, (2) heat denaturation at 100° C for 30 min (this procedure presumably produces single-stranded DNA[3]), (3) denaturation by the addition of glacial acetic acid to a final concentration of 0 2 *M*, (4) denaturation by addition of 19 *M* potassium hydroxide to a final concentration of 1 *M* (the solution was allowed to stand for 1 hr)

To the acid and alkali denatured aliquots, 2 vol of absolute ethanol were added and the solution was equilibrated at 0° C for 2 hr The resulting precipitates collected by centrifugation were washed with 70 per cent ethanol and re-sedimented The denatured DNA was then redissolved in 0 14 *M* sodium chloride to give a 0 4 per cent solution and the *p*H adjusted to 7 0 with a small amount of either 0 2 *M* acetic acid or 0 2 *M* potassium hydroxide

In a system the total volume of which was 5 0 ml , indium precipitation of the 4 different species of DNA at *p*H 5 (Fig 1) was examined as a function of concentration of sodium chloride The system contained the following 0 5 ml DNA solutions, 3 5 ml 0 2 *M* acetate buffer, *p*H 5 0, containing sodium chloride in the range 0 14–5 7 *M*; and 1 0 ml of 0 05 *M* indium chloride in distilled water (freshly made) rapidly blown in and mixed After standing at room temperature for 30 min , the tubes were centrifuged at 1,400*g* for 10 min and the absorbance of the supernatant solutions at 260 mμ determined The presence of 260 mμ-absorbing material indicates that the nucleic acid has not been precipitated Results

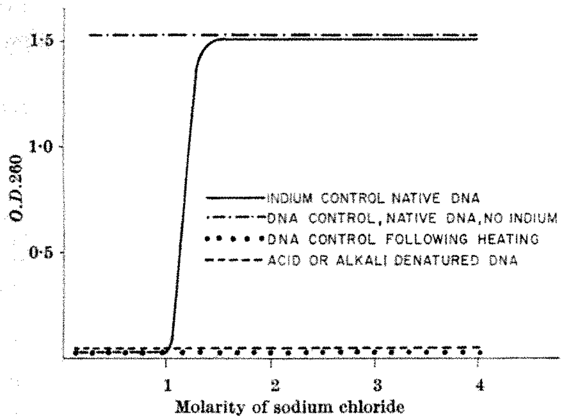

Fig. 1. DNA remaining in the supernatant solution after addition of indium chloride to solution of DNA treated in the manner indicated in the text. Only 'native' double-stranded DNA becomes unprecipitable in 1·5 and higher molarities of sodium chloride at pH 5. Measurements were made on the basis of increments of 0·1 M sodium chloride in the range 0·1–4 M

obtained in this manner on the four DNA aliquots are plotted in Fig. 1.

From these results it is apparent that native, double-stranded DNA was not precipitated by indium chloride at pH 5 in the presence of 1·5 M or higher molarities of sodium chloride. Acid, alkali and heat denatured (presumably single-stranded) DNA was precipitated regardless of the concentration of sodium chloride.

Furthermore, it has been found that precipitation of pneumococcal transforming DNA by indium chloride and subsequent removal of indium with versene does not alter the pneumococcal transforming activity. Pneumococcal DNA had properties similar to those shown in Fig. 1 for calf thymus DNA. The indium precipitates of DNA have also been found to be insensitive to digestion by DNase.

Indium complexes of yeast and rat liver RNA (like those of RNA of tobacco mosaic virus[4]) were also found to be insensitive to digestion by RNase.

It is possible to examine different molecular species in a nucleic acid preparation using the basic information presented here. As an example, nucleic acids isolated from rat liver and separated according to the indium technique[1] always yield an RNA fraction contaminated with a small amount of DNA. This DNA can be examined by placing the precipitate (DNA and RNA) in a solution of DNase I (1 mgm./ml.) and RNase (1 mgm./ml.) in 0·14 M sodium chloride plus 0·002 M magnesium sulphate buffered at pH 5 with acetate. These enzymes will digest completely only the nucleic acid not present as the indium complex. In order to digest indium complexed RNA, sodium duodecyl sulphate is then added to a concentration of 1 per cent. This completely inhibits DNase I activity but only about 60 per cent of the RNase activity. By adding versene, the indium is removed from the nucleic acids and only RNA is digested on incubation for 2 hr. at 37° C. The mixture is heated to 50° C. for 30 min. to denature completely both enzymes, and solid sodium chloride is added to a concentration of 1·5 M to precipitate the detergent and detergent-protein complex. Following centrifugation, the solution is dialysed against several portions of 0·14 M sodium chloride at 4° C. to remove low molecular-weight degradation products of RNA. The resulting dialysed solution of DNA may now be further studied.

Some of this work was done while I was the recipient of a National Institutes of Health post-sophomore research fellowship. It was supported, in part, by a contract with the U.S. Atomic Energy Commission (AT(30–1)–1286) and, in part, by the Ernest L. Woodward Fund of the University of Rochester School of Medicine and Dentistry.

WILLIAM G. ALDRIDGE

Departments of Experimental Radiology
and Anatomy,
University of Rochester School
of Medicine and Dentistry,
Rochester,
New York.

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Multiple Nature of Acetylcholinesterase in Nerve Tissue

WITHIN recent years much evidence has been presented on the multiple nature of enzymes. Such enzyme systems as dehydrogenases[1], esterases[2], phosphatases[3], etc., have been shown by various kinetic, electrophoretic and column separation techniques to exist as more than one molecular specie. It is too early to assess the significance of variations in properties of these similar substances because of the paucity of information correlating their biochemical nature and physiological functions. However, a large body of knowledge has accumulated on the physiological function of acetylcholinesterase relating it to the propagation of the nerve impulse. Data on the unitary or multiple nature of acetylcholinesterase in nervous tissue might lead to specific information on the relation of the enzyme to the nerve conduction process, or might shed light on the general problem of the physiological meaning of the occurrence of multiple molecular enzyme species. Bergmann and Segal[4] have already shown that more than one acetylcholinesterase may be present in dog tissue and the multiple nature of serum cholinesterases in a variety of animals has been reported[5,6].

In order to obtain an adequate source of the enzyme from nervous tissue, the caudate nucleus and putamen of human brain were dissected from post-mortem material. These nuclei have been shown to possess high enzymatic activity[7], and were obtained 4–8 hr. after death. The homogenate was assayed by the conventional manometric technique and was found to hydrolyse 1,375 μmoles acetylcholine/gm. brain/hr. The dissected tissue was homogenized with an equal volume of isotonic saline and the homogenate was then frozen and thawed six times, and centrifuged at 20,000g. The supernatant was pipetted, placed in a 'Visking' bag, and the volume reduced to 1/3–1/4 of the original by passing a current of air across the bag in a refrigerator at 4° C. (ref. 8). The concentrated extract was then subjected to starch-gel electrophoresis according to Smithies[9] for 16–18 hr. at an e.m.f. of 4·5 V./cm. in a 13° C. incubator. After the separation was completed, the starch block was cut longitudinally and incubated in the appropriate medium using acetylthiocholine or butyrylthiocholine iodides as substrates by a method previously described[6] and derived from that of Gomori[10].

The acetylcholinesterase activity obtained at *p*H 6·2 and *p*H 5·0 with acetylthiocholine as substrate is shown in Fig. 1. At *p*H 6·2, four zones of activity could be demonstrated. A dense zone, well delineated at its forward boundary but with some tailing at the trailing edge, was obtained proximate to the origin (*A*). In addition a zone practically as dense as (*A*) was present distal from the origin (*D*). Almost contiguous with (*D*), but of lighter intensity, another band of acetylcholinesterase activity was present (*C*), and a light band of activity intermediate between the dense zones could be shown (*B*).

When the starch block was incubated at a *p*H of 5·0, zones *A*, *C*, and *D* disappeared, but band *B* became intensified. The absence of activity of the enzyme at *p*H 5·0 is in agreement with the results of Wilson and Bergmann[11]. In addition, inhibitor experiments showed that bands *A*, *C* and *D* are completely inhibited by incorporation of physostigmine sulphate in the gel and afterwards in the substrate medium at a concentration of 1×10^{-5} *M*. Further evidence that the zones of activity at *A*, *C* and *D* are due to acetylcholinesterase is provided by the fact that the use of butyrylthiocholine (Fig. 1, II) results in a great diminution in intensity of bands *A*, *C*, and *D*, while band *B* has a density which is similar to that obtained with acetylthiocholine. Inhibition experiments with the specific acetylcholinesterase inhibitor mytelase (5×10^{-6} *M*) resulted in complete inhibition of zones *A*, *C*, and *D*.

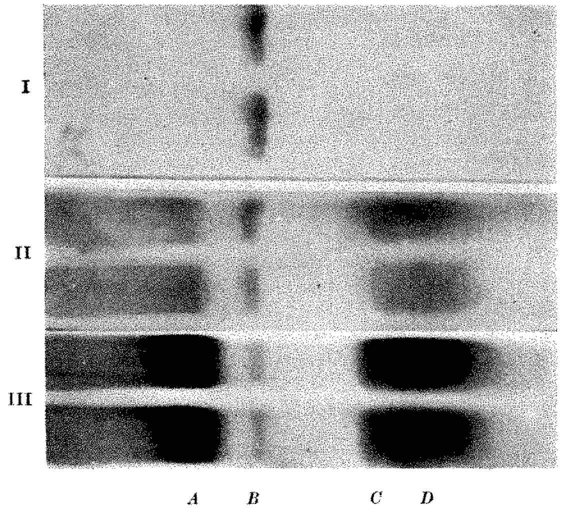

Fig. 1. Separation of acetylcholinesterase from brain (caudate nucleus and putamen) by starch-gel electrophoresis. The block letters refer to the bands of activity. (I) Acetylthiocholine as substrate at *p*H 5·0. (II) Butyrylthiocholine as substrate, *p*H 6·2. (III) Acetylthiocholine as substrate, *p*H 6·2

In view of the results obtained, namely, inhibition by physostigmine sulphate and mytelase, absence of activity at *p*H 5·0 and a greater activity for the 2-carbon acyl chain as compared to the 4-carbon chain, it would appear that zones *A*, *C* and *D* have enzymatic properties usually associated with acetylcholinesterase. Whether these 'isozymes' represent different molecular species is a problem of paramount interest in view of the physiological function ascribed to this enzyme. If they represent different molecular species, then it would seem that either the enzyme has more than one biochemical function in the mechanism of nerve conduction, or the variations in molecular structure reflected in the varying mobilities found in this work have no effect on the enzyme-substrate relationship in terms of the various parameters that have been studied by others. It is possible that the differences found here represent some multiple aggregate of a basic structure required for enzymatic activity. Our present knowledge of the active centre of the enzyme relates to the presence of an anionic and an esteratic site, spatially oriented so as to form an enzyme-substrate complex with acetylcholine. Information as to whether the intact enzyme is necessary for catalytic activity or whether some essential fragment can substitute for the whole protein molecule is lacking. It may be true that the different zones of activity obtained in this work represent enzymes with different functions, in which case the present concepts of acetylcholine metabolism may need some revision. This laboratory is at present engaged in characterizing the bands that have been resolved.

This work was aided by a grant from the National Multiple Sclerosis Society.

J. BERNSOHN
K. D. BARRON
A. R. HESS

Neuropsychiatric Research Laboratory,
Hines Veterans Administration Hospital,
Hines, Illinois, and
Department of Neurology and Psychiatry,
Northwestern University Medical School,
Chicago, 11.

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Non-Casein Proteins of Goat's Milk

EXISTENCE of genetically different types of β-lactoglobulin[1,2], α-lactalbumin[3] and β-casein[4] have been demonstrated in cow's milk; but no genetic polymorphism was detected in the non-casein proteins of buffalo's milk[5]. We have investigated the probable occurrence of genetically controlled proteins in the non-casein fraction of goat's milk from which the β-lactoglobulin and 'lactalbumin' have been crystallized and some of their physicochemical properties examined.

Twenty-three breeds of Indian goats are known to exist[6]. Of these Jamnapari, Black Bengal, Barbari and a few crosses between Saanen and Indian breeds were available for investigation. More than 75 samples of milk of individual goats were examined. Protein solutions for tests were prepared by the method of Aschaffenburg and Drewry[2] and subjected to paper electrophoresis in a hanging strip apparatus at *p*H 4·9 in acetate buffer, I = 0·05, using Whatman 3 *MM* paper and applying a voltage of 240 V. for 16 hr.

Fig. 1 shows a typical electropherogram. The number of protein components seem to be five, of which β-lactoglobulin, 'lactalbumin' and components III and III*a* migrate towards the cathode and com-

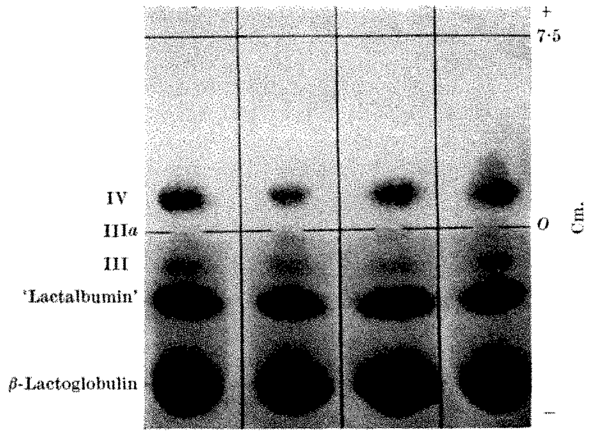

Fig. 1. Electropherogram of non-casein proteins of goat's milk from four individual animals (*O*, origin; bromphenol blue staining)

ponent IV to the anode. All the individual samples appear to give uniform results indicating the absence of any genetic factor in the occurrence of non-casein protein constituents of goat's milk in the breeds so far examined.

Two major protein constituents, β-lactoglobulin and 'lactalbumin', have been purified and crystallized. In Fig. 2*a* different forms of crystalline β-lactoglobulin are shown and in Fig. 2*b* crystals of 'lactalbumin', which appear to be rhombohedral. The diamond-shaped crystals of goat β-lactoglobulin seem to be similar to the monoclinic crystals of cow[1] and buffalo[5] β-lactoglobulin.

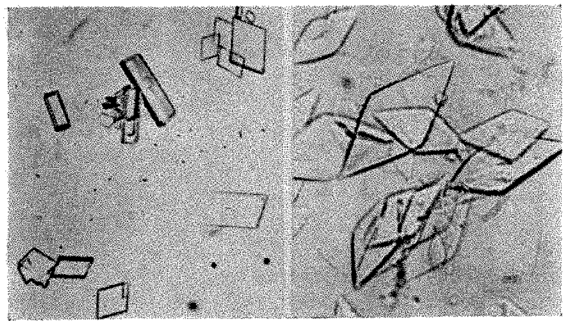
(*a*) (*b*)

Fig. 2. *a*, Crystals of goat β-lactoglobulin (× *c*. 150); *b*, crystals of goat 'lactalbumin' (× *c*. 360)

The separation and crystallization of β-lactoglobulin and 'lactalbumin' were carried out by the method of Aschaffenburg and Drewry[7] suitably modified by us for goat's milk. According to this procedure, a filtrate containing the non-casein proteins, obtained by addition of sodium sulphate (20 gm./100 ml.) to the whole milk, is adjusted to *p*H 3 when all the protein constituents with the exception of β-lactoglobulin are precipitated. From the filtrate, β-lactoglobulin is precipitated by the usual method[7]. The final concentrated solution of β-lactoglobulin is adjusted to *p*H 5·9 and dialysed against distilled water. A heavy oily layer separates from which the crystals of β-lactoglobulin (Fig. 2*a*) appear in 10–12 days. The β-lactoglobulin precipitate obtained by this method can also be crystallized in the form of hexagonal bipyramids[8].

The acid precipitated proteins are dissolved in water by adding dilute ammonia and freed from any residual β-lactoglobulin by reprecipitating at *p*H 3 in the presence of sodium sulphate (15 gm./100 ml.). This precipitate is dissolved in dilute ammonia, and the *p*H adjusted to 4 with hydrochloric acid. The solution is centrifuged, and from the supernatant 'lactalbumin' is precipitated with ammonium sulphate between 45 and 80 per cent saturation. The precipitate is dissolved in water and the solution after being adjusted to *p*H 6 is rendered slightly turbid by adding ammonium sulphate. This solution when allowed to evaporate slowly gives crystals of 'lactalbumin' (Fig. 2*b*). A salt-free concentrated solution of lactalbumin yields needle-shaped crystals when left in the cold (2°–4° C.).

In preliminary free electrophoresis experiments 'lactalbumin' shows single symmetrical peaks at *p*H 5·2, and its isoelectric point appears to be close to this *p*H. Its sedimentation coefficient, S_{20w}, measured at the same *p*H in a Spinco analytical ultracentrifuge, is $2\cdot 0 \times 10^{-13}$ sec., which is close to the S_{20w} valeus for cow[9,10] and buffalo[11] α-lactalbumins. With β-lactoglobulin, the hypersharp ascending and skew descending electrophoretic patterns obtained at *p*H 5·5 and the S_{20w} value of $2\cdot 9 \times 10^{-13}$ sec. at *p*H 5·7 are similar to those reported by Askonas[8].

We thank the staff of the Haringhata Farm for supplying the milk samples and identification of breeds.

A. SEN
SHILA CHAUDHURI

Chemistry Department,
Bose Institute,
93/1 Acharya Prafulla Chandra Road,
Calcutta 9.

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Screening Bacterial Colonies for Penicillinase Production

RECENT syntheses of novel penicillins[1,2] have led to revived interest in the detection of species and strains of bacteria able to destroy penicillin; it has also become important to identify the destructive agent. Screening methods based on substantial diminution of the antibiotic activity of penicillin against Gram-positive bacteria[3,4] or on changes in *p*H associated with the formation of extra carboxyl groups[4,5] have always had a range of application limited by their susceptibility to interference from general metabolic products. It has now become evident that such methods are also unsuitable for distinguishing penicillinase from penicillin acylase[6-8]. But penicillinase converts penicillin to penicilloic acid which, being a thiazolidine with a non-acylated imino group, reacts with iodine; whereas the 6-amino-penicillanic acid formed by penicillin acylase has an intact β-lactam ring and is as inert towards iodine as penicillin itself.

A series of iodometric methods for the specific detection and assay of penicillinase have been described by Perret[9]. We have since developed two simple and flexible modifications which permit the rapid screening of large numbers of bacterial colonies for the aerobic and anaerobic production of both constitutive and induced penicillinase. The exact details of the tests depend to some extent on the nature of the bacteria being tested and on the level of enzyme activity being sought. The particular versions given here were originally devised for the routine screening of strains of *Staphylococcus aureus* in mixed cultures plated-out from clinical material, but they have since proved suitable for both taxonomic and genetic studies of many other species of heterotrophic bacteria.

The test on solid medium is performed by touching selected colonies with a straight wire and transferring each of them to marked positions about 1·5 cm. apart on one or more plates of defibrinated-horse-blood-agar about 0·5 cm. deep. The plates are then incubated at 37° C. for 4 hr., after which time small colonies have formed at the sites of inoculation; if desired, the plates may at this stage be replicated by the velvet-pad technique. Strips of Whatman No. 1 filter paper previously impregnated with fresh 1 per cent starch solution and dried are now immersed in a solution of penicillin *G* (10,000 I.U./ml. in 0·05 *M* phosphate buffer at *p*H 6·5), drained, and laid carefully over the colonies so that air-bubbles are excluded. After 30 min. at room temperature 1 ml. of 0·04 *M* iodine is pipetted on to the paper and the results read up to 30 min. later. Colonies of penicillinase-producers form white spots which increase in area with time; the paper remains blue over non-producers. The limit of sensitivity of the method has not yet been determined, but a strain of *Bacillus cereus* producing only 70 molecules of penicillinase/cell/generation gives clear positive results. When it is important to exclude the possibility of specific enzyme induction the organisms are killed by exposure to chloroform vapour or ethylene oxide before applying the penicillin-impregnated paper.

For some purposes a simpler and quicker procedure may be used. Single colonies are removed from the surface of the primary isolation medium with a loop, emulsified in 0·2 ml. of the penicillin solution in 3 in. × 0·25 in. test tubes and left for 1 hr. at room temperature. One drop (about 0·02 ml.) of equal parts of 1 per cent sodium starch glycollate and 0·04 *M* iodine is then mixed with the contents of each tube. Decolorization ensues rapidly with most penicillinase-producing strains, but the test is recorded as positive if all colour disappears within 5 min.

Both tests should theoretically be accompanied by controls from which penicillin has been omitted; however, we have now tested about 200 strains and species of bacteria from 21 different genera and no false positive results have been detected. The controls therefore seem unnecessary during preliminary screening.

The tests were developed primarily in order to extend the search for naturally occurring penicillin-resistant strains of *Staph. aureus* which do not produce penicillinase[10]. They were therefore initially applied to 151 strains of coagulase-positive staphylococci isolated from clinical sources; 124 strains were penicillinase-positive by both methods, which gave identical results. The strains were then tested for penicillin-sensitivity by means of impregnated disks laid on the surface of spread plates, and by the inoculation of serial two-fold dilutions of penicillin in meat-infusion broth with about 10^7 organisms/ml. All 124 penicillinase-positive strains were resistant to disks bearing 1·5 I.U. penicillin and gave visible turbidity after 18 hr. at 37° C. in concentrations of 0·5 I.U. penicillin/ml. or more: all 27 penicillinase-negative strains were sensitive to the disks and gave no visible turbidity in concentrations of penicillin above 0·25 I.U./ml.

J. M. FOLEY
C. J. PERRET

Department of Microbiology,
University of Western Australia,
Perth.

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Elastase Activity in Human Pancreatic Secretions

IN 1949, Balo and Banga[1] first reported the isolation of the pancreatic enzyme elastase, which was unique in its ability to render soluble elastin (an important structural component of arteries, veins, skin and lung). This enzyme was first believed to be mucolytic in action, but it is now established that it is also a potent proteolytic agent[2]. Kokas, Foldes and Banga[3], in a series of acute experiments, cannulated the main pancreatic duct of dogs and reported demonstrable elastolytic activity in canine pancreatic secretions. Grant and Robbins[4] in a similar experiment reported that elastase in the pancreas is inactive. They hypothesize that the inactive 'proelastase' must be activated (by small amounts of crystalline trypsin or enterokinase) before elastolytic activity can be demonstrated.

Recently, we have had the opportunity to study a 46-year-old Negro male with a persistent external pancreatic fistula. Approximately 1,000–1,500 ml. of pure pancreatic secretion was discharged daily through the fistula as a colourless, slightly turbid fluid, which ranged in *p*H from 8·15 to 8·52. The amylase concentration of this secretion was consistently high, ranging from 2,000 to 24,000 Somogyi units per 100 ml.

In studies on this patient, secretions were collected at 30-min. intervals. After two fasting collections, the patient was fed a standard meal and collections were carried out for 5 hr. Utilizing a colorimetric method developed in this laboratory[5], 1-ml. aliquots of the pancreatic secretion were assayed for elastase activity. Ordinarily, the freshly collected pancreatic secretion had negligible elastolytic properties. However, when 0·2 mgm. of crystalline trypsin was added to each aliquot, considerable elastolytic activity was demonstrated. Using a previously prepared nomogram[5], correlating optical density with concentration of crystalline porcine elastase, the activity of elastase in each sample was determined.

Table 1 contains the results from a representative 6-hr. secretion study with this patient. There was a prompt increase in elastolytic activity in the 30-min.

Table 1. ELASTOLYTIC ACTIVITY IN HUMAN PANCREATIC SECRETION

Time (min)	Activated elastase activity (μgm /ml)	Non-activated elastase activity (μgm /ml)	Volume of secretion (ml /30 min)	Total elastolytic action (μgm /30 min)
Fasting				
30	12 5	0 0	22 0	275 0
60	14 0	0 0	15 0	210 0
Post-prandial				
30	18 0	<1 0	53 0	954 0
60	13 0	<1 0	43 5	565 5
90	12 0	0 0	34 5	414 0
120	6 0	0 0	26 5	159 0
150	19 0	<1 0	25 2	479 0
180	22 5	0 0	26 0	585 0
210	22 0	0 0	21 4	471 0
240	16 0	0 0	27 0	432 0
270	19 0	0 0	24 5	465 0
300	12 0	0 0	23 5	282 0

samples immediately following the meal, followed by a decline Later, the elastase concentration again rose and finally returned toward the fasting level

To our knowledge, this is the first instance that elastase activity has been demonstrated in human pancreatic secretion Further studies are in progress to relate and quantify the relationship of elastase secretion with that of trypsin and amylase

S TINDEL
I J SCHNEIDER *
D SHAPIRA
D STATE

Department of Surgery,
Albert Einstein College of Medicine,
Eastchester Road and Morris Park Avenue,
New York 61

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* Gastro-Intestinal Trainee Supported in part from the U S Public Health grant No 2A-5200 (C1)

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Detoxification of Heartwood Toxins and Chlorophenols by Higher Fungi

THE heartwood of many conifers and of some broadleaved trees is well protected against attack by fungi and insects by specific and very toxic heartwood-toxins such as pinosylvin, pinosylvin-monomethylether (PSM), thujaplicins (TH), nootkatin, etc [1] These compounds have proved to be uncoupling toxins which inhibit oxidative phosphorylation in aerobic organisms[2] They have in this respect the same effect as the synthetic poisons 2,4-dinitrophenol, pentachlorophenol and other chlorinated phenols which are used for technical impregnation of less-durable timbers and of the sapwood of conifers[3] PSM has, besides its main function, a strong activity towards SH-groups of several enzymes[4]

In spite of the toxicity of these compounds it is a well-known fact that several fungi are able to destroy the heartwood even in the living tree and that impregnated timber is destroyed after some time by wood-rotting fungi There is no reason for believing that the heartwood-rotting fungi are insensitive to the heartwood toxins, because their respiration is bound to the cytochrome system and oxidative phosphorylation is the main source of energy

My most recent investigations show that pinosylvin-mono-methylether and β-thujaplicin as well as the chlorophenols lose their toxicity after oxidation by fungal oxidases such as laccase, tyrosinase and peroxidase This is shown in Table 1 by the amount of secretion of inorganic phosphate in the yeast test under the influence of the toxins before and after an oxidation with laccase The other oxidases gave similar results Tyrosinase was even more active because it oxidizes monophenols more easily

Table 1 DETOXIFICATION OF CHLOROPHENOLS AND HEARTWOOD TOXINS AFTER OXIDATION WITH LACCASE

Measured by ability to uncouple oxidative phosphorylation (secretion of inorganic phosphate (μgm P/100 ml) in the yeast test (ref 2))

Compounds	2-CP		3-CP		4-CP		5-CP		PSM		β-TH	
	Days of oxidation											
Conc (mole)	0	10	0	9	0	9	0	9	0	3	0	3
10^{-4}	350	100	350	50	400	50	200	50	—	—	—	–
3×10^{-5}	300	100	350	50	400	50	250	50	400	100	350	0
10^{-5}	250	50	250	50	350	50	350	50	350	0	250	0
3×10^{-6}	150	0	200	0	250	0	250	0	300	0	200	0
10^{-6}	100	0	150	0	200	0	200	0	250	0	100	0
3×10^{-7}	0	0	0	0	50	0	50	0	150	0	50	0
10^{-7}	0	0	0	0	0	0	0	0	50	0	0	0

No acids have been found by paper chromatography, pointing to a ring cleavage. Detoxification is nearly complete after 72 hr in β-TH Characteristic changes in the ultra-violet spectrum of the compounds, which after some time are no longer reversible by reduction, show the change of the compounds due to oxidation Its nature is not yet clear In some cases a weak polymerization is probable The chlorinated phenols split off 6 per cent (pentachlorophenol (5-CP)), 11 per cent (2,3,4,6-tetrachlorophenol (4-CP)), 18 per cent (2,4,5-trichlorophenol (3-CP)) and 43 per cent (2,4-dichlorophenol (2-CP)) respectively of the bound chlorine after 14 days incubation with laccase or peroxidase plus hydrogen peroxide

Although heartwood toxins and chlorophenols are almost of equal toxicity in the yeast test, their stability against oxidative degradation is very different This may be estimated in relative values as follows 5-chlorophenol = 100, 4-chlorophenol = 95, 3-chlorophenol = 86, 2-chlorophenol = 60, PSM = 20, β-TH = 15 This stability against detoxification is a very important factor of quality for those toxins which were not yet recognized

Nearly all heartwood-rotting fungi possess one or more of these oxidases, and it seems reasonable to assume that they are active *in vivo* too, although here a ring cleavage as the second step of degradation is to be expected This would mean a new function for the oxidases, which must be regarded as multifunctional enzymes That does not hinder the participation of the ectoenzymes laccase and peroxidase in destruction of lignin For white-rotting fungi living in the heartwood the peroxidase system is more suitable for oxidations because it does not compete for oxygen with intracellular substrate-oxidations Tyrosinase may be found in nearly all brown-rotting fungi attacking the heartwood[5]

The tannins of the heartwood strongly inhibit oxidases[4] This may be a more important function of these compounds than their low toxicity against micro-organisms This fits well with the decrease of toxicity of the heartwood components after autoxidative polymerization of tannins in aged heartwood[6,7]

The enzymatic detoxification of phenolic toxins by fungal oxidases is important in the problem of heartwood destruction by fungi and shows some causal relations in ecological specialization Whether still other mechanisms of detoxification are involved will be investigated

H LYR

Institut fur Forstbotanik, Eberswalde,
Humboldt-Universitat,
Berlin

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PHYSIOLOGY

Relationship between Changes in Muscle Volume and Muscle Action Potential

THE method which was developed for measuring small rapid changes in volume occurring in a muscle during a single twitch[1] has been applied to the case of a frog sartorius muscle immersed in hypertonic Ringer's solution As reported by various authors, one of the principal effects of hypertonic Ringer's solution on a muscle is to eliminate the twitch tension on stimulation, but not to interfere with the propagation of the muscle action potential This provides a method of determining whether or not the previously reported muscle volume changes are a direct consequence of the action potential and are thus not connected with contraction as such

The method used to measure these volume changes has been described elsewhere[2] In this experiment, the same method was used except, after determining the changes in volume in normal Ringer's solution, hypertonic Ringer's solution (2 5 times the regular concentration) was added to the muscle chamber Since this addition took approximately 30 sec , the changes in volume were not measured until this point Thirty seconds after the addition of the 2 5 Ringer's solution the muscle was stimulated by a single stimulus and the volume changes recorded

After the addition of hypertonic Ringer's solution to the muscle, the magnitude of the tension slowly decreases In addition to the decrease in magnitude of tension, the rate of development of tension is greatly prolonged After the muscle has been in the hypertonic Ringer's solution for approximately 15 min , no tension can be developed on twitch stimulation Paralleling these changes in tension, the changes in volume slowly decrease in magnitude and increase in duration, as does the twitch tension After 15 min , when the twitch tension has been reduced to zero, the changes in volume are not detectable Both the previously reported increase in volume and the decrease in volume are no longer visible This effect is completely reversible If normal Ringer's solution is then added in place of the hypertonic Ringer's solution the changes in volume and the twitch tension return to normal within a few minutes All these experiments have been carried out at 20° C

These results indicate that the changes in volume relate to contraction in a muscle, and are not directly the result of the propagation of the action potential Previously, it has been postulated that the increase in volume may be an indication of the process linking excitation to contraction in the muscle Evidence has been presented by Hill (1957) indicating that, in hypertonic Ringer's solution, the activation heat is still present It must be pointed out that in order to obtain this activation heat one needs to stimulate the muscle with ten times the normal stimulus In the measurement of changes in volume this is not possible, since this quantity of stimulus would produce a change in volume due to heating

This work has demonstrated the close correlation between both the increase in volume and the decrease in volume and the actual contraction of the muscle It may be postulated that the changes in volume are a direct indication of the protein reorganization which occurs during the contraction of a muscle It must be emphasized that the action potential does not in itself appear to be responsible for any of the observed changes in volume which occur during a single twitch of a frog sartorius muscle

R J BASKIN

Department of Biology,
Rensselaer Polytechnic Institute,
Troy, New York

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Influence of Various Stimuli on Lactic Acid, Alanine and Gamma-Aminobutyric Acid Levels in Rat Brains

As has previously been shown[1,2], an increase of lactic acid, alanine and γ-aminobutyric acid in the brain of rats occurred when, after tying both carotids, stimuli were applied to the cortex which caused electroencephalographic depression This communication is concerned with the way in which the aforementioned metabolites are influenced after stimuli causing depression (3 and 0 3 *M* solutions of potassium chloride, rubidium chloride, cæsium chloride and ammonium chloride), after stimuli not causing depression (3 and 0 3 *M* solutions of lithium chloride and sodium chloride), after stimuli blocking the spreading of the depression (3 and 0 3 *M* solutions of magnesium chloride and calcium chloride[3]) and after stimuli causing the increased activity of the nervous system (1 and 10 per cent solutions of pentamethylentetrazol and 0 1 and 1 per cent solutions of strychnine), when both carotids were tied All stimuli were applied on the exposed cortex by placing a piece of filter paper dipped in the used solution on the cortex for 30 min The brains were then fixed in liquid air In ethanol extracts of the frozen brains the free amino-acids were determined by paper chromatography according to the method of Rindi and Ferrari[4] and in trichloroacetic acid extracts lactic acid was estimated according to the method of Barker and Summerson[5] All results are expressed as µmoles of the compound investigated per gram of protein nitrogen, which was determined by the micro-Kjeldahl distillation method For statistical evaluation Student's *t* test, unpaired variates, was used

As it was established that no differences could be found in the investigated metabolites between the experimental and the opposite intact hemispheres of the same amine, the latter were used as controls

The results showed that the 3 *M* chlorides of the alkali metals caused an increase of lactic acid alanine and γ-aminobutyric acid (Table 1) When 0 3 *M* solutions of the same compounds were applied, the increase of lactic acid, alanine and γ-aminobutyric acid occurred only after the solutions causing electroencephalographic depression, namely, chlorides of

Table 1 Lactic acid, alanine and γ-aminobutyric acid in the brains of the rats expressed as μmoles per gram of protein nitrogen On the exposed cortex locally applied 3 M solutions of chlorides of the alkali metals for 30 min

	LiCl		NaCl		KCl		RbCl		CsCl		NH_4Cl	
	C	E	C	E	C	E	C	E	C	E	C	E
Lactic acid	112±31	250±41	140±49	283±52	153±43	433±53	160±43	368±77	108±16	379±84	105±11	318±69
	$n=5$		$n=6$		$n=6$		$n=5$		$n=6$		$n=6$	
	$P<0\ 01$		$P<0\ 05$		$P<0\ 01$		$P<0\ 01$		$P<0\ 01$		$P<0\ 02$	
Alanine	43± 6	52±13	54± 9	84±22	46±15	89±23	45± 6	74± 7	49±16	79±15	44± 6	76±12
	$n=10$		$n=17$		$n=40$		$n=6$		$n=6$		$n=13$	
	$P<0\ 1$		$P<0\ 01$		$P<0\ 01$		$P<0\ 01$		$P<0\ 02$		$P<0\ 01$	
γ-amino-butyric acid	122±15	150±35	98± 9	138±25	125±57	206±62	120±10	175±21	111±14	168±24	128±14	228±49
	$n=10$		$n=13$		$n=40$		$n=6$		$n=6$		$n=13$	
	$P<0\ 05$		$P<0\ 01$		$P<0\ 01$		$P<0\ 01$		$P<0\ 01$		$P<0\ 01$	

For statistical evaluation the t test, unpaired variates, was used C, control hemisphere, E, experimental hemisphere

Table 2 Lactic acid, alanine and γ-aminobutyric acid in the brains of the rats expressed as μmoles per gram of protein nitrogen On the exposed cortex locally applied 0 3 M solutions of chlorides of the alkali metals for 30 min

	LiCl		NaCl		KCl		RbCl		CsCl		NH_4Cl	
	C	E	C	E	C	E	C	E	C	E	C	E
Lactic acid	129±24	143±40	139±40	178±47	148±36	368±82	109±36	251±86	145±52	201±36	151±61	270±41
	$n=6$		$n=6$		$n=9$		$n=5$		$n=5$		$n=6$	
	$P<0\ 5$		$P<0\ 2$		$P<0\ 01$		$P<0\ 02$		$P<0\ 2$		$P<0\ 01$	
Alanine	50± 9	52± 7	54± 5	58± 9	52±16	91±30	48± 8	65± 6	54± 9	67± 7	55±10	106±33
	$n=5$		$n=5$		$n=10$		$n=5$		$n=6$		$n=6$	
	$P<0\ 5$		$P<0\ 5$		$P<0\ 01$		$P<0\ 01$		$P<0\ 05$		$P<0\ 02$	
γ-amino-butyric acid	117±17	124±21	106±14	116±16	104±22	177±28	112±10	171±29	121± 6	156±28	99±10	124±27
	$n=5$		$n=5$		$n=10$		$n=5$		$n=6$		$n=6$	
	$P<0\ 5$		$P<0\ 2$		$P<0\ 01$		$P<0\ 01$		$P<0\ 02$		$P<0\ 02$	

For statistical evaluation the t test, unpaired variates, was used C, control hemisphere, E, experimental hemisphere

Table 3 Recovery period after the electroencephalographic (EEG) depression caused by 3 M potassium chloride solution for 30 min, lactic acid alanine, γ-aminobutyric acid expressed as μmoles per gram of protein nitrogen

	30 min EEG depression		After 30 min EEG depression 30 min recovery period carotids tied		After 30 min EEG depression 90 min recovery period carotids tied		After 30 min EEG depression 30 min recovery period carotids free		After 30 min EEG depression 90 min recovery period carotids free	
	C	E	C	E	C	E	C	E	C	E
Lactic acid	153±43	433±53	174±10	404±91	296±89	403±180	152±25	242±45	140±35	179±52
	$n=6$		$n=6$		$n=5$		$n=6$		$n=5$	
	$P<0\ 01$		$P<0\ 01$		$P<0\ 2$		$P<0\ 01$		$P<0\ 3$	
Alanine	46±15	89±23	47±12	91±10	199±77	238±143	53±10	76±16	42±11	45±12
	$n=40$		$n=10$		$n=7$		$n=7$		$n=10$	
	$P<0\ 01$		$P<0\ 01$		$P<0\ 3$		$P<0\ 02$		$P<0\ 3$	
γ-aminobutyric acid	125±57	206±62	120±35	208±59	241±95	261±84	120±31	149±45	117±26	128±29
	$n=40$		$n=10$		$n=7$		$n=9$		$n=10$	
	$P<0\ 01$		$P<0\ 01$		$P<0\ 3$		$P<0\ 2$		$P<0\ 3$	

For statistical evaluation the t test, unpaired variates, was used C, control hemisphere, E, experimental hemisphere.

potassium, rubidium, cæsium and ammonium (Table 2)

All these changes were reversed when the blood supply to the brain cortex was renewed (Table 3)

3 M solutions of chlorides of the alkali earths caused similar changes of the metabolites like 3 M solutions of the alkali metals When 3 M magnesium chloride was used, an increase of lactic acid from 138 ± 36 to 273 ± 41, alanine from 53 ± 4 to 71 ± 13 and γ-aminobutyric acid from 101 ± 10 to 126 ± 29 μmoles per gram of protein nitrogen was observed In the same way 3 M calcium chloride solution caused an increase of lactic acid from 146 ± 32 to 281 ± 58, alanine from 52 ± 5 to 71 ± 17 and γ-aminobutyric acid from 100 ± 25 to 157 ± 38 μmoles per gram of protein nitrogen (mean of 6 experiments) 0 3 M solutions neither of magnesium chloride nor of calcium chloride influenced the level of the metabolites in the brain

When 1 per cent strychnine solution was applied to the cortex, an increase of lactic acid from 97 to 342, alanine from 51 to 96 and γ-aminobutyric acid from 107 to 140 μmoles per gram of protein nitrogen was observed 10 per cent solution of pentamethylentetrazol caused an increase of lactic acid from 136 to 334, alanine from 52 to 81 and γ-aminobutyric acid from 111 to 146 μmoles per gram of protein nitrogen All changes are of statistical significance

Neither 1 per cent pentamethylentetrazol nor 0 1 per cent strychnine solutions influenced the level of the metabolites

0 15 M lactic acid solution caused an increase of alanine from 52 ± 7 to 82 ± 11 and that of γ-aminobutyric acid from 97 ± 9 to 134 ± 26 μmoles per gram of protein nitrogen

The increased alanine production during oligæmia of the brain, as has been shown previously[2,6], is related to the utilization of the free amino-acids in metabolic processes and to the increased amination of pyruvates produced during enhanced glycolysis Increased glycolysis and the decrease of pH can change the balance between the glutamic acid decarboxylase and γ-aminobutyric acid transaminase so that the predominance of decarboxylase is reached[7] and more

γ-aminobutyric acid can be produced Together with this phenomenon a decrease of α-ketoglutarate during oligæmia of the brain can be expected and so the transamination of γ-aminobutyric acid slowed down owing to lack of the substrate With respect to the increased formation of γ-aminobutyric acid and its decreased transamination the increased level of γ-aminobutyric acid in the experimental hemispheres was found

The increase of lactic acid, alanine and γ-aminobutyric acid is accompanied by some changes in the structure of the nerve cells The sections of the brain cortex after stimulation showed vacuolization and chromatolysis of the cytoplasm, thickening of the nuclear membrane, shrinking of the nuclear chromatin and the disintegration of the nucleolus[8] Therefore it is supposed that the metabolic reactions described are not only due to the altered functional activity of the nerve elements (that is, electroencephalographic depression, stimulation with pentamethylentetrazol and strychnine), but also to the rebuilding of the damaged cell structures

MICHAL RUŠČÁK

Slovak Academy of Sciences,
Bratislava

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Starvation and Renal Uptake of Vitamin B_{12}

Rosenthal and Cravitz have reported[1-3] that rabbits subjected to starvation accumulated remarkably large amounts of vitamin B_{12} in the kidney as compared with normally fed controls They also found similar, but less pronounced, effects of starvation in some animal species. No satisfactory explanation has been offered, however Our investigations with rats demonstrated that the renal uptake of vitamin B_{12} labelled with cobalt-60 following an oral administration was much greater in animals which had received vitamin B_{12} in their diet than in those which had been deprived of it[4] This finding together with other results[5] strongly suggest that the kidney is a kind of storage organ of vitamin B_{12}, and that accumulation of the vitamin in this organ is indication of a highly saturated state of the body tissue with respect to vitamin B_{12} In view of the discrepancy between Rosenthal's and our findings, the starvation experiment described in his recent paper[3] was followed, except that a special measure was taken for the prevention of coprophagy

Female rabbits weighing about 2 kgm were housed in individual cages with a wide screen bottom They were divided into 6 groups, 3 animals in each Three control groups (*A*, *B* and *C*) were fed *ad lib* with fresh and dried green vegetables and soybean-curd (Okara) for 6 days and then given labelled vitamin B_{12} by intramuscular injection, 0 05 μgm (0 04 μc)/kgm body-weight After the injection, group *A* was fed continuously, groups *B* and *C* were fasted, and all were killed 3 days later During the fasting period, each animal in group *C* was kept in a metal cylinder-type rabbit holder in the daytime and was released for the night The holder kept the animal in a stretched position, preventing it from reaching the fæces The three starved groups (*D*, *E* and *F*) were given no food for 6 days, the same dose of labelled vitamin B_{12} was injected, and the animals were fasted for 3 more days before death Groups *D* and *F* were kept in the holder during the night, and group *E* was kept in the holder during the daytime Group *F* received, besides, 3 doses of 1 mgm vitamin B_{12} by subcutaneous injection every other day during the pre-injection period At the end of 3 days after the administration of labelled vitamin B_{12}, the liver and the kidneys were removed, a portion of the former and all the latter were digested with 30 per cent potassium hydroxide and the radioactivities measured using the technique already described[6] The uptake of cobalt-60 by these organs expressed as a percentage of the dose per wet gram tissue is presented in Fig 1 It is evident that group *D*, which was fasted without having access to the fæces during the night, retained considerably less radioactivity in the kidneys as compared with other groups The kidneys of groups *E* and *F*, which were fasted but had access to the night fæces or received vitamin B_{12} by injection, accumulated more cobalt-60 Thus, the effect of starvation in rabbits with controlled coprophagy was quite contrary to that obtained without such a measure

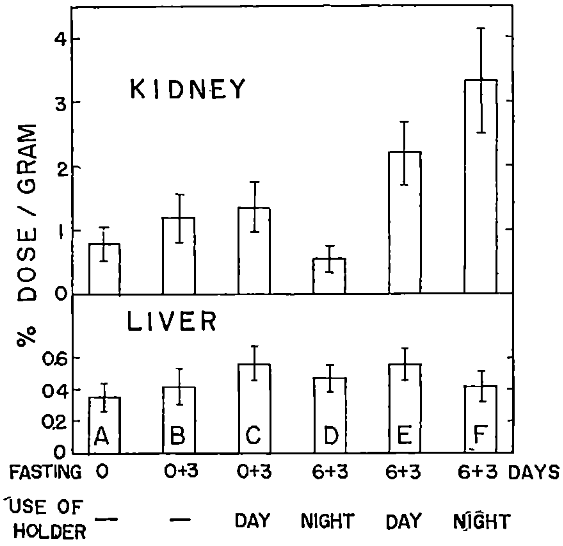

Fig 1 The effect of starvation on tissue uptake of injected vitamin B_{12} labelled with cobalt-60 in rabbits The vertical bars represent *S E* The numbers before and after (+) indicate days of fasting before and after the administration of labelled vitamin B_{12} Animals were killed three days after the injection of labelled vitamin B_{12}

Southern[7] and Eden[8] have observed that the rabbit voids soft fæces during the night which are consumed as they are excreted Kulwich[9] collected the soft fæces for three days by fitting a collar to the rabbit to prevent coprophagy, and found that the fæces

Table 1 EFFECTS OF STARVATION AND VITAMIN B_{12} ADMINISTRATION ON TISSUE DISTRIBUTION OF VITAMIN B_{12} LABELLED WITH COBALT-60 IN RATS

Group	Liver (per cent/organ)	Kidneys (per cent/organ)
(*A*) Non-fasted	13 ± 1 2	14 ± 1 5
(*B*) Non-fasted + B_{12}*	7 ± 0 8	41 ± 3 6
(*C*) Fasted	15 ± 1 4	11 ± 0 9
(*D*) Fasted + B_{12}*	6 ± 0 8	39 ± 4 2

Animals were killed three days after the administration of 20 mμgm of labelled vitamin B_{12} intraperitoneally

* Three doses of 50 μgm were given subcutaneously prior to labelled vitamin B_{12} Each figure is average of three rats ± *S E*

made a significant contribution to the vitamin economy of the animal. It seems that this characteristic behaviour is responsible for the exceptionally high contents of vitamin B_{12} in the rabbit[10]. Recently, Hirabayashi *et al.*[11] have demonstrated, using the collar technique, that normally the soft fæces voided during the night are completely consumed and that the mucous film separated from such fæces is an almost pure mucoprotein-B_{12} complex. According to Y. Sahashi (personal communication), use of a screen bottom is of little value and even the collar does not afford absolute arrest of coprophagy.

It may be that restricting food without a special measure for absolute prevention of coprophagy will simply increase coprophagic activities of the rabbit, and will result in a greater intake and therefore accumulation of vitamin B_{12} in the kidney. The uptake of a test dose of vitamin B_{12} labelled with cobalt-60 by the kidney depends more on the amount of vitamin B_{12} than food taken prior to the test. This fact is also clearly borne out in rats by the following experiment. Twelve young adult male rats which had been raised on a stock diet were placed in individual cages with a screen bottom, and were divided into 4 groups. Two groups (*A* and *B*) were fed *ad lib.* the same diet and the other two (*C* and *D*) were fasted for 5 days. Groups *B* and *D* received 50 μgm. of vitamin B_{12} by injection daily for the first 3 days. All animals were given 20 μgm. of labelled vitamin B_{12} intraperitoneally at the end of the 5th day. They were either fed continuously (*A* and *B*) or fasted (*C* and *D*) for 3 more days after the administration of labelled vitamin B_{12} and then killed. The amounts of cobalt-60 measured in the liver and the kidneys (Table 1) demonstrate that the uptake of cobalt-60 (most likely as the labelled vitamin B_{12}) by the kidney reflects the preceding intake of vitamin B_{12} rather than the amount of food fed.

KUNIO OKUDA

Department of Medicine,
Yamaguchi Medical College,
Ube, Japan.

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Initiation of Anaerobic Metabolism of Mammalian Spermatozoa by Carbon Dioxide

LITTLE is known about the effect of the absence of carbon dioxide on the metabolism of spermatozoa. In 1930 Harvey[1] suggested that the inability of *Arbacia punctulata* spermatozoa to survive during a 4-hr. incubation period in an atmosphere of hydrogen might be due to the absence of carbon dioxide. To our knowledge this suggestion has not been investigated nor has a similar observation and suggestion been reported for mammalian spermatozoa.

The beneficial use of diluters containing carbon dioxide to lengthen the *in vitro* survival of bovine spermatozoa has been demonstrated[2,3]. The increased survival presumably is due to the fact that anaerobic glycolysis[4,5] and the motility[6,7] of mammalian spermatozoa are markedly inhibited by relatively high $p\mathrm{CO}_2$. On the other hand, respiration[8,9] and aerobic glycolysis[10] of spermatozoa have been reported to be stimulated by low $p\mathrm{CO}_2$. In our work[9], it was observed that with some washed spermatozoan samples there was less carbon dioxide present in the flasks after 1 or 2 hr. of incubation than was present initially. Although earlier work has suggested an involvement of carbon dioxide in spermatozoan metabolism and survival, an actual need for carbon dioxide for these responses has not been reported. This communication reports results of an investigation implicating carbon dioxide as a necessary factor in initiating anaerobic glycolysis and for extending the *in vitro* survival time of bovine spermatozoa.

The anaerobic metabolism of epididymal-like spermatozoa[11] diluted in a sodium citrate, sodium bicarbonate (IVT) diluent[4] containing fructose was studied under gaseous atmospheres of 100 per cent nitrogen, 5 per cent carbon dioxide + 95 per cent nitrogen, and 100 per cent carbon dioxide in a Warburg respirometer at 37° C for 4 hr. Epididymal-like spermatozoa are obtained by collecting semen into an inhibitory diluent which renders the sperm cells non-motile and prevents the uptake of exogenous substrates from seminal plasma. The sperm cells are washed free of inhibitory diluent and re-suspended in 0.9 per cent sodium chloride solution to the original volume of the semen sample. The carbon dioxide evolved by the reaction of metabolically produced acid with bicarbonate (corrected for acid and carbon dioxide retention), the fructose disappearance, the lactic acid accumulation, and the recovery of motility on exposure to air were used as criteria of measurement. The methods of analysis have been reported previously[10].

The results presented in Table 1 show that of 59 spermatozoan samples examined the response to 100 per cent nitrogen could be divided into two distinct groups. The one group including 49 samples demonstrated little if any glycolytic activity, whereas the other group of 10 samples demonstrated substantial glycolytic activity under the nitrogen atmosphere.

Spermatozoa from the same 49 samples not metabolizing under nitrogen demonstrated normal glycolytic activity in a nitrogen atmosphere containing 5 per cent carbon dioxide. When the $p\mathrm{CO}_2$ was increased to 100 per cent, glycolysis by the sperm cells was markedly inhibited and was similar to that of the sperm cells under pure nitrogen. Although there was essentially no glycolysis occurring under both pure nitrogen and pure carbon dioxide in these 49 samples the recovery of motility on exposure to air, as determined by microscopic examination at the end of the 4-hr. incubation period, was markedly improved by the presence of carbon dioxide in the incubation atmosphere. There was essentially no sperm cell motility observed in samples which had been incubated under nitrogen, in contrast to good recovery of sperm cell motility in samples incubated under 100 per cent carbon dioxide (Table 1).

As already stated, substantial glycolysis did occur under 100 per cent nitrogen in 10 of the 59 samples studied, but even in these samples there was a stimulation of glycolysis when 5 per cent carbon dioxide was included in the gaseous atmosphere. It should be noted that these 10 spermatozoan samples contained more spermatozoa per unit volume than

Table 1 EFFECT OF CARBON DIOXIDE ON THE ANAEROBIC METABOLISM OF EPIDIDYMAL-LIKE BOVINE SPERMATOZOA IN A CITRATE–BICARBONATE MEDIUM CONTAINING FRUCTOSE AND THE RECOVERY OF MOTILITY ON EXPOSURE TO AIR AFTER INCUBATION

Diluent	n	Sperm conc × 10^8	Gas	CO_2* evolved	−Fructose*	+L A *	Motility † (per cent)
IVT	49	2 162	100% N_2	8 6	38 1	40 7	0 8
			5% CO_2+95% N_2	139 6	715 7	417 8	30 0
			100% CO_2	8 6	29 3	62 2	38 0
IVT	10	3 543	100% N_2	110 4	430 4	268 3	17 0
			5% CO_2+95% N_2	132 9	723 4	437 2	37 0
			100% CO_2	16 7	178 9	23 8	42 0

* μl or μgm /10^8/4 hr
† Mean initial percentage motility was 50 and 57 for the 49 and 10 samples respectively

did the 49 samples which did not metabolize under nitrogen Even in these more concentrated samples glycolysis was markedly inhibited by 100 per cent carbon dioxide Although some motility was observed on aeration at the end of the incubation period in these samples metabolizing under nitrogen, the percentage of motile spermatozoa was much greater after incubation in the carbon dioxide atmosphere The reason for glycolysis occurring in 10 samples under nitrogen in contrast to the lack of it in 49 samples is not known, but it is suspected that due to the relatively higher sperm cell concentration enough carbon dioxide was retained by the spermatozoa to initiate glycolysis and maintain motility It was observed that in flasks containing a sperm cell concentration above 4 0 × 10^8 spermatozoa per ml glycolysis did occur under nitrogen, and in flasks containing less than 2 0 × 10^8 spermatozoa per ml glycolysis did not occur With sperm cell concentrations between 2 0 and 4 0 × 10^8 spermatozoa in the flasks the results were not predictable in that some samples did glycolyse and some did not

The results presented here are the first to show an apparent need for carbon dioxide in the metabolism and survival of spermatozoa and investigations are being continued to determine the exact part which carbon dioxide plays in the metabolic process

J R LODGE
G W SALISBURY

Department of Dairy Science,
University of Illinois,
Urbana

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Effect of Ouabain on the Resting Tension and the Rate of Net Entry of Calcium-45 in Isolated Perfused Rabbit Ventricles

RECENT work in this laboratory has shown that in isolated rabbit atria the contracture induced by ouabain is probably the result of an increased calcium uptake[1] Furthermore, it was shown that ouabain only affected calcium exchange that accompanies the process of activation[2] Temperature studies revealed a high temperature coefficient (Q_{10}) of 4–6 for both the effects on contracture and calcium-45 entry[3] In this report we present the effects of ouabain on entry of calcium-45 and contracture in isolated perfused rabbit ventricles The results indicate that contractures induced by the cardiac glycosides in both rabbit atria and ventricles are associated with an increased calcium entry

Young rabbits were used Hearts freshly excised were perfused through a cannula in the ascending aorta, the normal perfusion fluid containing sodium chloride 7 7 gm , potassium chloride, 0 42 gm , calcium chloride, 0 24 gm , sodium bicarbonate, 2 1 gm , crystalline sodium dihydrogen phosphate, 0 143 gm , dextrose, 2 0 gm , sucrose, 4 5 gm , distilled water to 1 litre The solution was saturated with 95 per cent oxygen and 5 per cent carbon dioxide Perfusion was performed by means of a special apparatus in which the temperature of the solution entering the heart could be kept constant at 37° C , regardless of the perfusion-rate Tension changes were recorded on a Sanborn twin-channel recorder using a strain gauge by means of a thread attached to the tip of the left ventricle

The rate of net entry of calcium-45 was determined by perfusing the heart with the isotope and with and without ouabain (10^{-6} M) for 25 min This was followed by perfusion with non-radioactive solution for 5 min The ventricular tissue was then dried, weighed and homogenized in distilled water The total radioactivity of an aliquot of this solution was determined and the rate of net entry of calcium-45 calculated

Table 1 shows the effects of ouabain on both the rate of net entry of calcium-45 in c p m /gm of ventricular tissue and the percentage increase in resting tension Both the mean and individual paired experiments done simultaneously are presented Ouabain significantly increased the mean rate of net entry of calcium-45 116 per cent ($P < 0\ 02$) In addition ouabain increased the mean resting tension 45 per cent In experiment seven only a positive inotropic effect was recorded It is interesting to note that in this experiment there was an 11 per cent increase in the net rate of entry of calcium-

Table 1 EFFECTS OF OUABAIN (10^{-6} M) ON THE RATE OF NET ENTRY OF CARBON-45 AND PERCENTAGE INCREASE IN RESTING TENSION IN ISOLATED PERFUSED RABBIT VENTRICLES PAIRED EXPERIMENTS WERE PERFORMED SIMULTANEOUSLY

Experiment No	Rate of net entry of Ca^{45} c /m /gm /20 min Control	Ouabain	Percentage increase in rate of entry	Percentage increase in resting tension after ouabain
1	5,869	11,714	99	12 5
2	6,130	11,411	86	12 2
3	6,815	20,726	204	63 0
4	6,414	22,072	244	48 0
5	12,313	25,622	108	66 0
6	5,022	7,433	48	11 0
7	6,987	7,817*	11	—
8	13,778	31,524	129	100
Mean	7,916	17,289	116	45
S E	±1,190	±3,370		
P		< 0 02		

* Positive inotropic effect only

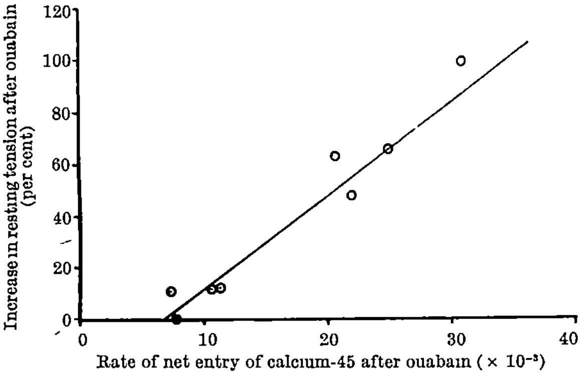

Fig 1 Relation between changes in resting tension and the rate of net entry of calcium-45 after treatment with ouabain (10^{-6} *M*)

45 in the ouabain-treated heart when compared with its paired control However, there was no significant difference when compared with the mean control value

In Fig 1 we have attempted to correlate changes in resting tension with the rate of net entry of calcium-45 in each individual heart after ouabain It can be seen that there is an essentially good positive correlation between resting tension at 20 min and the rate of net entry of calcium-45

Thomas indicates that the contractures induced by ouabain in frog hearts are associated with an increased uptake of calcium-45 (ref 4) However, in his experiments there was only a slight correlation between the changes in resting tension and uptake of calcium-45 In addition, the percentage changes in uptake of calcium-45 were small (25 per cent) In our experiment a good positive correlation was obtained between the increase in resting tension and the rate of net entry of calcium-45 Furthermore, the increase of the rate of entry of calcium-45 was large (116 per cent) These differences could be due to several factors In our experiments we used a wash-out period in non-radioactive solution in order to decrease extracellular calcium-45 and accentuate cellular changes In addition, our experiments were carried out at 37° C as compared with 21°–24° C Recent investigations in this laboratory indicate that the contracture and increased entry of calcium-45 induced by ouabain are both temperature-sensitive (Q_{10}s of 4–6)

Since calcium-45 efflux measurements were not attempted, the net effect of calcium could not be determined However, studies in several laboratories of both skeletal and heart muscle have revealed that ouabain has no effect on calcium outflow[1,5] In addition, Lee has demonstrated an actual net increase of cellular calcium during ouabain intoxication in the papillary muscle of the cat[6]

These results indicate that the ouabain-induced contractures in both rabbit atria and ventricles are basically similar and probably result from increased calcium concentration in the cardiac cell

The exact mechanism by which this increased concentration of calcium in the myoplasm results in contracture is not known Evidence obtained from isolated fibre systems indicates that calcium is the only physiological ion which causes contraction when injected directly into the myoplasm[7] However, in isolated glycerol-extracted fibres calcium does not cause contraction unless relaxing factor or factors are present and active[8] This suggests that under physiological conditions ouabain-induced contractures could result from an inhibition of relaxing factor(s) due to an increased concentration of calcium in the vicinity of the contractile apparatus

Investigations in this laboratory are now in progress to determine in what sub-cellular fraction(s) this increased calcium is located and also whether ouabain has any direct effect on calcium metabolism in these fractions

This work was supported by Public Health Service grants *H*–5612, *H*–5151 and *H*–4265

A H Briggs
W C Holland

Department of Pharmacology,
University of Mississippi Medical Center,
Jackson, Mississippi

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PHARMACOLOGY

Effect of Salicylate on Plasma Magnesium-Levels

In this laboratory a recent examination of the distribution of electrolytes in rats following the repeated administration of both sodium and potassium salicylate has shown that a marked decrease in the excretion of urinary magnesium occurs Evidence of a less well-defined increase in the level of plasma magnesium was also found[1]

To examine this point in greater detail, plasma was collected from a group of 23 untreated laboratory rats and from 13 animals which had been given either sodium or potassium salicylate by gavage for at least seven days The drug was given as an aqueous solution in a dosage of 30 mgm salicylate radical/100 mgm body-weight/day All the treated rats exhibited clear symptoms of salicylate intoxication prior to the collection of blood samples, that is, hyperventilation, body wastage and hæmorrhage Heparinized blood was collected within 30 min of administration of the drug in order to obtain near maximum levels of plasma salicylate[2]

Plasma magnesium was estimated by the method of Orange and Rhein[3] and salicylate by Trinder's method[4]

It can be seen from the results in Table 1 that there is a substantial elevation in the plasma magnesium-levels of those rats receiving salicylate (mean 3 7 mgm per cent *SD* ± 0 23 mgm per cent) compared

Table 1 EFFECT OF SALICYLATE ON PLASMA MAGNESIUM-LEVELS IN THE RAT

Group	No	Plasma constituent	Mgm (per cent)					Mean
Control	23	Mg++	2 1	2 7	2 3	2 4	3 0	
			2 8	3 0	3 3	3 0	3 1	
			2 6	2 9	3 0	3 3	3 2	2 9
			3 0	3 1	3 2	3 3	2 4	
			2 9	2 9	2 8			
		Salicylate	—					
Treated	13	Mg++	3 0	3 8	3 1	3 8	4 6	
			4 2	4 0	4 5	3 1	3 2	3 7
			4 3	3 9	2 9			
		Salicylate	39	23	52	48	82	
			58	65	40	33	45	51
			56	56	75			

Salicylate given as the sodium or potassium salt by gavage in dosage of 30 mgm /100 gm body-weight/day for at least seven days with the levels estimated in the untreated group (mean 2 9 mgm per cent *S D* ± 0 63 mgm per cent) By calculating a common variance, comparison of the mean values for each group reveals a significant difference at the 0 1 per cent level The elevated plasma magnesium-level in the treated group is associated with a mean plasma salicylate-level of 51 mgm per cent (*S D* ± 16 8 mgm per cent)

Taken with the finding of decreased excretion of Mg^{++} under similar conditions of intoxication[1] it is apparent that a retention of magnesium ions is produced by the administration of salicylate

Since the early observations of Winter and Barbour[5] magnesium has been used both experimentally and clinically to reduce body temperature under a wide variety of conditions[6] The rise in plasma magnesium which is associated with high plasma salicylate-levels may therefore explain the antipyretic effect of this drug

The support of the National Health and Medical Research Council of Australia is gratefully acknowledged

J S Charnock
R Lockett
B S Hetzel

Department of Medicine,
Queen Elizabeth Hospital,
University of Adelaide,
Woodville, South Australia

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Protective Action of γ-Aminobutyric Acid against Oxygen Toxicity

The effects of the inhalation of oxygen at greater than normal pressures have assumed greater importance as man's penetration into the depths of the sea has increased It was Bert[1] who for the first time revealed that, although oxygen at low pressures sustains life, the gas is toxic at high pressures In their review of the subject, Stadie *et al* [2] conclude that the dominant symptoms of the action of oxygen at 0 8–2 0 atmospheres are respiratory, while at higher oxygen pressures central nervous system symptoms predominate Several theories have been put forward to explain the toxic effects of oxygen at high pressures[3], but no one theory is completely satisfactory

Of the animal tissues and fluids which have been examined for γ-aminobutyric acid (GABA), only those from the central nervous system contain the amino-acid in detectable amounts[4], and it has been suggested that GABA may play an important part in some aspects of the regulation of physiological activity in the brain[5] More recent investigations have indicated that treatment of animals with GABA affords some protection against convulsions induced by hydrazides[6] and that human beings with various types of seizures experience significant improvement in their condition as a result of treatment with oral doses of GABA[7]

In view of these findings it was deemed worth while to investigate the influence of GABA on the toxic effects of oxygen at high pressures The results of this investigation are presented here

Table 1 EFFECT OF AN ORAL DOSE OF γ-AMINOBUTYRIC ACID ON THE RESISTANCE OF RATS TO OXYGEN TOXICITY

Exp No	No of rats convulsing		No of rats with severe convulsions		No of rats dying within 24 hr	
	Control	GABA	Control	GABA	Control	GABA
1	3	0	2	0	2	1
2	2	3	1	1	0	1
3	3	3	2	0	3	0
4	3	2	2	0	2	0
5	3	1	0	0	0	0
6	3	2	3	0	2	1
7	3	2	1	0	1	0
8	3	1	2	0	2	0
9	3	1	1	1	1	1
10	3	3	1	1	1	0
11	3	3	2	2	3	3
12	3	3	2	1	2	1
13	3	2	2	1	2	2
14	3	2	0	0	2	1
15	3	2	2	0	2	0
16	3	3	2	1	2	1
Total	47	33*	25	8†	27	12*

* Probability that difference from control rats is significant, P < 0 01

† Probability that difference from control rats is significant, P < 0 001

Male Wistar rats weighing 190–220 gm were used in the experiments Two ml of 0 9 per cent aqueous sodium chloride containing 2 m moles of GABA were injected intraperitoneally 30 min prior to exposure of the animals to oxygen at high pressure Control rats were treated similarly except that the GABA was omitted from the saline solution Three control and three treated rats were studied simultaneously using the procedure of Walker[8], which entails placing the animals in lucite compartments contained in a pressure chamber After the apparatus had been flushed well with oxygen for approximately 1 min the pressure was increased steadily during a 1-min interval to a gauge pressure of 75 lb /in [2] This pressure was maintained for 40 min followed by decompression to atmospheric pressure over a period of 2 min During the exposure to oxygen at high pressure a fan outside the pressure chamber circulated the oxygen and passed the respired gas through a screen of soda-lime to remove the carbon dioxide exhaled by the animals

The effect of oxygen at high pressure on control and GABA-treated animals is shown in Table 1 A convulsion was scored when an involuntary movement of the body occurred and the seizure was considered to be severe when there was gross and violent movement of the body as a whole It is evident that GABA reduced significantly the effects of oxygen at high pressure on the treated rats, not only in the number of deaths resulting from oxygen at high pressure but also in the number and severity of the convulsions Of the control rats having seizures, 53 per cent suffered violent convulsions whereas the corresponding figure for the treated rats was 24 per cent

J D Wood
W J Watson

Defence Research Medical Laboratories,
P O Box 62, Postal Station 'K',
Toronto, 12

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HÆMATOLOGY

Acid Phosphatase Activity in Human Blood Cells

An improved azo dye method[1] has been applied to human blood cells by which acid phosphatase activity could be demonstrated in all peripheral leucocytes and platelets. This technique is based on the use of phosphate esters of 'Naphthol *AS*' derivatives[2] as substrates and hexazonium *p*-rosanilin as a coupler[3,4].

Blood smears, air-dried for 10–20 min. at room temperature, were fixed for 10 min. in cold (4° C.) 10 per cent formalin containing 1 per cent calcium chloride. The formalin solution was adjusted to *p*H 7.0 with *N* sodium hydroxide and controlled weekly. (A 10 per cent formalin solution in 0.1 *M* sodium acetate adjusted to *p*H 7.0 can be substituted with similar results.) Following fixation, the smears were rinsed well with distilled water and incubated for up to 3 hr. (generally 2 hr.) at room temperature. The incubation time can be reduced to about half with similar results by incubating at 37° C. The incubation solution was freshly prepared from four stock solutions: (1) Michaelis veronal–acetate buffer stock solution: 9.71 gm. sodium acetate. $3H_2O$ plus 14.71 gm. sodium barbiturate dissolved in carbon dioxide free distilled water made up to final volume of 500 ml. (2) Substrate stock solution: 'Naphthol *AS–BI*' phosphate (Nutritional Biochemical Co., Cleveland, Ohio) dissolved in *N*,*N*-dimethyl-formamide in 10 mgm./ml. concentration. (3) 4 per cent sodium nitrite in distilled water. (4) 2 gm. 'Pararosanilin-hydrochloride' (National Aniline Division, Allied Chemical and Dye Corporation, New York) added to 50 ml. 2 *N* hydrochloric acid and gently heated. After cooling, the solution was filtered. Solutions 1, 2 and 3 were stored at 4° C. The working solution was prepared as follows: 5 ml. of solution No. 1 and 1 ml. of No. 2 were mixed with 13 ml. of distilled water in a beaker. To this was added 1.6 ml. of hexazonium *p*-rosanilin solution obtained by adding 0.8 ml. of solution No. 3 to 0.8 ml. of solution No. 4 in a test tube. After mixing, the *p*H was adjusted to 5.0 with *N* sodium hydroxide.

Following the incubation, the smears were rinsed in distilled water and counterstained with methyl green (1 per cent methyl green in phosphate or veronal–acetate buffer, *p*H 4) for 30–60 sec., rinsed well in distilled water, quickly dehydrated through alcohol and mounted via xylene in synthetic mounting medium. The sites of enzyme activity were marked by deposits of amorphous red azo dye. (The microscopic observation of the red azo dye precipitate is facilitated by using a balanced light source obtained by combining a didymium and Wratten 82 *C* filter.) The nuclei were stained green.

During the incubation period some precipitate developed in the test medium. Nevertheless, changing the solution midway during the incubation produced no improvement in the staining reaction. Acid phosphatase activity could be demonstrated in cells in unfixed smears. The fixation used, however, improved the morphological preservation of the cells. From the point of view of both morphology and preservation of enzyme activity neutral formalin was found to be the superior fixative.

Acid phosphatase activity could also be demonstrated in blood cells by using other 'Naphthol *AS*' substrates. The intensity of the staining reaction decreased with the various substrates tested in the following order: 'Naphthol *AS—BI*', '*AS—AN*', '*AS—TR*', '*AS—CL*' and '*AS—MX* phosphate'. Practically no staining occurred after a 3-hr. incubation with 'Naphthol *AS—GR*' phosphate. With these phosphate esters no substrate specificity or preferential staining was observed in the blood cells tested. α-Naphthyl phosphate with hexazonium *p*-rosanilin (1 at *p*H 6 or 6.5 sec., ref. 4) gave less satisfactory results.

After incubation for 2 hr., acid phosphatase activity was demonstrated in all types of leucocytes. Every cell showed some degree of activity. Basophiles, however, were not definitely identified. Eosinophiles and monocytes revealed the most intense reaction followed by neutrophiles and lymphocytes. Staining was also observed in platelets but not in erythrocytes. In each cell series variations of the enzyme activity were seen. The nature of the staining reaction was generally very finely granular and diffuse. However, in each of the cell series there were cells which revealed coarser granules of azo dye scattered in their cytoplasm. (The demonstration of acid phosphatase activity in human blood cells using Gomori's lead sulphide method, as well as the relationship of enzyme activity to lysosomes, was discussed elsewhere[5].) The technique reported here can be applied to bone marrow smears (for example, very high acid phosphatase activity was found in multiple myeloma cells).

Since the simple technique reported here gave reproducible and artefact-free localization, it is hoped that it will facilitate the investigation of acid phosphatase activity in both blood and bone marrow cells in various pathological conditions.

'Naphthol *AS—AN*' and '*AS—CL*' phosphates were synthesized and kindly supplied by Dr. M. S. Brustone, whom we thank.

This work was supported by grants *A*-3846 and *C*-5987 from the U.S. Public Health Service.

Arthur F. Goldberg
Tibor Barka

Department of Hematology and Pathology,
The Mount Sinai Hospital, New York 29.

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Two Human Blood Group Antigens, St^a (Stones) and Ri^a (Ridley), closely related to the MNSs System

A series of screening tests has been undertaken with a serum Ath. known to contain powerful saline-agglutinating antibodies against the rare Wright (Wr^a) and Swann (Sw^a) blood group factors. Control reagents were prepared by absorbing separate aliquots of the serum with known Wr(a +) and Sw(a +) cells. Samples found positive with the native serum were tested afterwards with these control reagents, so that Wr(a +) and Sw(a +) individuals were readily identified.

The cells of one such positive reactor, Mrs. Stones, were found to react with both the absorbed sera, and further tests have shown that a third antibody is present in serum Ath. This is clearly separable from the other two antibodies by absorption with Mrs.

Stones's cells, thus yielding a third control reagent for subsequent tests

The propositus' cells were grouped as

A_2, cde/cde, NS/Ns, P_1 + +, Lu(a − b +), Le(a − b +), K − k +, Kp(a − b +), Fy(a + b +), Jk(a + b −)

and negative reactions were obtained with anti-V, -$\mathrm{C^x}$, -$\mathrm{Js^a}$, -$\mathrm{p^k}$, -$\mathrm{Di^a}$, -By, -$\mathrm{Be^a}$, -Good, -$\mathrm{Bi^a}$, -Levay, -$\mathrm{Mi^a}$, -Vw, -Hu, -He, -Vr, -$\mathrm{M^g}$, -Wb [1], -$\mathrm{Mt^a}$ [2], -Mu [3], -$\mathrm{Tr^a}$[4] The cells reacted strongly with anti-Vel, -Ge, -$\mathrm{Yt^a}$, -$\mathrm{Tj^a}$ (− P + P_1), -$\mathrm{Lu^b}$ and -H, and the I-i status was normal No relationship was established with some 19 assorted un-solved 'private' groups, some of which are admittedly of rather doubtful standing

It is considered that this is clear evidence of the presence of a hitherto unrecognized blood group antigen on Mrs Stones's cells It is proposed to call this $\mathrm{St^a}$ (Stones), and the gene responsible for its transmission, St^a, the antibody, anti-$\mathrm{St^a}$, recognizing phenotypes St(a +) and St(a −)

A total of 17,013 random samples, including 830 normal cord bloods, has now been tested with serum Ath , with results as follow

Wr(a+)	Sw(a+)	St(a+)	Total
16	2	20*	38

* 2 related

The frequencies for $\mathrm{Wr^a}$ and $\mathrm{Sw^a}$ are in good agreement with those previously reported by me[4,5], and support the belief that the sample was random One St(a +) propositus was identified by testing cord blood, as was 1 Wr(a +), which indicates that both these antigens are well developed at birth

A study of the families of 19 unrelated St(a +) individuals is in progress Inheritance of $\mathrm{St^a}$ is clearly that of a Mendelian dominant character, and all of 25 sibships containing St (a +) individuals have been shown to stem from the mating St(a +) × St(a −) The antigen has been demonstrated in at least 2 generations of 12 of the families tested, and in 4 generations of 3 of them

Independent segregation of St^a and the genes for the ABO, Rh, P, Lutheran, Kell, Lewis red cell-ABH secretor, Duffy and Kidd systems has been established, and sex-linkage excluded

Information on the relationship of $\mathrm{St^a}$ to the MNSs system derives from 12 matings in 10 families, and linkage appears to be very close In 39 segregations observed between St^a and the MNSs genes, there were 38 non-cross-overs and 1 cross-over ($\Sigma\lambda = +73$, $\Sigma\varkappa = 83$ $P = \gg 0\ 01$) If the apparent cross-over is accepted at face value, and there seems to be no reason for doubting its authenticity, then it would appear likely that the linkage is not of the very close type like that between D, C and E in the Rh system, but that St^a and the MNSs genes occupy closely adjacent sites on the same chromosome Considerably more family material will have to be tested before this point can be settled conclusively

The MNSs alignment of St^a has been established by family study in 12 instances, and is

MS	*Ms*	*NS*	*Ns*	Total
0	1	2	9	12

In one other family, alignment was with either *MS* or *Ms*

While this work was in progress, the presence of yet another antibody in serum Ath was established by these methods, recognizing another 'new' factor, $\mathrm{Ri^a}$ (Ridley), of great rarity, only one propositus being found in the 17,013 random samples tested His blood groups were

A_2, CDe/cde, MS/M$\bar{\mathrm{s}}$, P_1 + + +, Lu (a − b +), Le(a − b −), K − k +, Kp(a − b +), Fy(a + b +), Jk(a + b +),

results similar to those with Mrs Stones being obtained with the 'private' and 'public' antisera listed here

The family study so far has shown that the character is inherited as a Mendelian dominant, segregating independently of the ABO and Rh genes Here again, strong evidence is building up for linkage with the MNSs system, alignment being with *MS* Independence counts[6] of 3 0 and 5 0 have been obtained, as well as 2 additional single segregations with *MS* Further, in a sibship of 7 in the first generation, 4 members with *MS* are all Ri(a +) while 3 without *MS* are Ri(a −)

It is tempting to speculate on the possibility that anti-$\mathrm{St^a}$ and -$\mathrm{Ri^a}$ in serum Ath are related as are anti-$\mathrm{Mi^a}$ and -Vw in antisera of the 'Miltenberger' type[7] On the other hand, their presence together may be entirely fortuitous, as was, presumably, that of anti-$\mathrm{Wr^a}$ and -$\mathrm{Sw^a}$, which have both been excluded from any connexion with the MNSs system

T E Cleghorn

South London Transfusion Centre,
Sutton,
Surrey

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HISTOCHEMISTRY

Histochemical Localization of β-Glucuronidase in Healing Wounds of the Natterjack Toad

β-Glucuronidase the enzyme related to cell proliferation and the formation of connective-tissue ground substance, has been observed in basal layers of the oral mucous membrane and the epidermis, the blood vessels of the normal derma, and healing wounds of the skin of the back, the palate and the tongue in the rat[1–3] and healing wounds of the dorsal skin and the tongue in the iguana[4] In addition to these experiments I have examined the activity of β-glucuronidase in healing wounds of the dorsal skin and the tongue in an amphibian species, the natterjack toad (*Bufo calamita*), the animals being killed by decapitation after 4, 7, 14, 21, 28, 35 and 42 days

After excision of the wound with an extensive zone of normal surrounding tissue and fixation in formalin/chloral hydrate, the enzymatic activity was determined by the method of Fishman and Baker[5] on frozen sections with different incubation times A negative reaction was obtained in the superficial necrotic band of connective tissue in the 4-day animals, whereas immediately beneath this negative zone there was an intensely positive zone due to the presence of inflammatory exudate, and particularly leucocytes As the healing progressed, the fibroblastic proliferation zone appeared to react moderately but consistently. The multinucleated giant cells and histiocytes reacted more intensely. In the 21, 28, 35 and 42-day animals the epidermis, which had proliferated over the margins of the wound, showed a significant increase in enzymatic activity.

The epithelial proliferation appears to be closely related to β-glucuronidase activity. In the connective tissue this enzyme is associated with tissue formation and macrophage function. Since these results are in good agreement with those obtained in the rat[1] and the iguana[4] the distribution of β-glucuronidase in the epidermis no doubt has wider implications.

A. Stolk

Histological Laboratory,
Free University, Amsterdam.

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Arrangement of Cholesterol Molecules in the Myelin Sheath

Polarized light studies[1,2] have indicated that most or all lipid moieties of the myelin sheath are radially arranged. Finean[3,4] correlated the X-ray diffraction patterns of fresh and lipid-solvent extracted myelin with known data about the form and size of lipid molecules and suggested that a phospholipid–cholesterol complex forms a part of myelin. According to this notion the long chain of the phospholipid moiety is lying radially with curling of the polar end group, so that the phosphate might serve to bind the lipid to protein, whereas the positively charged part of the molecule is closely associated with the hydroxyl group of cholesterol. The complex would have, therefore, the form of a U, with the cholesterol and the hydrocarbon chain of the phospholipid situated in a parallel fashion and in a radial direction.

In this laboratory extraction with acetone at 0° to + 4° of human peripheral nerve and of cerebral white matter yielded similar results to those reported by Elkes and Finean[5], that is, acetone at this temperature (two 1 hr. changes) extracted practically all the cholesterol and less than 10 per cent of the phospholipids. In addition it was found that cold acetone extracted no hexosamine-containing compounds and negligible amounts of cerebroside. The estimation of the amount of total extractable lipids was done by 6 hr. extraction in boiling chloroform/methanol, 2 : 1.

A comparative histochemical study was made on fresh frozen sections extracted with acetone and on control (not extracted) sections. Human corpus callosum and peripheral nerve were frozen rapidly in isopentane chilled to about – 65° and 9μ thick sections were cut in the cryostat. Acetone extraction was done as above.

Cold acetone extraction was found to have no appreciable effect on the intensity of staining with the following procedures: sudan black *B* (for total lipids), plasmal and performic acid–Schiff (for unsaturated lipids) and the periodic acid–Schiff procedure which stains mainly carbohydrates and carbohydrate-containing lipids. No staining with the Schultz procedure for cholesterol was obtained in the extracted sections. Staining by Baker's procedure for phospholipids and by the *Einschlussfaerbung* of Feyrter with toluidine blue revealed some reduction of the intensity of staining in the extracted sections. These findings indicate that cholesterol was wholly extracted, whereas the total amount of lipids and the amount of cerebrosides were not markedly affected. The reduced intensity of staining with Baker's method and the reduced metachromasia indicates that acidic phospholipids were more markedly affected by the solvent than other phospholipids. The fact that no increase in metachromasia was noted in the extracted sections supports the idea of Finean according to which the negatively charged group of the phospholipid plays no part in binding cholesterol.

The effect of cold acetone extraction on the birefringence of myelin was examined as follows:

Longitudinal and transverse cryostat sections of human peripheral nerve were mounted in glycerine (in order to minimize form birefringence) and the intensity of birefringence was determined at certain marked sites. For low intensities the Brace-Köhler compensator was used, and for high intensities the Köhler compensator. After removing the coverslip and rinsing with water to remove the glycerine, the sections were extracted with acetone as above and then again mounted in glycerine. The intensity of birefringence was again determined at the same sites.

It has been found that in longitudinal sections the negative birefringence of myelin was decreased by about 40 per cent in the extracted sections. In transverse sections the positive birefringence was decreased by about 60 per cent of the value in untreated sections. As it is known[6] that cholesterol is positively birefringent it is obvious that its molecules are radially arranged and their presence contributes to the negative birefringence in longitudinal sections and to positive birefringence in transverse sections.

The evidence for a radial arrangement of the cholesterol moieties in myelin corroborates the scheme which has been suggested by Finean's X-ray diffraction studies.

This work was supported by grant No. 138 from the National Multiple Sclerosis Society, New York.

J. J. Bubis
M. Wolman

Department of Pathology,
Government Hospital,
Tel-Hashomer, Israel.

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PATHOLOGY

Ependymomas produced after Intracerebral Inoculation of *SV40* into New-born Hamsters

Eddy *et al.*[1] have reported that extracts of rhesus monkey kidney cell cultures produce subcutaneous sarcomas when inoculated into new-born hamsters. Sweet and Hilleman[2] have shown that a simian virus (*SV*40) is present in most rhesus monkey kidney cell cultures although it causes no cytopathogenic effect in them. Cytopathogenic effect is produced, however, in cell cultures of kidneys from African green monkeys. Later, Eddy *et al.*[3] found that similar subcutaneous

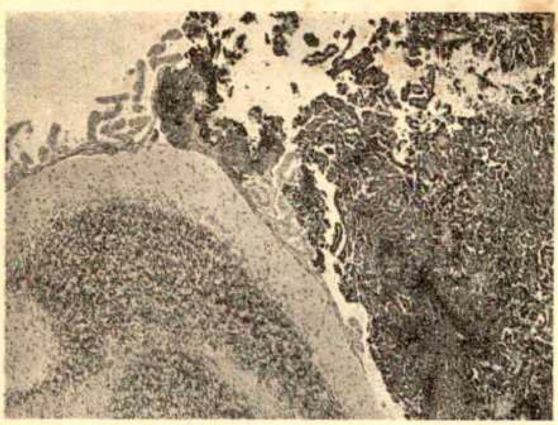

Fig. 1. Ependymoma of lateral ventricle of hamster inoculated with *SV*40. Transition from normal ependyma to tumour is seen. Hæmatoxylin and eosin (× *c*. 39)

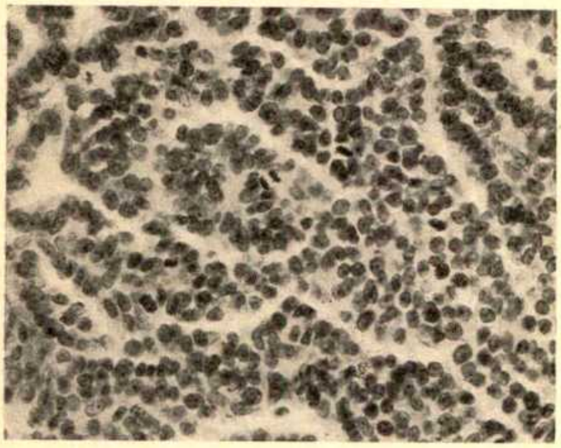

Fig. 2. Numerous mitotic figures in a cellular area of the ependymoma. Hæmatoxylin and eosin (× *c*. 530)

sarcomas in hamsters can be induced after inoculation of preparations containing *SV*40.

Since it has been previously shown that new-born hamsters are markedly susceptible to the induction of tumours after intracerebral inoculation of polyoma virus[4], studies were undertaken to determine the effect of intracerebral inoculation of large doses of *SV*40 into new-born hamsters.

Strain 777 of *SV*40 used in this work has been isolated in this laboratory. The virus pool was prepared in cell cultures of African green monkey kidneys in serum-free medium 199 (ref. 5). This virus preparation contained $10^{8\cdot7}$ $TCID_{50}$/ml.

Pregnant Syrian hamsters (*Cricetus auratus*) were obtained from the animal production unit of the National Institutes of Health and the litters were used for intracerebral inoculation. One litter of 11 animals was inoculated when less than 24 hr. old with 0·02 ml. of virus fluid. One litter of 9 animals of the same age was inoculated with 0·02 ml. of medium 199. The inocula were injected into the mid-portion of the right cerebral hemisphere using tuberculin syringes and No. 27 gauge ¼-in. needles. The animals were observed daily for evidence of neurological dysfunction. At the time of death, complete autopsies were performed and the tissues were fixed in formol–mercuric bichloride (sublimate). Sections for microscopic study were stained with hæmatoxylin and eosin and phosphotungstic acid hæmatoxylin.

Two of the 11 baby hamsters inoculated with virus fluid were eaten by the mother and 9 remained by the seventh day. Daily observation revealed no abnormalities until 100 days after inoculation. At that time 2 of the 9 appeared weak and lethargic. This weakness rapidly progressed to complete paralysis of all limbs, and these 2 animals were killed on the 100th and 101st days respectively. A third animal developed paralysis on the 111th day and was killed. Three more animals were killed on the 124th day even though there were no signs of weakness. At the time of this report (156 days) the remaining three animals appear well clinically.

Of the 9 control animals, 2 were eaten by the mother. Seven remain well clinically at 156 days.

At autopsy, all three paralysed hamsters had grossly discernible tumours of the brains. These were white and appeared to be present in the anterior horns of the lateral ventricles. The tumour of the third animal killed on the 111th day was very large and had caused marked dilatation of the lateral ventricles. One of the 3 normal appearing animals also had a tumour.

All 4 tumours had the same histological appearance. They filled the lateral ventricles of the cerebrum. They consisted of cords and rosettes of cuboidal cells with dark-staining nuclei and scant cytoplasm. Transition from the normal ependymal lining of the ventricle to tumour was seen (Fig. 1). In many areas, the tumour cells were arranged in a papillary fashion. In others, the cells were compact and arranged in sheets. Mitotic figures were numerous (Fig. 2) and the tumours were quite vascular.

These tumours have the characteristics of ependymomas, tumours arising from the ventricular lining cells[6].

The stroma of the tumour of the third animal had, in addition to normal connective tissue, areas of sarcomatous tissue and numerous multinucleated giant cells. These areas are similar histologically to the subcutaneous sarcomas described by Eddy *et al.* after inoculation of extracts of rhesus monkey kidney cell cultures[1].

Although the tumours produced by intracerebral inoculation of polyoma virus in hamsters are sarcomas and resemble the subcutaneous tumours produced by this agent, the tumours produced after intracerebral inoculation of *SV*40 are ependymomas. Such tumours are true gliomas and thus are of ectodermal origin. Ependymomas have been produced after implantation of carcinogens into the brains of experimental mice[7]. This, however, represents the first report of the production of a true glioma after virus inoculation.

RUTH L. KIRSCHSTEIN
PAUL GERBER

Division of Biologics Standards,
Laboratory of Viral Immunology,
National Institutes of Health,
Bethesda, Maryland.

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IMMUNOLOGY

Immunization of the *A/Jax* Mouse with Irradiated Cells of its Indigenous Tumour, Sarcoma I

One of the transplantable tumours widely used in investigations of tumour immunology is Sarcoma I (SaI), a tumour that originated in 1947 in a mouse of the Strong *A* strain that had been treated with dibenzanthracene[1] We, as well as others, have maintained the tumour by transplantation in mice of the *A/Jax* strain, a sub-line of the Strong *A* strain, and utilize animals of this strain as the susceptible host in various experiments Since any immunological response, even though it may be insufficient to cause rejection of the tumour, may influence the results of experiments with this tumour–host system, it appeared desirable to determine whether an immunological response to SaI occurs in the *A/Jax* mouse From work of others it appeared that the possibility of an antigenic disparity between the tumour and the *A/Jax* mouse is favoured by the likelihood of genetic change in the mouse or the tumour during the prolonged period of transplantation and by the fact that the tumour was induced by dibenzanthracene[2,3]

Experiments were designed to demonstrate immunity to SaI in adult *A/Jax* animals by treatment with irradiated SaI ascites tumour (SaI-AT) prior to challenge with a lethal dose of SaI cells (The *A/Jax* mice used were obtained in 1958 from the Roscoe B Jackson Memorial Laboratory, Bar Harbor, Maine They were maintained in our laboratory by colony inbreeding for seven generations before use)

The irradiation of SaI-AT cells was accomplished by X-ray treatment of adult *A/Jax* mice bearing an ascites tumour transplanted seven days earlier Irradiation was delivered by a cobalt-60 source at a rate of 200 r /min and a distance of 35 cm The field size was 10 cm × 10 cm and the total exposure doses ranged from 18,000 r to 36,000 r (Dr R. G Parker assisted in the radiology)

Table 1 Immunity to Sarcoma I induced in *A/Jax* Mice with Irradiated Tumour Cells

Route of administration of irradiated cells	Days after challenge with live SaI-AT cells 10	20	30	40	50	100	MST*
Intraperitoneal	0/12†	1/12	6/12	7/12	8/12	8/12	27 6
Intradermal	0/12	2/12	6/12	9/12	10/12	10/12	28 7
Untreated controls	0/14	14/14					17 1

* Mean survival time

† The numerator designates the total number of animals dead of tumour at the interval shown and the denominator the total number of animals tested

Immediately after irradiation, the ascites fluid was removed with a 26-gauge needle and the tumour cells were counted Ten million irradiated cells were injected into each of 12 female and 12 male adult *A/Jax* mice The cells were deposited either intraperitoneally or intradermally in multiple sites of the skin and foot pads

Fifty days after the administration of the immunizing dose of irradiated tumour cells all animals were given an intraperitoneal challenge dose of 1,000 viable ascites tumour cells This number of tumour cells was equal to at least 100 lethal doses

The data presented in Table 1 show that whereas all the control mice died of tumour before the 21st day many of the immunized mice survived for longer periods Six of the 24 animals in the latter group were alive and healthy on the 100th day following challenge and at death showed no indication of tumour The mortality-rates and survival-times of the immunized mice were not significantly different in female and male animals or in animals immunized by the two routes

The results show that irradiated SaI-AT cells are immunogenic for the *A/Jax* mouse and indicate that antigenic differences exist between the *A/Jax* mouse and its indigenous tumour Sarcoma I

This work was supported in part by a graduate traineeship (*CRT*-5040) and a research grant *C*-2668(*C*4) from the National Cancer Institute, U S Public Health Service

John W Jutila*
Russell S Weiser
Charles A Evans

Department of Microbiology,
School of Medicine,
University of Washington, Seattle

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* Postdoctoral research trainee (*CRT*-5040)

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Staphylococcal Infection and Blood Groups

Many workers have shown similarities between blood group substances and a variety of bacterial and non-bacterial antigens Illchmann-Christ and Nagel[1] found that anti-A antibody was removed from B and O sera by *Staphylococci* and reasoned that *Staphylococci* possessed an A-like receptor This suggests that persons of blood group A may have an increased susceptibility to staphylococcal infection due to an inability to elaborate an antibody which may cross-react with their own red blood cells

Anderson, Coulter and Keynes[2] reported an extensive epidemiological investigation into staphylococcal infection and nasal carriage among mothers, babies and staff in a maternity hospital No attempt was made at the time to correlate blood group with other inquiries, but this has now been done retrospectively

All blood groups of mothers were recorded Each mother was nose swabbed five times between the 36th ante-natal week and the 6th post-natal week At the visit on the 36th week, each mother was questioned about previous staphylococcal infection and at the post-natal visit inquiries were made concerning infection following discharge from the hospital The results are presented in Table 1

Table 1

	Blood group of mother A	B	AB	O
Nasal carrier	127	22	7	135
Not nasal carrier	207	62	19	193
Positive history of staphylococcal infection	77	22	7	98
Negative history of staphylococcal infection	178	42	13	149
Staphylococcal infection in baby	29	15	0	37
No staphylococcal infection in baby	170	36	13	158

This table may be summarized nasal carriage, O > A* > AB > B, history of staphylococcal infection, O > AB > B > A* < O

* Indicates significance (χ^2 test $P < 0\ 05$)

Group O persons are seen to be at a slight disadvantage both as regards nasal carriage and overt infection It is doubtful whether these slight differences are of any clinical significance

J R Coulter

Institute of Medical and Veterinary Science,
Adelaide

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RADIOBIOLOGY

Immediate Effects of X-Irradiation on the Heart of the Frog

Recent work has shown that ionizing radiations increase the excitability of peripheral receptors[1], nerves[2–4], nervous centres[4], muscles[4], and embryo heart of the chick[5]

In the work reported here, the influence of whole-body irradiations on the heart of a frog was studied according to the following technique The frogs (*Rana temporaria*) were pithed through brain and cord, after opening the thorax and pericardium, the ventricle basis was pinched with a small clip and kept still, while the apex, pinched in the same way, was connected to a strain gauge and electric recorder (Philips 'Cardiopan') This system allows a continuous record, before, during and after irradiation, of ventricular isometric contractions In some cases, the electrocardiogram was taken simultaneously

Irradiations were carried out with a Siemens 'Stabilipan' X-ray tube, for a period of 5 min (195 kV, 20 m amp, filter 0 2 mm copper, focal distance 17 cm, dose-rate 950 r /min)

Fig 1 shows the effect of X-rays on heart beat The strong bradycardia observed after 1–5 min of irradiation is reversible within 1 min after irradiation and often followed by a transient acceleration This effect can be repeated several times on the same animal by successive irradiations In some instances, the cardiac frequency was reduced to half, the electrocardiogram, however, did not reveal any auricular-ventricular block, while in three experiments, a sino-auricular block was noticed by direct observation during the first minute after irradiation

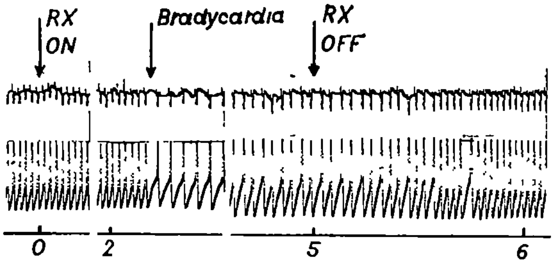

Fig 1 Reversible bradycardia by X-irradiation Upper trace electrocardiogram which shows *P* and *R* waves, lower trace isometric contractions, time is indicated in minutes

In other cases, no modification of cardiac rhythm was obtained even after a much longer period of irradiation This lack of response is not related to the sex of frogs, but seem dependent on seasonal variations, in October, 9 of the 18 frogs tested did not respond, while during the colder months, November–January, bradycardia appeared in two cases only out of 13, though the animals were acclimatized at laboratory temperature for a week When the frequency was unchanged during irradiation, a reversible decrease of contractile strength (10–20 per cent) occurred in the non-hibernating frogs (October)

These negative chronotropic or inotropic effects suggest a vagal participation in the phenomenon Consequently, the action of atropine and eserine solutions, spread upon the heart area 5 min before irradiation, was investigated in the animals, showing a good chronotropic effect

As shown in Fig 2, atropine sulphate solution (0 1 per cent) almost completely inhibits bradycardia induced by X-rays, after washing the preparation it is still possible to induce by a new irradiation the characteristic heart rate decrease Accordingly, physostigmine salicylate solution (0 1 per cent) increases the bradycardia as compared to a preceding irradiation without drug, but washing the preparation does not restitute the primitive state These two drugs seem to act in the same way on the X-rays induced decrease of contractile strength

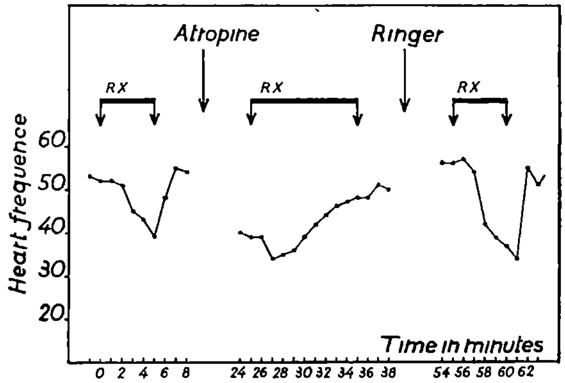

Fig 2 Successive irradiations of the same frog, effect of atropine sulphate, 0 1 per cent

High dose-rate X-irradiations induce in the spinal frog's heart a negative chronotropic or inotropic action These effects are inhibited by atropine and potentiated by eserine Hibernating frogs, as a rule, do not react The present observation suggests the occurrence of a vagal excitation or acetylcholine liberation under the influence of irradiation

Cl Onkelinx

Laboratoire de Pathologie
et de Thérapeutique générales,
Université de Liège
Laboratoire pour la Protection des
Populations Civiles, Liège

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Sensitivity of Interphase Cells to Chromosome Breakage by X-rays

Several previous studies have dealt with the sensitivity of interphase cells to chromosome breakage by X-rays In the root tips of beans (*Vicia faba*) Revell[1] observed that the sensitivity reaches its maximum about 8 hr prior to mitosis According to Howard and Pelc[2] this time lies in the post-DNA-synthetic period (G_2) The results obtained by Sparrow *et al* [3] in *Trillium* microspores are also similar to those of Revell In a recent investigation of the survival of HeLa cells Terasima and Tolmach[4] have found that the irradiation of interphase cells, which are presumably in the process of synthesis of DNA (S), reduces their capacity to form colonies more markedly than when they are irradiated at other stages of interphase In the present work, using tritiated-thymidine to identify directly DNA-synthesizing cells, it is shown that these cells, which are in the S-phase at the time of irradiation, are more sensitive to chromosome breakage than cells at other stages of interphase (G_1 and G_2)

Table 1. MEAN FREQUENCIES/ROOT OF LABELLED AND UNLABELLED ANA- AND TELO-PHASES, CONTAINING CHROMOSOME ABNORMALITIES (ONION)

Time (hr.) after X-rays	Labelled ana- and telo-phases		Unlabelled ana- and telo-phases	
	No. scored	Per cent abnormal	No. scored	Per cent abnormal
3½	0	0	234	17.4 ± 3.8
6½	25	40.0	83	26.2 ± 10.3
9	23	52.2	65	32.6 ± 21.3
20½	114	64.1 ± 3.8	88	33.2 ± 3.4
24	40	44.5 ± 5.6	83	19.9 ± 5.2
32½	42	30.3 ± 10.3	122	13.8 ± 7.1
Total	244	46.2 ± 2.6	675	23.8 ± 4.2

No. of roots: 3 at 3½ to 9 hr.; 2 at other times. Only one root at 6½ and 9 hr. contained labelled ana- and telo-phases. Mitotic rate reached control-level at 20½ hr.

Onion and bean root meristems were exposed to tritiated thymidine (1 μc./ml., spec. act. 1.9 c./mM) for 16 min., during the last 4 min. of which they were irradiated with 200 r. (for irradiation procedures see our earlier work[5]). After irradiation, roots were allowed to grow in tap water containing unlabelled thymidine (0.05–0.1 mgm./ml.). Controls with unirradiated and irradiated roots were run in order to determine the effect of tritiated thymidine on chromosome breakage. Samples were fixed in acetic alcohol at various times after irradiation. Stripping film autoradiographs of Feulgen-stained squash preparations were made and the frequencies of labelled interphase cells and labelled and unlabelled ana- and telophases containing chromosome aberrations (fragments and bridges) were determined. In control samples, not covered with stripping film, the overall frequencies of abnormal cells (both mitotic and in interphase, possessing chromosome fragments in the form of micronuclei) were observed.

The frequency of radioactive nuclei (37.5 per cent) observed in onion roots which were exposed to tritiated thymidine for 16 min. was about the same as that (37.6 per cent) found in roots grown for 3½ hr. longer in unlabelled thymidine. Furthermore, in the latter samples no increase in silver grain density over labelled nuclei is apparent. Similar results were also obtained in the bean root tip cells. These findings show that the pools of tritiated thymidine in both onion and bean roots are diluted very rapidly after the roots are transferred to unlabelled thymidine (cf. Wimber[6]). Therefore almost all labelled cells that are detected after irradiation represent those which underwent synthesis of DNA at the time of exposure to X-rays.

It can be seen in Tables 1 and 2 that during the entire experimental periods in both onion and bean root the frequencies of labelled ana- and telo-phases containing chromosomal aberrations are much higher than the frequencies of unlabelled aberrant mitoses. The results in Tables 1 and 2 are presented in slightly different ways, because a high variability of aberrations among individual roots in each sample was observed in the onion, but not in the bean; in either case, however, the differences between frequencies of aberrant mitoses in labelled and unlabelled cells are highly significant. The increase in the frequency of labelled aberrant mitoses is not brought about by a synergistic effect of tritiated thymidine and X-rays, since the data in Table 3 clearly show that there is no difference between the per cent abnormal cells obtained from onion roots irradiated in the presence and absence of tritiated thymidine. Moreover, an exposure of unirradiated roots to tritiated thymidine for only 16 min. does not produce any abnormal cell (Table 3, last column). These results, therefore, demonstrate that, among interphase cells in both onion and bean, the sensitivity to chromosome breakage by X-rays is at its maximum in cells which undergo DNA synthesis at the time of irradiation. (This observation is in agreement with that of S. Mitra (*Genetics*, 43, 771; 1958) on X-rayed meiotic cells of lily.) Such chromosomal damage may account for the marked reduction in colony-forming capacity of HeLa cells irradiated at the time of synthesis of DNA[4].

The present data do not permit us to determine clearly any difference in the sensitivity between post-synthetic (G_2) and presynthetic (G_1) cells. The total duration of the mitotic cycle in these materials is fairly well known[2,7]. It normally requires more than 12 hr. for cells in the G_1 stage to reach the next mitosis; consequently, the unlabelled mitoses which appear in the earliest samples (up to 9 and 12 hr.) of the present work were furnished by cells which had been in G_2 during irradiation. Since one of the immediate effects of radiation is to block cells in the G_2 stage[8,9] without greatly affecting the process of synthesis of DNA[5], the unlabelled mitoses in later samples consist of a mixture of cells that had been in G_2 and G_1 at the time of exposure to X-rays.

Although the reason for a high sensitivity of cells in the S-period, compared to those in G_2 to X-irradiation, is not apparent on the basis of "target theory"[10], it is possible that the susceptibility of chromosomes to breakage by X-rays increases when the DNA double helix is opened up at the time of replication. It is also possible that chromosome damage occurring during

Table 2. FREQUENCIES OF LABELLED AND UNLABELLED ANA- AND TELO-PHASES, CONTAINING CHROMOSOME ABNORMALITIES (BEAN)

Time (hr.) after X-rays	Labelled ana- and telo-phases (A)			Unlabelled ana- and telo-phases (B)			Test of significance between A and B
	No. scored	Per cent abnormal	Homogeneity within samples	No. scored	Per cent abnormal	Homogeneity within samples	
4	0	0	—	105	66.7	$\chi^2_2 = 2.27$ $P > 0.30$	—
12	0	0	—	69	68.1	$\chi^2_2 = 3.11$ $P > 0.20$	—
16	112	83.9	$\chi^2_3 = 1.94$ $P > 0.50$	146	63.7	$\chi^2_3 = 1.19$ $P > 0.70$	$\chi^2_1 = 11.96$ $P < 0.001$
20	63	79.4	$\chi^2_1 = 2.02$ $P > 0.30$	185	55.1	$\chi^2_2 = 5.45$ $P > 0.05$	$\chi^2_1 = 10.66$ $P < 0.01$
24	119	83.2	$\chi^2_2 = 3.83$ $P > 0.10$	146	62.3	$\chi^2_2 = 3.40$ $P > 0.10$	$\chi^2_1 = 13.09$ $P < 0.001$
28	103	83.5	$\chi^2_3 = 1.55$ $P > 0.30$	155	52.3	$\chi^2_2 = 0.50$ $P > 0.70$	$\chi^2_1 = 25.02$ $P < 0.001$
Total	397	82.5	$\chi^2_9 = 9.34$ $P > 0.30$	806	61.4	$\chi^2_{13} = 15.92$ $P > 0.20$	$\chi^2_4 = 60.73$ $P < 0.001$

No. of roots: 3 at each time, except at 16 hr. when 4 roots were used. Mitotic rate reached the control-level at 16 hr. χ^2-test of significance was used, since there were no marked differences among roots within each sample; Yates's correction was used in the last column.

Table 3 FREQUENCIES OF ABNORMAL CELLS (INTERPHASE AND MITOTIC) IN UNIRRADIATED AND IRRADIATED ONION ROOTS GROWN IN THE PRESENCE OR ABSENCE OF TRITIATED THYMIDINE

	Roots exposed to					
Time (hr) in unlabelled thymidine	200 r alone		^{3}H-thym +200 r		^{3}H-thym alone	
	No of cells	Per cent abnormal	No of cells	Per cent abnormal	No of cells	Per cent abnormal
9–10	1,354	3 2	1,259	1 3	1,293	0
24	1,234	5 0	819	4 2	1,244	0

No of roots 3 at each time

the *S*-period does not "heal" as readily as damage occurring at other stages of interphase, the chromosomal abnormalities observed in this and in other studies of this type represent only that fraction of damage which remains and becomes observable during the next mitotic division

This work was supported by grants from the National Science Foundation (*G*–6129) and from the U S Public Health Service (*RG*–6025)

NIRMAL K DAS
MAX ALFERT

Department of Zoology,
University of California, Berkeley, 4

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BIOLOGY

Antagonism by Deoxyribonucleic Acid Extracts of the Growth Inhibitory Effects of 8-Azaguanine on Mammalian Cells in Culture

IN a series of *in vitro* experiments involving attempts to transfer resistance to 8-azaguanine (AzG), a non-specific effect of cell preparations rich in deoxyribonucleic acid (DNA) has been observed

The continuous cell line used, *P*–388[1,2], was maintained in Eagle's basal medium supplemented with L-serine (0 2 m*M*), sodium pyruvate (1 m*M*) and calf serum (5 per cent) This cell line shows a consistent behaviour with respect to inhibition of growth by 8-azaguanine and the other inhibitors used in this investigation[3]

In these experiments a suspension of cells was inoculated into glass Petri dishes (6 cm diam) containing 8-azaguanine and DNA, then incubated at 37° C in an atmosphere of 5 per cent carbon dioxide–95 per cent air After 7 days incubation, the contents of the dishes were stained with crystal violet and the number of colonies per dish was counted using a dissecting microscope[2]

Extracts rich in DNA were obtained by the method of Kay, Simmons and Dounce[4] DNA precipitates were never dried but taken into solution in either 0 0014 *M* or 0 14 *M* sodium chloride solution

As shown in Table 1, the presence of the DNA extract leads to the appearance of large numbers of colonies at concentrations of 8-azaguanine which completely inhibit growth in the absence of the DNA With a constant concentration of 8-azaguanine, decreasing the amount of the DNA preparations per dish results in a graded decrease in the number of colonies per dish (Table 2)

Table 1 EFFECT OF DNA EXTRACT ON 8-AZAGUANINE INHIBITION OF GROWTH OF *P*388/*P*

AzG concentration	Control	No 4 DNA*
Nil	251† (81 8 per cent)‡	236 (76 9 per cent)‡
1×10^{-7} *M*	228	249
3×10^{-7}	159	243
5×10^{-7}	22	242
8×10^{-7}	0	211
1×10^{-6}		204
3×10^{-6}		126
5×10^{-6}		95
8×10^{-6}		37
1×10^{-5}		31
3×10^{-5}		0

* DNA extract from *P*388/AzG^{r-3} (sub-line 55) resistant to 8-AzG, 0 1 ml per dish = 10 μgm /ml DNA
† Each value is the average number of colonies for 3 dishes
‡ Plating efficiency, 307 cells inoculated per dish

Table 2 EFFECT OF VARYING DNA CONCENTRATION ON THE GROWTH INHIBITION OF 8-AZAGUANINE ON *P*388/*P*

AzG concentration	No 7 DNA*/ml /dish	Average No colonies per dish‡
—	—	74 (34 4 per cent)†
1×10^{-6} *M*	—	0
1×10^{-6}	0 03125	0
1×10^{-6}	0 0625	1
1×10^{-6}	0 125	35
1×10^{-6}	0 25	69
1×10^{-6}	0 5	65

* DNA extract from *P*388/*P*, 120 μgm /ml DNA
† Each value is the average from 4 dishes
‡ Plating efficiency, 215 cells inoculated per dish

This effect has been observed with several preparations of DNA from different sources, for example *P*388/*P*, parental cell line (2 preparations), *P*388/AzG^{r-3} (sub-line 55) resistant to 8-azaguanine (4 preparations), *L*1210/*S*, transplantable lymphocytic leukæmia in *DBA*/2 mice, Sprague-Dawley rat spleen, *C*57*BL*/*Ka* mouse liver

Table 3 shows that the presence of the DNA extract also diminishes the inhibitory activity of 6-mercaptopurine, 8-azaguanylic acid and 8-azaguanosine

Table 3 EFFECT OF DNA EXTRACT ON VARIOUS GROWTH INHIBITORS OF *P*388/*P*

		Average no colonies per dish*	
Drug	Concentration	Control	No 6 DNA†
—	—	97 (32 9 per cent)‡	
8-azaguanine	1×10^{-6} *M*	0	75
6-mercaptopurine	3×10^{-8} *M*	2	73
	5×10^{-8} *M*	1	54
	1×10^{-7} *M*	0	26
	3×10^{-7} *M*	0	26
8-azaguanylic acid	8×10^{-7} *M*	66	78
	1×10^{-6} *M*	43	74
	2×10^{-6} *M*	0	69
8-azaguanosine	5×10^{-7} *M*	79	80
	8×10^{-7} *M*	60	88
	1×10^{-6} *M*	44	81
	2×10^{-6} *M*	0	82

* Each value is the average from 3 dishes
† DNA extract from *P*388/*P* , 0 1 ml per dish = 100 μgm DNA
‡ Plating efficiency , 295 cells inoculated per dish

These results can possibly be explained in the following ways (1*a*) The DNA-rich preparations contain as impurities substances capable of binding the 8-azaguanine, etc , thus lowering the effective concentration of the drug, (*b*) in the presence of the cells, under these experimental conditions, substances can be released from the DNA extracts which effectively bind the 8-azaguanine, etc , and/or (2) the DNA extracts contain, or release on incubation, a compound or compounds which inhibit the uptake of the 8-azaguanine, etc , into the cells

There is evidence (Table 1) that the presence of the DNA extract does not merely increase the plating efficiency of the cells The regularly graded effect found with increasing concentrations of 8-azaguanine (Table 1) and also the non-specificity with respect to the source of the DNA extract strongly suggest that the colonies found are not truly resistant cells

The nature of this effect is being investigated further since, if it is a property of some substance released from highly purified native DNA under the experimental conditions used, it will be difficult to assess experiments of this nature with compounds such as 8-azaguanine and 6-mercaptopurine

However, if the activity is due to some contaminant carried through, or arising during the procedure of extracting, DNA then it may be possible to remove it prior to experimental use of the DNA extract

T R Bradley*
R A Roosa
L W Law

National Cancer Institute, Bethesda, Md ,
and Wistar Institute, Philadelphia, Pa

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* Visiting associate, National Cancer Institute Present address Department of Physiology, University of Melbourne, Parkville, Victoria, Australia

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'Messenger RNA' in a Fission Yeast

It is now widely believed that one fraction of the ribonucleic acid (RNA) of a cell, the 'messenger RNA', is responsible for the transmission of genetic information from the deoxyribonucleic acid (DNA) of the nucleus to the cytoplasm[1] This messenger RNA has been identified with that fraction of the total RNA which shows some or all of the following properties a high rate of turn-over, a physical difference from the ribosomal RNA, a base composition similar to the DNA, and a capacity to interact specifically with the DNA Evidence for the existence of this RNA has come from phage-infected bacteria[2,3], normally growing bacteria[3], and mammalian nuclei[4,5] It has also come from bacteria[6] and budding yeast[7] in a 'step-down , where growing cells are transferred from a rich medium to a poor one For a period after the step-down it seems that messenger RNA is the main kind of RNA made by the cells We have investigated this situation in the fission yeast *Schizosaccharomyces pombe* with the object both of identifying messenger RNA and also of following by autoradiography its location in the cell and its relation to the cell-cycle

When yeast cells growing exponentially in malt extract broth at 32° C were transferred to a minimal medium containing 4 75 per cent ethanol at the same temperature, the optical density at 595 mμ remained constant for 20–30 min At the end of this plateau following the step-down, the optical density rose exponentially for several generations with a mean generation time of 6 hr The mean generation time in the normal minimal medium was 3 hr and in the malt extract broth was 2½ hr Total RNA remained constant for a longer period (1 hr) after the step-down These results are similar to those found with bacteria[8,9] except that the mean cell size of the yeasts did not change appreciably after the step-down

Continuous and pulse labelling with $^{32}PO_4$ and ^{14}C-adenine showed that RNA continues to be synthesized after the step-down The initial rate of incorporation was 3–7 per cent of that in control cells growing in normal minimal medium The kinetics of labelling with phosphorus-32 followed by an excess of phosphorus-31 as a 'chase' showed that a substantial proportion of this RNA was turning over The results from two experiments are shown in Fig 1 The decline in counts after the chase indicates instability of the labelled RNA In the control, which is not illustrated, the phosphorus-32 in RNA did not fall after the chase but rose at the expense of phosphorus-32 in the acid-soluble pool Similar experiments with ^{14}C-adenine gave somewhat different results The control behaved in the same way as that with phosphorus-32 the acid-insoluble label (RNA) rose after the chase while the acid-soluble declined After a step-down and chase, however, the acid-insoluble label only rose slightly and the acid-soluble label stayed constant. The difference between the results with phosphate and adenine is presumably because the former can exchange with the medium while the latter cannot The results with adenine after a step-down are compatible with an unstable RNA exchanging with an internal pool of nucleotides This pool would only be slowly accessible to adenine entering the cell and it would not exchange with adenine in the outside medium

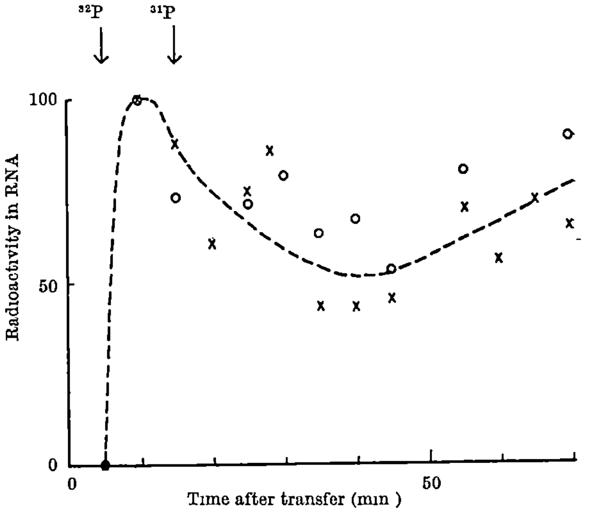

Fig 1 Phosphorus-32 label in RNA after step-down Cells transferred at zero time from malt extract broth to minimal medium with 4 75 per cent ethanol At 5 min , cells were labelled with carrier free $^{32}PO_4$ At 15 min., excess $^{31}PO_4$ was added (1,000 × conc in medium) Acid washed cells were counted at intervals on membrane filters before and after digestion with RNAase Difference in counts was taken as measure of radioactivity in RNA Two experiments with different labelling levels (0 33 and 1 0 μc /ml) normalized to 100 after 5 min labelling ○, 0 33 μc /ml , ×, 1 0 μc /ml

RNA nucleotide ratios are shown in Table 1 Those for the bulk (largely ribosomal) RNA are the means of the ultra-violet absorption data from all the experiments They do not differ significantly from those for *Saccharomyces cerevisiae*[7] Control cells growing in minimal medium were labelled for a generation time (3 hr) with phosphorus-32 The radioactive base ratios were very similar to those from the bulk, except that the CMP was somewhat lower With control cells labelled for 10 min , the ratios were disturbed, both UMP and GMP being depressed relative to AMP This tendency was even more marked in a step-down culture (10-min label started 5 min after transfer) Here the GMP is severely depressed and is nearly equal to the CMP. Only the low UMP stops these ratios being similar to those for the DNA of *S cerevisiae*[7] Base ratios for the DNA of *S pombe* have not yet been found, but this work is going forward

It is clear that the RNA made immediately after a step-down has a very different composition from the

Table 1 RNA Nucleotide Ratios

	Bulk (ribosomal) ultra-violet	Control Phosphorus-32 for 3 hr	Control Phosphorus-32 for 10 min	Step-down Phosphorus-32 for 10 min
AMP	10	10	10	10
UMP	9 9	9 5	8 8	7 0
GMP	10 2	9 1	7 6	6 5
CMP	7 1	5 6	6 5	6 1

RNA extracted by hot 10 per cent sodium chloride, and deproteinized with chloroform-octanol Nucleotides separated by electrophoresis on cellulose acetate strips after alkaline hydrolysis Radioactivity measured by scintillation counting Acid eluates measured in ultra-violet

ribosomal RNA and that the deviation is in the direction of DNA (with U replacing T) RNA labelled with a brief pulse of phosphorus-32 during exponential growth also deviates in the same direction, though not so markedly These results are consistent with the view that messenger RNA is a significant fraction of the RNA synthesized during a brief interval[3,7], though they suggest that this fraction is larger after a step-down than in normal growth

^{14}C-leucine was incorporated into acid-insoluble material at a relatively high rate for the first $\frac{1}{2}$ hr after a step-down As judged by pulse labels, this rate was about 30 per cent of that in a control growing in normal minimal medium This, coupled with the constant optical density, suggests that there is a considerable amount of protein turn-over after a step-down

Autoradiographs were made of cells labelled for 10 min with ^{3}H-adenine at various periods after a step-down, and were analysed by a method described elsewhere[10] Preliminary results show that the label is present both in nucleus and cytoplasm, though the nuclear concentration is higher They also show that cells of different length and therefore at different stages of the cell-cycle incorporate approximately equal quantities of the label This latter result differs from that with normal growing cells where the rate of incorporation rises steadily throughout the cell-cycle[11]

In summary, this work shows that fission yeasts, after a step-down in medium, produce for a time RNA which is unstable and which has a base composition unlike ribosomal RNA and approaching DNA We would therefore identify it tentatively as messenger RNA It also seems that its pattern of synthesis during the cell-cycle differs from that in normal growth

This work was supported by a grant from the Department of Scientific and Industrial Research

P R Gross[*]
J M Mitchison

Department of Zoology,
University of Edinburgh,
West Mains Road, Edinburgh, 9

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[*] Senior Postdoctoral Fellow, National Science Foundation (U S A), 1961–62 Permanent address Department of Biology, Brown University, Providence 12, Rhode Island

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Pathways of Adenosine Triphosphate Production by Calf Lens Homogenates

In previous work we investigated the phosphorylations occurring in homogenates of various zones of the crystalline lens of a calf incubated with glucose-6-phosphate[1] The transformation of adenosine diphosphate (ADP) into adenosine triphosphate (ATP) was measured by isolation of the nucleotides by column chromatography 'Dowex 1 × 8' 200–400 mesh (formate form) The application of such a method is rendered possible by the very feeble ATPase activity of the lens We saw that the intensity of phosphorylation diminished on passing from the anterior zone, constituted by the epithelium and young fibres, towards the older fibres of the cortical and central zone The aim of the present work was to determine the extent of phosphorylation due to anaerobic glycolysis, the Krebs cycle and possibly to the pentose cycle by a transdehydrogenation reaction reduced coenzyme 2 + coenzyme 1 → reduced coenzyme 1 + coenzyme 2

Our experiments were carried out on homogenates of the cortex of calf lenses in a solution of 0 12 M potassium chloride + 0 02 M sodium bicarbonate, 1 gm wet tissue being used per 2 ml solution The conditions of incubation and the results are summarized in Table 1

Table 1

Substrates	Quantity of ATP formed (μmoles)
Homogenate + oxygen	0 2
Homogenate + glucose + oxygen	2 6
Homogenate + galactose + oxygen	0 2
Homogenate + ribose-5-phosphate + oxygen	1 6
Homogenate + fructose-1,6-phosphate + oxygen	9 9
Homogenate + 3-phosphoglycerate + oxygen	3 4
Homogenate + α-cetoglutarate + oxygen	0 2
Homogenate + glucose-6-phosphate + oxygen	5 3
Homogenate + glucose-6-phosphate + nitrogen	5 2
Homogenate + glucose-6-phosphate + CH_2ICOOH	0 5
Homogenate + glucose-6-phosphate + TPN	0 6
Homogenate + glucose-6-phosphate + TPN + DPN	0 6

Incubation medium Homogenate, 3 ml All substrates at a concentration of 0 01 mM, ADP, 0 012 mM, monoiodoacetate, 0 1 mM, coenzyme 2 (TPN), 3 μM, coenzyme 1 (DPN), 2 7 μM Final volume 8 ml Incubation, 15 min , at 37°, stopped by rapid cooling plus addition of 2 ml of 1 2 N perchloric acid

The results show that, in our experimental conditions, the amount of phosphorylation involving galactose is almost nil, that involving glucose is high Glucose-6-phosphate was more efficiently used, but most phosphorylation was obtained with fructose-1,6-diphosphate We observed that the production of ATP was the same whether or not oxygen was present, a fact indicating that the Krebs cycle can contribute only to a very small extent to the production of ATP in the lens This does not mean that the Krebs cycle pathway is not utilized in the lens *in vivo*, one of us has in fact noted a true respiration of the mitochondria of the lens cortex even though these are scarce[2]

The addition of monoiodoacetate to the incubation medium blocks almost completely the phosphorylation of ADP The production of a small quantity of ATP was, in fact, observed, but at the same time the amount of AMP increased so that the ATP formed could be attributed to the myokinase activity which we have shown to exist in the lens[3] The observed excess of AMP in relation to ATP indicates that a part of the ATP formed by the myokinase activity was utilized in the transphosphorylation reactions which we have found in similar homogenates It is noteworthy that this augmentation of AMP does not occur in conditions in which phosphorylation is active and that, in this latter case, the myokinase activity does not appear

The possibility of production of ATP by the pentose cycle and by the route involving transdehydrogenation of reduced coenzyme 2 was investigated by the addition of coenzyme 2 to the incubation medium containing glucose-6-phosphate and monoiodoacetate, the latter to block glycolysis We observed the formation of a considerable quantity of adenosine diphosphate–ribose–phosphate, the product of acid decomposition of reduced coenzyme 2 This corresponds to an almost complete utilization of coenzyme 2 In these conditions, only a supplementary production of ATP which represents about 1 3 per 100 of the ATP formed by simple anaerobic glycolysis was observed This fact indicates that, if the pentose pathway does produce ATP in the lens, it can only give very small quantities It was also observed that the addition of coenzyme 1 which would favour transdehydrogenation was quite ineffective, no reduced coenzyme 1 and no more ATP being formed This observation shows that the possibility of energy production involving transdehydrogenation cannot be demonstrated in the lens *in vitro* Therefore, in our *in vitro* conditions at least, anaerobic glycolysis seems to be the sole source of ATP in the lens The Krebs cycle or the pentose cycle seems to provide ATP in quantities negligible by comparison with those provided by anaerobic glycolysis The preferential utilization of glucose-6-phosphate and particularly of fructose-1,6-diphosphate indicates either the fragility of enzymes forming hexose diphosphate or that these enzymes represent limiting factors

Summarizing, the most efficient production of ATP from ADP by lens homogenates was obtained through fructose-1,6-diphosphate The addition of coenzyme 2 with or without coenzyme 1 caused an almost quantitative production of reduced coenzyme 2 Monoiodoacetate blocked the phosphorylation of ADP almost entirely, whatever substrate was used The same quantity of ATP was produced with or without oxygen The pentose cycle seems capable of producing only very small quantities of ATP

P MANDEL
J KLETHI

Institut de Chimie Biologique,
Faculté de Médecine,
Strasbourg

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Dividing Cells as the Locus for Vernalization

IN an earlier communication[1] I described the "leaf vernalization" of leaf cuttings of *Lunaria biennis* After cold treatment, such cuttings from leaves of adult plants regenerate and yield flowering plants, while without a cold treatment no flowering occurs It was left undecided exactly where in the leaf cutting the vernalization takes place—in the leaf as a whole or in meristematic cells only Evidence has been obtained now that the locus of vernalization consists of cells which are in the process of mitosis

First of all, this concept explains some of the former results which were not clear originally Vernalization of leaf cuttings requires much longer cold treatment than is needed for intact plants The explanation which offers itself now is that for vernalization mitosis should start and that this takes considerable time at the low temperature of 5° C , which time must be subtracted from the total period of cold treatment to find the actual time of vernalization On the other hand, when leaf cuttings were allowed to start rooting by keeping them at ordinary greenhouse temperature for 2 weeks before the cold treatment, a much shorter period of cold treatment was sufficient for vernalization In this case mitosis had set in when the plants were exposed to low temperature

Secondly, the results of an experiment with root cuttings completely confirm those with leaf cuttings *Lunaria biennis* forms a number of thick 'contractile' roots, and these regenerate as cuttings better and even much better than leaf blades with petioles Groups of 25 root cuttings were exposed to 5° C for 0, 8, 12, 16 or 20 weeks respectively and were afterwards grown at a greenhouse temperature of about 20° C Flowering of the regenerated root cuttings occurred for 0, 8, 80, 100 and 100 per cent respectively When the root cuttings were kept at greenhouse temperature for 2 weeks before cold treatment, the percentages of flowering were 0, 80, 100, 100 and 100 per cent respectively The mean numbers of days from the end of the cold treatments to the appearance of the first visible flower bud were inversely proportional to the duration of the cold treatment In itself these results would point to the existence of 'root vernalization', but in comparison with the results of vernalization of leaf cuttings it seems more appropriate to speak of 'vernalization of dividing cells'

Thirdly, there are at least two possibilities for a direct test of this hypothesis As a matter of fact, they have led to a reconsideration of the former results and to an interpretation of the results with root cuttings as discussed here Hagemann[2] found that leaf cuttings from naturally overwintered plants regenerated into flowering plants In repeating this experiment, originally leaf cuttings were made from plants immediately after a prolonged artificial cold treatment, and no flowering occurred This means that intact leaves on their plants cannot be vernalized, while isolated leaves + petioles can be vernalized, as already stated The difference lies in the fact that in the latter case mitosis occurs during cold treatment When, in a second attempt Hagemann's experiment was repeated under identical conditions, the leaf cuttings indeed regenerated into flowering plants In this case the cuttings were made of leaves which were still growing during the exposure to cold, while in the first case the leaves were already full-grown when cold treatment was begun

The second test consists of removing the lower part of the petioles (where the mitoses occur) of leaf cuttings immediately after cold treatment, to make them re-regenerate and to see whether flower formation will take place In anticipation of an extensive experiment a preliminary test was made with rather poor plants during the bad growing conditions of the winter After cold treatment for 20 weeks, 16 cuttings re-regenerated after removing the lower portion of their petioles over a length of 0 5 cm None of them flowered, while 5 out of 24 cuttings, which were left intact, have flowered The number of flowering cuttings in the last group is rather small and this makes the total results not yet fully conclusive However, they do not disprove my hypothesis

Taking into consideration all the arguments discussed here, the conclusion may be drawn that they present evidence that vernalization occurs only in cells which are in the process of mitosis Of course the well-known fact that seed vernalization occurs only in germinating seeds supports this view

S J Wellensiek

Laboratorium voor Tuinbouwplantenteelt
Landbouwhogeschool,
Wageningen,
The Netherlands

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Effect of Temperature on Hatching in the Cereal Root Eelworm

Unlike most other species of *Heterodera*, the larvæ of the cereal root eelworm (*Heterodera avenae* Wollenweber, 1924) do not hatch in response to diffusates of the host plant root[1] The mechanism controlling hatching in this species has never been demonstrated, although it has been known for many years that in the field larvæ invade the roots of the host plant in the greatest numbers in spring and early summer[2] Experiments carried out at the Welsh Plant Breeding Station during the summer and winter of 1961/62 indicate that, in the laboratory, hatching of the cereal root eelworm occurs in response to a rise in temperature after a period of low temperature

In the first experiment cysts obtained from soil in July were stored in tap-water at 5° C and at room temperature (12°–22° C) for 90 days At the end of that period both lots of cysts were set up to hatch in tap-water at 21° C and over 14 days hatching was strongest among those which had been stored at 5° C (Table 1)

Table 1. Mean Daily Hatch per 100 Cysts at 21° C

After 90 days at room temperature	After 90 days at 5° C.
10 9	72 3

In a second experiment, cysts obtained from soil in October were set up to hatch in tap-water at 5° C and at 21° C Over 62 days there was no difference between the hatching under the two treatments, for the cysts at 5° C , the mean hatch was 9 4 per 100 cysts per day, while for the cysts at 21° C the mean hatch was 8 8 However, when the cysts hatching at 5° C were transferred to 21° C the hatch during the next four days was 510 larvæ per 100 cysts, which was almost as great as the hatch for the whole of the previous 62 days During this 4-day period only 2 8 larvæ per 100 cysts emerged from those cysts which were continued at 21° C throughout

These results appear to show that hatching occurs as a result of a rise in temperature after a period at low temperature, results of experiments still in progress agree with this view It has been found, for example, that plants grown in infested soil which previously had been stored out of doors during the winter bore large numbers of mature cereal root eelworm females, while plants grown in the same soil which had been stored at 21° C over winter bore almost none It is possible, however, that the viability of the larvæ had been affected by storage at the higher temperature and this point is being investigated

Clearly, and for the first time, larvæ of the cereal root eelworm have been shown to hatch in response to an external influence The results appear to show that a period at low temperature is essential for the subsequent spring hatch of the cereal root eelworm, and that this occurs in response to a rise in temperature In the field, cysts would not meet the abrupt rise in temperature to which they were subjected in these experiments, nor is it likely that they would ever be subjected to a temperature of 21° C , except perhaps for short periods in the surface layers of the soil It is possible, however, that a smaller rise in temperature may induce hatching, this question is under investigation

This work was supported by an Imperial Chemical Industries Ltd research fellowship

John Cotten

Welsh Plant Breeding Station,
Plas Gogerddan,
Nr Aberystwyth

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Endogone Spores in Cultivated Soils

Using nematological methods of wet sieving and decanting with agricultural soils in Illinois, Gerdemann[1,2] extracted two types of large one-celled spores which gave endotrophic mycorrhiza of the phycomycetous type in inoculated plants The larger of these spores, 183–812µ diam , produced arbuscules but not vesicles in infected roots Clusters of echinulate one-celled spores were formed on external hyphæ[1] A smaller spore, 133–288µ diam , induced typical vesicular-arbuscular infections and, in addition, produced on hyphæ surrounding the roots an *Endogone* sporocarpic stage and chlamydospores[2] The chlamydospores were identical with those extracted from field soil and the sporocarps were similar to those produced by the unnamed mycorrhizal species of *Endogone* described by Mosse[3]

Using the same technique in the examination of cultivated soils from the Dundee area, numerous large one-celled spores have been observed They range in size from 130 to 400µ diam , and also vary in shape, which may be spherical to irregular, or oblong These spores are considered to be either chlamydospores or zygospores of species of *Endogone* When extracted from soil they occur singly (Fig 1) and do not appear to have been formed in sporocarps Samples containing an abundance of spores have also been examined for the presence of sporocarps, but only a few small specimens have been found

The spores can be divided into 6 distinct types which are recognized by differences in average size, in wall thickness and in the morphology of the hyphal attachment In addition, each spore type has a characteristic colour which may be various shades of yellow or brown, and black In some soils only one

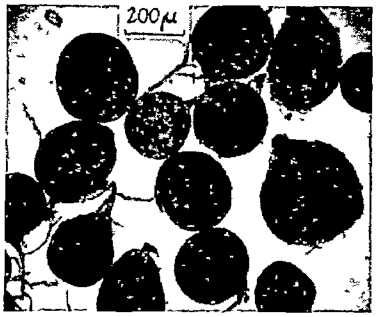

Fig 1 Miscellaneous collection of spores extracted from soil of a wheat field

type of spore is represented In other cases, up to four of the types have been noted in the same sample

Investigations are proceeding to determine whether these spores will induce mycorrhizal infection on various hosts grown in sterilized soil and to determine how many species are represented However, it is now apparent that spores of *Endogone* are abundant in certain cultivated soils These can be detected by direct microscopic examination of soil fractions obtained by wet sieving and decanting and are not revealed by methods normally utilized in soil mycology

This work was supported in part by a grant from the U S National Science Foundation

J W GERDEMANN*
T H NICOLSON

University of St Andrews,
Department of Botany,
Queen's College,
Dundee

* On sabbatical leave from the Department of Plant Pathology, University of Illinois

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MICROBIOLOGY

Inhibition of Endotrophic Sporulation by Antibiotics

ENDOTROPHIC sporulation occurs when an organism forms spores in a medium lacking substances necessary for growth It was of interest to determine whether a group of growth-inhibitory substances also inhibited sporulation

A strain of *Bacillus cereus* var *mycoides* was isolated from soil The organism was grown for 18 hr in shaken flasks at 32° C in a peptone–yeast extract–glucose medium The cells were washed twice in distilled water and suspended at a concentration of 9×10^8 cells/ml in 0 1 *M* potassium phosphate buffer (*p*H 7 4) containing 1 mgm /ml glucose Antibiotics were added in a graded series of concentrations to 10 ml aliquots of cell suspensions and these were then placed in 125-ml flasks and incubated with shaking at 32° C overnight Sporulation was determined by microscopic examination of smears stained with a malachite green spore stain In the controls, greater than 90 per cent of the cells sporulated Where inhibition of sporulation occurred, the minimum concentration of antibiotic that completely inhibited sporulation was then determined

All the antibiotics used were able to inhibit growth of the organism at concentrations below 50 μgm /ml The effect of the antibiotics on sporulation is shown in Table 1 It can be seen that only three antibiotics inhibited sporulation at growth-inhibitory concentrations It is interesting that these three antibiotics, tetracycline, chloramphenicol and erythromycin, are the only ones in the table that are known to act by inhibiting protein synthesis[1].

Table 1

Antibiotic	Minimum concentration to completely inhibit sporulation
Tetracycline	10 μgm./ml
Chloramphenicol	10 μgm /ml
Erythromycin	25 μgm /ml
Penicillin *G*	1,000 μgm /ml
Novobiocin	> 1,000 μgm /ml
Neomycin	> 1,000 μgm /ml
Streptomycin	> 1,000 μgm /ml
Polymyxin *B*	> 1,000 μgm /ml

Concentrations of antibiotic used 1,000, 500, 250, 100, 50, 25, 10 5, 2 5 and 1 μgm /ml

It should be emphasized that the sporulation process is occurring in the absence of growth Several of the other antibiotics listed in the table are known to be active primarily against growing cells, that is, penicillin[2], novobiocin[3], and streptomycin[4]. The present results then seem to confirm that endotrophic sporulation does occur in the absence of growth In addition, these results suggest the value of testing other growth inhibitors for their effects on sporulation, both as an aid in the understanding of the sporulation process, and to help shed more light on the mode of action of antibiotics

THOMAS D BROCK

Departments of Bacteriology and Microbiology,
Indiana University,
Bloomington, Indiana

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Withdrawal of the Concept of the Occurrence of Classical Mitosis in Bacteria

I WISH to retract the concept previously presented by me[1], and to which I have been committed, that classical mitosis occurs in bacteria This interpretation of cytological material was developed on the basis of routine microscopy on stained preparations The configurations observed were interpreted using instrumentation of inadequate resolution to solve the problem of the mechanism of division of this group of organisms It seems likely that this interpretation of structures by white-light microscopy does in fact depend on the presence of not only nuclear material which can assume a multiplicity of forms, but also on the presence of membranous bodies in the cytoplasm which accept stain and at low resolution could be misinterpreted as centrioles The application of the electron microscope in the hands of many others[2,3], as well as more recently by us[4,5], has demonstrated that a classical form of mitotic division in these organisms cannot occur Details of the ultrastructure of bacteria are now becoming resolved, and what is presently known precludes the possibility of a classical mitotic mechanism

It seems appropriate, therefore, on the basis of work of others as well as of ourselves, to acknowledge the error in interpretation which was made, and in so doing perhaps remove some of the confusion in the literature contingent on such an interpretation By acknowledging the error of interpretation, however, I do not mean to imply that I consider that the interpretations of some of my critics concerning the structure of the bacterial nucleus are necessarily correct in the present state of our knowledge There is much yet to be done by means of high-resolution instruments to resolve the ultrastructure of the bacterial cell The work of Kleinschmidt *et al.*[6] is perhaps the most revealing so far, and correlates well with the genetic studies of Wollman and Jacob[7].

It should also be emphasized that much of the work done still remains valid The staining and dehydration procedures[8] developed during these investigations are accepted and used by many as routine[9] More recent efforts at isolation of bacterial nuclei by chemical dissection, as well as the chemical evaluations of the procedures, appear sound[4] Preliminary electron microscopic studies of dissected cells promise to be most revealing[4,5] Electron microscopic studies of the isolated bodies have yet to be completed, and these likewise may contribute to our understanding of the ultimate organization of bacterial 'nuclei' The various suggestive configurations of these isolated bodies as seen by routine microscopy seem likely not to have organizational significance

I am glad of this opportunity to aid in resolving the confusion resulting from the presentation of the interpretation of material with instruments not adequate for the purpose

This investigation was supported in part by research grant *PHS* No *E*–2203 from the National Institutes of Health, U S Public Health Service

Edward D DeLamater

Section on Cytology and Genetics,
School of Medicine,
University of Pennsylvania,
Philadelphia 4

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PSYCHOLOGY

Effects of Frontal Cortex Lesions on Object Discrimination Learning by Monkeys

For a long time it has been known that monkeys with bilateral dorso-lateral frontal cortex lesions are impaired on delayed response problems At the same time there does not generally appear to be any impairment on visual discrimination learning Recently, Rosvold and Mishkin[1] have suggested that, in certain circumstances, the discrimination learning of monkeys with frontal lesions may be influenced by various preference factors Specifically, Brush *et al* [2] reported that monkeys with frontal lesions are impaired, as compared with intact animals and others with inferotemporal lesions, on a series of object discrimination problems Each individual problem was presented for ten trials Bait was placed under both objects on the first trial of half the problems, and the object chosen by the monkey became the positive (rewarded) cue on the subsequent trials On the first trial of the remaining problems bait was not placed under either object, the chosen object became the negative (unrewarded) cue on the subsequent trials The frontal monkeys were impaired particularly on this latter category of problem—that is, where a reward could be obtained only by responses to the initially non-preferred object It was this finding which led Rosvold and Mishkin to their conclusion that preference factors were an important feature of the frontal deficit

If the performances of frontal monkeys are dominated by stimulus preference factors, then it would be expected that this should become apparent in a testing situation designed specifically to provide data in a form amenable to mathematical analysis of the frequency with which various strategies are adopted Such a method has been described by Levine[3] The experiment described here was undertaken with this object in mind Six young monkeys (*Macaca mulatta*) were tested in a modified version of the Wisconsin general testing apparatus Initially they were trained on three two-choice (simultaneous) object discrimination problems, each problem was run to a criterion performance-level of 90 per cent correct in 50 trials before the next problem was introduced Two of the monkeys then received bilateral ablations of the dorsolateral frontal cortex anterior to the arcuate sulcus Another two received bilateral ablations of the interior temporal cortex It was intended that lesions on both hemispheres should be symmetrical The remaining two monkeys served as intact controls Seven days later the monkeys were retrained on the second and the third of the original problems to a criterion performance level of 23 correct responses in 25 trials

Object discrimination training suitable for detailed analysis then began 16 separate problems were presented during each testing session and each problem was presented for 3 trials On the first trial of half the problems food reward was placed under both cues (first trial positive problems), on the first trial of the remaining problems neither cue was baited (first trial negative problems) During trials 2 and 3 the same reward contingencies held as in the study of Brush *et al* [2] Each testing session contained 8 problems of each type The use of three trials per problem makes the data amenable to the method of analysis suggested by Levine[3] Each pair of cues was drawn at random from approximately 500 multi-dimensional objects bought in Woolworth's and similar stores After each group of 250 problems the objects were re-paired, the only condition being that no pair should appear more than once during the course of the experiment Such 'learning set' training was continued until 1,600 problems had been completed Finally, each monkey was trained on a standard positional 5-sec delayed alternation task until a criterion performance-level of 90 per cent correct responses in 100 trials had been achieved or, if this was not achieved, for a maximum of 1,000 trials

Table 1

Animal	Problem No 1	2	3
Normal No 10	170	82 (0)	0 (0)
Normal No 12	157	117 (0)	18 (0)
Frontal No 8	108	36 (47)	4 (0)
Frontal No 9	390	143 (0)	0 (0)
Temporal No 11	209	123 (23)	45 (21)
Temporal No 13	161	26 (69)	0 (24)

The number of trials required to learn the initial object discriminations are recorded in Table 1, these scores do not include the criterion trials The retraining scores are shown in brackets As would be expected, there was some indication that the temporals were impaired post-operatively Fig 1 summarizes the performances on the 1,600 object discrimination problems Each problem block consisted of 200 problems (100 first trial positive and 100 first trial negative) There is no evidence of any overall frontal impairment Indeed, the performances of the two frontal monkeys were consistently rather better than those of the other subjects The average performance of the two temporal monkeys has been plotted One of these was severely impaired and did not achieve 60 per cent correct responses on any one problem

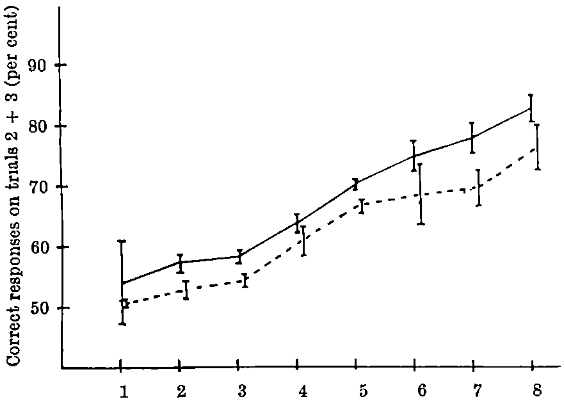

Fig 1 ---, Normals, ——, frontals, , temporals

block However, the other one reached a score of 80 per cent correct on the seventh block

Fig 2 has been constructed from the performances on trials 2 and 3 within each problem block by measuring

$$\frac{\text{(correct responses on first trial positive problems)} \atop \text{minus (correct responses on first trial negative problems)}}{\text{total correct responses}}$$

If the frontal monkeys had been handicapped on first-trial negative problems, this score should have been consistently in the positive rather than the negative range Although this was to some extent true during the earlier problem blocks, there was no evidence to suggest that the frontals differed significantly from the normals A full analysis of the monkeys' performance along the lines suggested by Levine[3] will be published later

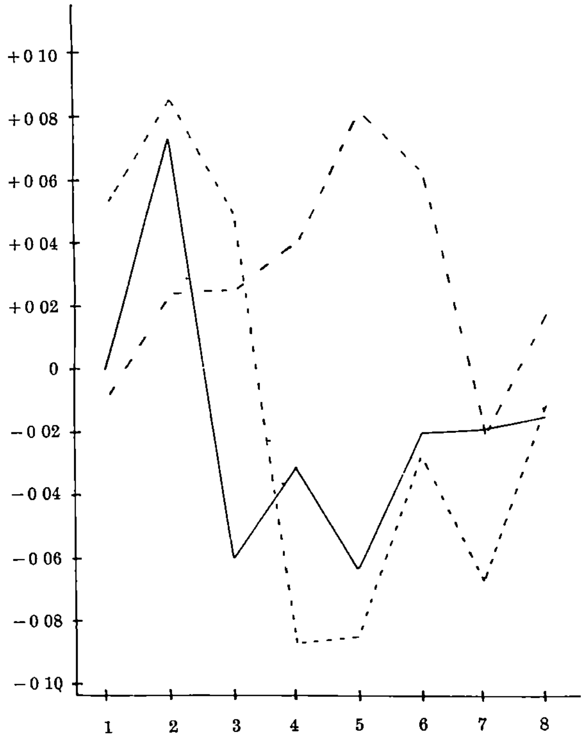

Fig 2 ——, Frontal, No 8, ---, frontal, No 9, , normal, No 10, — —, normal, No 12

Finally, the performance on the delayed alternation task was consistent with previous reports of a frontal impairment on this particular task Neither of the frontal monkeys achieved the criterion within 1,000 trials The scores for the other two groups were Normals, 497 and 117, Temporals, 693 and 111 (criterion trials have not been included in these scores)

There appears to be a discrepancy between the results presented here and those of Brush *et al* [2] It is possible that this might be due, among other reasons, to the difference in the number of trials per problem Although Levine *et al* [4] compared the performances of normal monkeys in situations where the problems were presented for three trials each and where they were presented for twelve trials, and found that the rate of learning set development, considered as a function of the total number of trials, was approximately equal in both groups, this might not apply to frontal monkeys According to a hypothesis recently proposed by one of us[5], frontal lesions may result in an inability to maintain adequate selective attention Along these lines it may be argued that when the cues are presented for only three trials they may retain a degree of novelty value which they lose when they are presented for larger numbers of trials A falling off in the frontals' attention in the latter situation might result in their behaviour deteriorating, one manifestation of which might be the adoption of a stimulus preference strategy This is a primitive response pattern in the sense that both Harlow[6] and Levine[3] have shown that it is particularly liable to occur in normal monkeys during the early stages of learning set development, and is usually eliminated as the training continues

We should like to acknowledge our gratitude to Miss Sheila Hopkins, who tested the animals on the learning set problems, and to Miss Ruth Fishel, who tested them on the delayed-alternation task The research was supported by the U S Air Force under contract *AF* 61 (052)–185 and grant 62–20

JOHN OXBURY
LAWRENCE WEISKRANTZ

Psychological Laboratory,
Cambridge

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Time Uncertainty and the Estimation of Time-intervals

DURING the past decade an increasing number of experimental psychologists have returned to the problems raised by a human subject's reaction-time, the interval between the occurrence of a signal and the initiation of a response to it Many recent investigations, such as those of Klemmer[1,2], Karlin[3], Drazin[4], Bertelson and Boons[5], Kay and Weiss[6], have been concerned with the way in which reaction-time to a signal varies with the duration of the foreperiod, the interval between a warning signal and the signal to respond

As Klemmer pointed out, two different kinds of effect may determine a subject's reaction-time in these situations. The first effect depends on the subject's 'expectancy' that the warning interval will be of a certain length, this being determined by the range of foreperiods used and the probability of particular foreperiods within this range. The second effect depends on the subject's time-keeping mechanism, on the accuracy with which he can estimate an interval of a certain length, when other factors, such as the probability of its occurrence, are held constant. Whereas the first of these effects has been extensively investigated in a recent research, the second has been relatively neglected and there has been little systematic investigation of conditions which influence a subject's accuracy in estimating temporal intervals since Woodrow's[7] work on the "indifference interval".

It has been assumed by most workers that the variability or uncertainty in a subject's estimate of an interval will increase monotonically with the length of the interval in a manner corresponding to the Weber Law, or some variation of it. However, experiments which I recently performed at the Laboratoire de Psychologie Expérimentale, University of Paris, indicate that this is not so and that intervals in the region of 0·5 sec. are estimated with less variability than intervals either smaller or larger than this value. The experiments were also designed to investigate the accuracy with which a subject is able to estimate time-intervals using different rates of counting, and the results showed that a frequency in the region of one count every 0·5 sec. produced much more accurate estimation of longer time-intervals than any other rates either faster or slower.

The experiments were carried out in the following manner. Subjects listened to a regular series of clicks presented by a small loudspeaker, with an interval i between clicks. A series of n clicks was presented and the subject counted these as they occurred. He then had to imagine that the series continued and had to press a key at the estimated moment of occurrence of the $2n-1$th click. In each experimental session the numbers n and $2n-1$ were always specified, and instructions to the subject were of the following form: "You will hear a series of [for example] 5 clicks. Imagine the series to continue and press the key at the moment you estimate the 9th click would have occurred". The error of estimation was recorded to the nearest 1/100th sec. on a continuous pen recorder. In this manner the interval I between the first and last click of the stimulus series should be the same as the interval I' between the last click of the stimulus series and the subject's response. Five values of i (frequency of counting) were used, 0·25 sec., 0·5 sec., 1·0 sec., 2·0 sec. and 4·0 sec., in combination with three values of I, 2 sec., 4 sec., and 8 sec.

Seven subjects attended for three sessions. At each session the various combinations of i and I were presented in random order, and subjects were given practice under each condition before any results were recorded. The final analysis is based on thirty responses for each subject under each combination of i and I.

Typical results are given in Fig. 1, which shows the standard deviations of the estimates of the seven subjects for each value of i when $I = 4$ sec.

Variability in estimation is clearly a minimum when the value of i lies in the region 0·5–1·0 sec. The results for the other conditions are similar except that, when subjects estimate the longest interval $I = 8$ sec. using values of i greater than 2 sec., they tend to interpolate their own count in between the clicks given by the loudspeaker. Hence the value of i does not represent the actual rate of counting for values of i greater than about 2 sec. When subjects did introduce their own method of counting it was again found that the frequency they spontaneously tended to adopt corresponded to $i = 0·5$ sec.

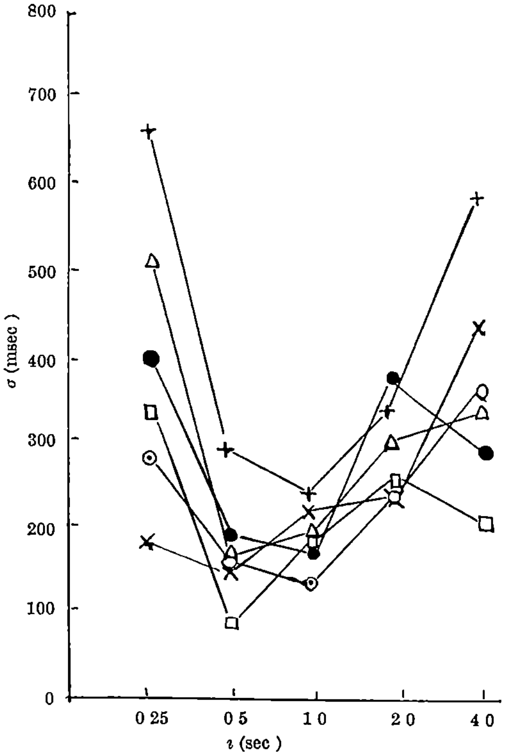

Fig. 1. Standard deviations (msec.) for seven subjects estimating $I = 4$ sec., using values of i from 0·25 to 4·0 sec.

It is worth emphasizing that the values of standard deviation given are absolute not relative values, and that the effect is so marked that, using the optimum frequency, there is less absolute variability in estimating an interval of 4 sec. than there is in estimating an interval of 2 sec. using a non-optimum frequency. Further details of these comparisons will be given by me elsewhere[8].

A general theoretical consequence of these results is that it is most important to take account of the units used, either overtly or implicitly, by a subject when one constructs psychophysical scales by extrapolation or interpolation methods. A practical consequence is that, in tasks having a rhythmic structure, synchronization of a response with an anticipated event should be more accurate at some frequencies than at others. As Fraisse[9] remarks, the value of 0·5 sec. appears in a variety of experiments too often for this to be mere coincidence, but the physiological significance of this value is as yet obscure.

R. Davis

Department of Psychology,
University of Reading

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FORTHCOMING EVENTS

Tuesday, July 24

Parliamentary and Scientific Committee, General Committee (in Committee Room 10, House of Commons, Westminster, London, S W 1), at 5 30 p m —Discussion on "Atomic Energy in this Country" introduced by Sir William Cook, Mr A J Sayers and Colonel G W Raby

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Sub-Editor (with practical experience of work in medical bibliography and/or scientific publishing, and a wide knowledge of medical terms and accuracy in detailed work) for the *British Medical Bulletin*, for duties which will include verifying references, marking copy for printers and checking proofs—The Director, Recruitment Department, British Council, 65 Davies Street, London, W 1, quoting Ref G3 N (62) (July 27)

Assistant Lecturers or Demonstrators (2) (with medical qualifications registrable in this country) in Pathology—The Registrar, The University, Manchester 13 (July 28)

Lecturer in Geochemistry and Metamorphic Petrology, for duties which will include initiating and administering the geochemical and spectrographic units in the newly completed Sedimentary Research Laboratory—The Registrar (Room 22, O R B), The University, Reading (July 30)

Plant Physiologist (preferably with research experience), for the investigation of effects of temperature, oxygen and carbon dioxide on the respiration of apples—The Director, Ditton Laboratory, Agricultural Research Council, Larkfield, Maidstone, Kent (July 30)

Assistant Lecturer in the Department of Geography—The Registrar, The University, Liverpool, quoting Ref CV/132 (July 31)

Lecturer or Assistant Lecturer in Bacteriology—The Registrar, The University, Manchester 13 (July 31)

Lecturer in Civil Engineering—The Secretary of University Court, The University, Glasgow (August 4)

Associate Professor in the Department of Geography, University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, August 11)

Temporary Lecturer in Mathematics (Pure or Applied)—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (August 11)

Chair of Western Asiatic Archæology at the Institute of Archæology—The Academic Registrar, University of London, Senate House, London, W C.1 (August 15)

Professor of Botany in the School of Biological Sciences, The University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, The University of New South Wales, Box 1, Kensington, New South Wales, Australia (August 20)

Professor of Zoology in the School of Biological Sciences, The University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and the Appointments Section, The University of New South Wales, Box 1, Kensington, New South Wales, Australia (August 20)

Medical Research Fellow (preferably with experience in pædiatrics and nutrition) in the Department of Chemical Pathology, University College, Ibadan, Nigeria, to take part in nutrition studies —The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (August 22)

Senior Tutor/Demonstrator (with research interests in socio-economic geography (for preference in the geography of settlement), and preferably field experience in the African or Asiatic tropics) in Geography at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, August 24)

Lecturer or Assistant Lecturer in the History of Science, and a Lecturer or Assistant Lecturer in Zoology at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (August 31)

Senior Lecturer in Biochemistry—The Registrar, The University of Adelaide, Australia, or The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia, August 31)

Lecturer in Veterinary Pathology—The Registrar, The University, Liverpool, quoting Ref No CV/129 (September 15)

Professor and Head (with experience in teaching and research) of the Department of Pharmacy—The Executive Secretary, School of Pharmacy, Hebrew University of Jerusalem, P O Box 1172, Jerusalem (September 15)

Professor of Agricultural Chemistry—J P MacHale, Secretary and Bursar, University College, Earlsfort Terrace, Dublin 2, Republic of Ireland (September 15)

Senior Lecturer (with an honours or higher degree in science or engineering, with teaching and/or research experience in the field of physical metallurgy) in Physical Metallurgy at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, September 30)

Assistant Lecturer in Geology—The Registrar, The University, Nottingham

Experimental Officers or Senior Experimental Officers (metallurgists, physicists or physical chemists, preferably, but not essentially, with a degree), for work on direct generation involving cathode preparation and testing—Prof J G Ball, Imperial College of Science and Technology, London, S W 7

Graduate (interested in population genetics, and preferably with appropriate postgraduate research experience), to study the effects of radiation-induced mutations on mouse populations—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berkshire

Lecturer (graduate of a British university, preferably with experience in the field of virology) in Microbiology—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting Ref CST 62/43

Lecturer (with a good honours degree in geology and experience in the application of photogeological techniques) in Photogeology, for undergraduate and postgraduate instruction and research—Prof W D Gill, Geology Department, Imperial College of Science and Technology, London, S W 7

Physics Master—The Headmaster, Carmel College, Wallingford, Berkshire

Research Assistant (graduate chemist or biochemist), to work on problems of protein analysis and structure—The Secretary, St Mary's Hospital Medical School (University of London), Paddington, London, W 2

Research Fellow or Lecturer (mathematical physicist, preferably possessing doctorate) in the Metallurgy Department, for work on problems of crystal plasticity with an established research group—Prof J G Ball, Imperial College of Science and Technology, London, S W 7

Research Physicist (with a higher degree in physics, and preferably experience of high vacuum technique and/or electronic counting equipment) in the J J Thomson Physical Laboratory, to carry out a programme of electron diffraction intensity measurements at high energies—The Registrar, The University, Reading

Research Students in the Department of Chemistry, for research in inorganic fluorine chemistry—Prof Panson, Department of Chemistry, The Royal College of Science and Technology, George Street, Glasgow, C 1

Senior Lecturer or Lecturer in Chemistry, and a Senior Lecturer or Lecturer (preferably with a special interest in geomagnetism or ionospheric physics) in Physics at Ahmadu Belo University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1

Tutorial Assistant in the Dfpartment of Geology—The Registrar, University College of Wales, Aberystwyth

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research Road Research Laboratory Road Research Technical Paper No 57 Soils and other Roadmaking Materials in Nigeria By K E Clare and P J Beaven Pp vi+54+4 plates (London H M Stationery Office, 1962) 4*s* 6*d* net [115

Royal Observatory Bulletins (Joint publications of the Royal Greenwich Observatory, Herstmonceux and the Royal Observatory, Cape of Good Hope) No 49 Standard Magnitudes in the E Regions By A W J Cousins and R H Stoy Pp El–58 8*s* net No 50 Photo-electric Magnitudes and Colours of Southern Double Stars By P A Wayman Pp E59–76 3*s* net (London H M Stationery Office, 1961) [115

Publications List of the Commonwealth Agricultural Bureaux Agriculture, Animal Health, Forestry Pp 36 (Farnham Royal Commonwealth Agricultural Bureaux, 1962) [115

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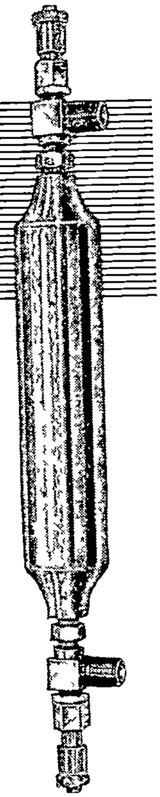

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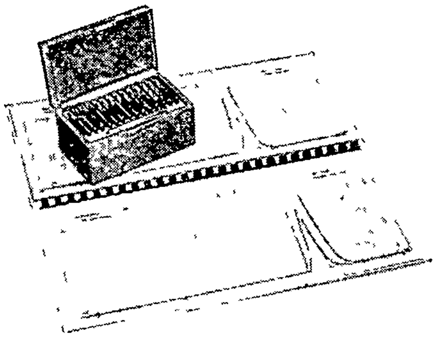

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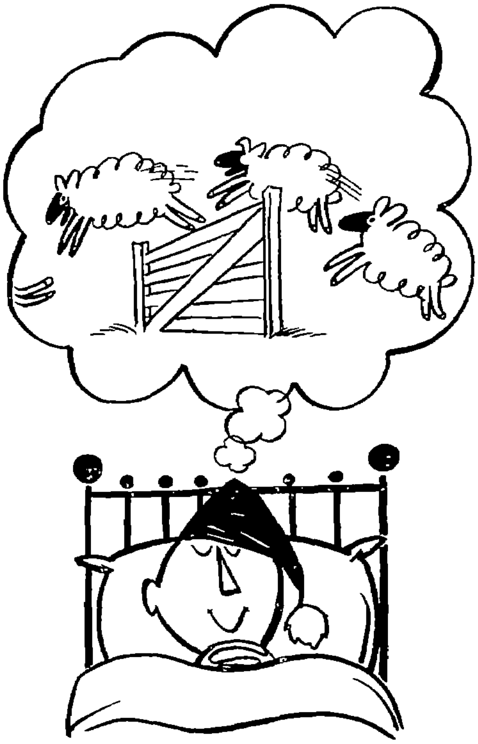

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Regd T M

TECHNICAL TRANSLATING

TECHNICAL translating must be regarded as a regrettable necessity If scientists knew their languages, or if translators knew their science, there would be no problem As matters stand, however, the flood of technical and scientific literature in all languages continues unabated, and the problem has been solved to date by producing a limited number of cover-to-cover translations from Russian into English and leaving all other articles and papers to the individual interest of scientists who can request translations privately

In this way the Copyright Act of 1956, which lays down that translating is legal only when done with the author's consent, is obeyed in the spirit and there the matter might rest were it not that in the final analysis technical translating is an essential service to scientists and should be improved within the limits of the possible

Staff translators can get help from their colleagues, and free-lance translators and translation agencies manage with their given resources, but officially no questions are asked whether the services provided as a whole are good enough Technical translators generally do not seem to appreciate that the literary aspect of their work should be subordinated to its technical aspect An expert translator in his particular technical field knows only too well how complex the subject-matter usually is and thereby does not throw the onus of understanding entirely on the reader, but the technical or scientific worker has in far too many cases to be extremely indulgent in puzzling out possible meanings in what passes for a technical translation

Provided a technical translator has had the necessary training and is sufficiently acquainted with the details in the two relevant languages, the translation itself should only be in plain technical English, which should be regarded as the professional standard to be aimed at in this kind of work The alternative —that scientists should learn languages and do their own foreign reading—is interesting in theory and would naturally broaden their cultural outlook, but in practice the vast majority of scientific workers will always have to rely on the help they can get from technical translators Another point is that translations in their nature are expensive It would therefore be in the interest of everyone concerned to see that a professional level were reached and possibly taken for granted

A step in this direction would be for some authorized and recognized body to produce a publication similar to *Technical Translations*, a fortnightly typescript-offset journal published by the U S Department of Commerce, Office of Technical Services, Washington, D C , at the reasonable price of 12 dollars per annum inside the United States (16 dollars abroad) The value of such a British *Technical Translations* would lie in the co-operative effort required to bring all the translators and translation agencies in Great Britain under one umbrella so that the service given to scientists and technologists would be no less prompt and comprehensive than that given to their colleagues in the United States

Translations not only have to be made, they also have to be made accessible

The difficulties of educating technical translators, however, should not be underestimated It takes years to learn science, and equally years to learn languages It follows, therefore, that technical translators cannot change or be produced overnight Similarly, it follows that if no academic courses of a suitable nature are provided, there will never be enough trained technical translators to do the work in the future, even if the demand for good workers does not increase

The Fédération Internationale des Traducteurs in Paris is drawing up a 'Translator's Charter' which is intended to put all translators, technical and literary, on a professional basis internationally, and the Institute of Linguists, 3 Craven Hill, London, W 2, is preparing plans for a diploma specially for technical translators in Britain It remains to be seen whether these measures, taken by themselves, will be sufficient to satisfy the demand for a rapid and accurate communication of technical and scientific papers published in languages other than English

The following suggestions, therefore, are put forward as a contribution to the study of a problem which perhaps deserves far more attention than it has so far received

The objective should be two-fold to provide courses for beginners and short refresher courses for practising translators All this would take time, but only a limited number of people would be involved, otherwise there might be a flood of translators to cope with a possibly static amount of work

Courses for beginners should be relatively easy to organize as the requirements would involve only basic languages and basic science or technology The Holborn College of Law, Languages and Commerce, Red Lion Square, London, W C 1, is running at present a German technical translation course and will have in the autumn also a similar course in French, while the Institut Français, Queensberry Place, London, S W 7, is offering a French technical translation course now

By adopting a scheme with a high-sounding title such as industrial scholarships, it might also be possible to transfer from the universities direct to industry a limited number of talented graduates who would rather combine science and languages than do either separately Again, the idea of industrial scholarships would have to come preferably from large firms or an organization such as the Federation of British Industries

With regard to refresher courses, translators need to keep up with the advancing terminology and developments in the scientific and technical fields every bit as much as their practising opposite numbers, that is, their potential clients Annual weekends could meet this requirement if the necessary backing from authoritative quarters were forthcoming The subject-matter for such refresher courses is more or less well known to every technical translator techniques and jargon

Beginners must naturally keep strictly to the original, but with experience a certain latitude becomes possible and desirable For example, every language has its typical grammatical constructions Normally, translating is done word for word with the minimum of rearrangement to give adequate readability Perhaps more acceptable results could be achieved faster if translators regarded it as part of their task to translate also grammatical constructions The substance of the original may come through even more clearly in this way

Another technique which sounds scarcely relevant, but is extremely important from a practical point of view, is typing The better a translator can type, the more time and energy he has for the actual work of translating A quarter of an hour, or even longer, looking for the right word is most uneconomical working, yet it is so easy for one translator to phone another who knows the answer and might reciprocate on some future occasion

The image of technical translating is small, pettifogging and 'uninteresting' All this it certainly is on occasion, especially when output alone is important for economic reasons.

In the wider picture, however, science and technology have many important small links in the chain from the original conception to the final achievement, whatever it happens to be, and technical translating is one such link in the information and communications field Perhaps this link is a more vital one than is generally appreciated because, in the past, too many amateurs have had to do it or it would, by and large, not have been done at all

THE SECOND MASER MILESTONE

Advances in Quantum Electronics

Edited by Prof Jay R Singer. Pp xvii+641 (New York and London Columbia University Press, 1961) 15 dollars, 120*s*

THE title of this book rather suggests that it is a comprehensive review of the present state of quantum electronics, and so it is, for the expert

The new field of quantum electronics was firmly established at its first international conference, held in September 1959 The second international conference was held in Berkeley, California, in March 1961, and the present volume is really the conference report It comprises the summaries and parts of the discussions of about seventy papers presented at the meeting The articles vary in length from a few paragraphs to about ten pages, depending presumably on the whims of the authors, and their contents are usually devoted to areas of the subject where recent advances have been made, with relatively little space devoted to giving the background of the topic Thus the book must be regarded as primarily for the worker in quantum electronics, and a newcomer will probably wish there were more review articles Even so, a casual reader can scarcely fail to be impressed by the picture which emerges, of a most exciting and rapidly developing field of activity.

It is natural to compare this book with the report of the first conference, published under the title of *Quantum Electronics* and edited by Dr C H Townes, to see how the ideas and work there reported have progressed Two main features emerge In 1959 it was rather novel to have a microwave amplifier or oscillator using the maser principle, but now this is so commonplace that relatively little space is devoted to masers The big step forward has come with the application of maser principles to optical frequencies, and has led to the laser, or the generation of coherent infra-red and visible light From the high proportion of papers devoted to lasers it is clear that interest has now swung away from microwave masers, for the very good reason that the new field provides all sorts of challenging problems and exciting possibilities To mention a few there is the possibility that modulated visible light can be used for communication purposes both terrestrially and in outer space, so intense can be the radiation from a laser that a wide variety of second order effects may be observable, such as the generation of higher harmonics of optical frequencies, and the production of far-infra-red radiation by the mixing of two optical frequencies, alternatively, just the study of the effects produced by subjecting materials to intense radiation fields should in itself be of considerable interest All these possibilities and many others were discussed at the conference, and there are papers describing the present state of progress As no developments of this magnitude can take place without accompanying advances in techniques there are papers dealing with all aspects of laser technology. materials, light sources, optical systems and so on But in nearly every case the relevant paper is short, and probably omits as much as it reveals

One section is entitled, "Some New Ideas and Accomplishments" I would have thought that these sentiments should have applied to the whole volume, and its publication will be widely welcomed by the considerable number of physicists and electronic engineers who are at present exploiting the field of quantum electronics K W H STEVENS

ASTRONOMY IN EVOLUTION

A History of Astronomy

By A Pannekoek Pp 521+24 plates (London George Allen and Unwin, Ltd , 1961) 65*s* net

THE main problem facing an author attempting to write about the history of any science is to decide on the amount of detail which should be included, too much detail and the reader loses the main gist of the story, too little and the story tends to become a string of generalizations The result must always be a compromise and especially is this so in a history of astronomy which must go back before the beginnings of civilization and the advent of written records The late Prof Pannekoek, well known as a practising astronomer, has managed to steer a middle course between too many facts and too much generalization On this important criterion his book scores a considerable success

Yet a balance between fact and general assessments is not enough, for the facts must be correct, there must be no gaping lacunæ and the historical generalizations should fit the evidence presented On the facts themselves, Prof Pannekoek is sound enough, even if occasionally an oversimplification tends to give one a wrong impression, and there are certainly no major omissions to be laid at his door. It is through some of the paragraphs giving the author's historical interpretations that the reader should, however, be prepared sometimes to tread rather warily, in a number of instances there is more than a tendency to apply a mid-twentieth century attitude in assessing the reasons for failures or changes made long before the Renaissance of Western Europe had come to alter men's outlook Yet these occur mainly in the earlier part of the book, and, in any event, they never amount to serious proportions

Prof. Pannekoek laid out the general scheme of his book in a thoroughly sound way, dividing it into three parts The first of these—astronomy in the ancient world—is concerned with the very beginnings of the subject from prehistoric times until the fifteenth century A D when Ulugh Beg made his famous star catalogue at Samarkand This occupies less than a third of the book The second part—astronomy in revolution—commencing with brief chapters on what is called "Dark Europe" and the renascence of science, passes on to discuss the contributions of Copernicus, Brahe, Kepler, Galileo and Newton New observational accuracy following on the invention of the telescope and refinements to celestial mechanics consequent on the application of gravitational theory bring the section to a close The ground is now prepared for part three which, beginning with the extraordinary observations of William Herschel, is concerned with the stellar universe With the title "Astronomy Surveying the Universe", the reader is brought, if not up to date, at least well into the twentieth century, and there is a brief mention of the work of Eddington and Einstein.

Prof Pannekoek shines most in the third part, when he deals with the more modern aspects of astronomical history In the second part, the era of dynamical and telescopic astronomy, his magnitude is scarcely perceptibly dimmed, and it is only in the first part of the book, when he deals with ancient astronomy and those whose outlook did not include any truly empirical approach, that he becomes partially eclipsed from time to time Clearly, his sympathies grow in inverse proportion to the time difference between his own age and that with which he is dealing Strange to relate, it is in the first part of the book that he expresses his willingness to believe that the Babylonians and others since their day were able to see the crescent of Venus with their eye alone and that even the satellites of Jupiter have been observed in this way such observations are equivalent to being able to read from a distance of more than 32 ft the sans serif capitals at the top of this review! No, Part 1 is not the happiest feature of the book, although it certainly contains a wealth of interesting and useful material.

Prof Pannekoek's book appeared first in 1951 in Amsterdam and the fact that it contains no reference to the steady-state theory is not, then, a cause for adverse comment, nor is the complete omission of radioastronomy Unfortunately, readers may nevertheless feel surprised, at least over radioastronomy, as the publishers in their wisdom have inserted a photograph of the 250-ft parabola at Jodrell Bank They must, however, assuage their disappointment by reading the useful appendixes or referring to the very full list of references It is also sad to record that the translation of Prof Pannekoek's original text is not all it might be indeed, at least two translators seem to have been at work, one whose style is far more pedantic than the other In consequence, tortuous sentences and infelicities of phrase which are not conducive to relaxed reading are all too frequent, but in spite of this, the book is something which can do no less than add to what was already a very worthy reputation COLIN A RONAN

REACTOR MATERIALS AND RADIATION DAMAGE

Properties of Reactor Materials and the Effects of Radiation Damage

Proceedings of the International Conference held at Berkeley Castle, Gloucestershire, England, from 30th May to 2nd June, 1961 General Editor D J. Littler (Central Electricity Generating Board: Berkeley Nuclear Laboratories) Pp xv + 562. (London Butterworth and Co (Publishers), Ltd, 1962) 126*s*

THIS book is a report of a conference held in May 1961 to mark the opening of the Berkeley Nuclear Laboratories of the Central Electricity Generating Board One hundred and fifty delegates were invited, and forty-seven papers were presented. There were three introductory lectures F Seitz spoke on the point defects introduced by irradiation, but as his lecture was based on work already published a summary only is given A H Cottrell summarized the theory of fracture, and L Rotherham gave an interesting account of the history of pile design and of the materials problems that arose in the development of the British gas-cooled reactors. The main Conference was organized in nine sessions, and dealt with the measurement of neutron flux in reactors, the theory of damage and the direct and indirect experimental methods used in its study, the effects of irradiation on mechanical properties and resistance to corrosion, and the phenomena of growth and swelling in reactor materials The term 'growth' was applied to the changes of shape and dimensions produced by thermal cycling and by the production of vacancies, interstitials and fission fragments within the material, while the term 'swelling' was confined to the effects of the generation of insoluble gases within the material, though the distinction was not always consistently maintained The papers were discussed in groups, the discussions were lively and informative they are fully reported but well edited The book as a whole is well printed and illustrated, and pleasant to read

The book does not really live up to its title, for it does not present a logical account of the properties of reactor materials in relation to their function in the reactor Questions of neutron absorption, and the conflict that so often arises between desirable nuclear properties and undesirable mechanical and chemical properties are scarcely mentioned Instead, a discussion of present knowledge and theories of radiation damage is presented Consequently, the book is immediately useful to the specialist concerned with the day-to-day handling of these problems rather than to the general reader. In due time it will be frequently used to discover what was thought about these subjects in 1961.

The picture is one of great activity and some confusion. The methods of calculating or measuring the neutron fluxes within a reactor are not yet finalized, so that it is still better to state that a sample was exposed for a given time at a certain point in a certain pile, with an estimate of the flux, than simply to give a figure for the total flux. A variety of direct methods for observing the effects of radiation damage have been developed, but often only the results of a few preliminary observations were available, showing that the method has promise. It is, however, apparent that the electron microscopy of thin films is a powerful method, and that much is to be gained by its use for observations of specimens systematically exposed to accurately measured and closely defined radiations.

The important practical problems included the structural changes and loss of strength in irradiated graphite and the release of the stored energy on heating, the hardening and changes of creep and fatigue strength in canning materials, the possibility of radiation increasing the chemical reactivity of graphite towards the constituents of cooling gases, the swelling and embrittlement of boron-containing control rods, and the control of swelling and distortion in uranium and uranium alloy fuel elements. The embrittlement of ferritic pressure vessel steels did not receive as much attention as might have been expected, but there was some discussion of the relation between twinning and the initiation of cleavage fracture in iron. A number of papers aimed at the elucidation of the physical processes associated with radiation damage but experiment yielded enough incalculable results to show that the processes are not yet understood, and that theory must for some time depend on the guidance of experiment.

Most of the papers were highly condensed, and some of them were written in a breathless shorthand, unnecessarily difficult for the reader. The authors of these papers would do well to study the Russian contributions, which were generally models of courteous and lucid English. N. P. Allen

POLITICS OF AGRICULTURE

The State and the Farmer

By Peter Self and Prof. Herbert J. Storing. Pp. 251. (London: George Allen and Unwin, Ltd., 1962.) 30s. net.

The Economics of Subsidising Agriculture

A Study of British Policy. By Gavin McCrone. (University of Glasgow Social and Economic Studies, New Series, No. 1.) Pp. 189. (London: George Allen and Unwin, Ltd., 1962.) 25s. net.

POLITICS 'contains' economics in the same sense that medicine contains pharmacology, or agriculture contains veterinary science; each of the latter sciences constitutes a legitimate branch of knowledge in its own right; but the actual application of the conclusions reached by these sciences can only be made by the legislator, the physician and the farmer, respectively (together with the scientists who advise him). These latter are synthesizers, who have to take into account the evidence from a variety of contributory sciences before they reach their conclusions.

So the economist must not claim priority; and it i philosophically correct to regard agricultural economics as a contributor of only part of the knowledg required for the solution of the problems of agricultural politics, the subject-matter of *The State and th Farmer*.

Peter Self, lecturer in political science at th London School of Economics, has mainly made hi name as a far-seeing writer on the acute problems o town planning and urban expansion; in this book collaborating with an American professor, he enter quite a new field. The book covers the subject mos comprehensively; I have searched diligently for th smallest inaccuracy, without success; the authors g out of their way to be scrupulously fair to ever party concerned; and all this is done without an sacrifice of the ease with which this book can b read, a most unusual feat in writing. My only complaint is that the study might have gone back a littl farther into history to mention Lloyd George's *Green Book* of 1925, which proposed all the ideas—security of tenure, low rents, and guaranteed prices—which were to become the policy of both the Conservativ and Labour Parties in the 'forties.

The essence of the book is a fascinating and rather alarming account of the extraordinary skill with which the National Farmers' Union was organized—"losing battles to win campaigns" as a political pressure group, to obtain such large concessions from a predominantly urban parliament. Governments of both parties were afraid of an open breach with so well-organized a minority.

In return for all these concessions, the Government took power to expel farmers judged incompetent from their farms and homes. The National Farmers' Union had no qualms about the Englishman's home no longer being his castle, and indeed protested when the Government repealed these powers in 1958. Attempts to exercise these powers had in fact been few and half-hearted. However, the decision to repeal them, the authors think, must be attributed to the hostile publicity so brilliantly planned by a small group of Liberal farmers, the Farmers' and Small Holders' Association.

The authors are moderately critical of the administration of these Acts by the Civil Service (as in the Crichel Down case), and by War Agricultural Committees, in which 'muddle' and 'gossip' too often took the place of efficiency and justice. However, they defend the Government, and criticize the Farmers' and Small Holders' Association, over the notorious case of the expulsion of Lady Garbett.

The National Farmers' Union, as *The State and the Farmer* points out, has always been impatient of political claims based on the preservation of farming as a way of life for those deeply attached to it, and of the beauty of the English countryside—though it is here, in fact, that the real strength of the Union's claims lies. It has always contended that its case was strictly economic, namely, the improvement of Britain's balance of payments by replacing imports. McCrone meets this claim head on. He argues the case in technical detail, but his central point, which is valuable and important, is that the imports displaced have been mainly from the Commonwealth (including some poor sugar-producing communities) and Denmark, all very good customers for British manufactures. Their economic situation requires that, when Britain buys less from them, they must, on the whole, react by buying less from Britain. Indeed, they go out of their way to foster new manufacturing capacity in order to make sure that they buy still less from Britain in the future. Meanwhile, the expansion of high-cost production of milk and pork in Britain has greatly increased imports of fodder grains, most of which come from the dollar area. Much of the grain fed to dairy cows, it appears, is wasted, but the author evades discussion of this question.

The author did some original calculations, comparing the price support received by farmers in European countries measured as a percentage of their gross sales, with Britain third on the list at 24 per cent (only Switzerland and Finland are higher), Germany 18 per cent, France 15 per cent, and the other Common Market countries lower again. If we reckon on 'net sales' (that is, excluding imported fodder grains and store cattle) the British proportion would come out very much higher again. Neglect of this point leads McCrone into a really serious error (although a great many others have fallen into the same error, including Government spokesmen) when he claims that British agriculture produces 43 per cent of the country's requirements. Debiting imported fodder and stores, the correct figure for home production as a proportion of all agricultural requirements is only about 25 per cent (a little higher if tropical products and textile fibres are omitted).

He is also wrong when he asks us to "take a look at farm buildings"—after all the tax-aided farm building of the past decade it is now probably true to say that too much money has been spent on farm buildings rather than too little.

Finally, however, the author has the courage to make himself unpopular by challenging the principle of security of tenure. Was it not precisely the lack of this security, he asks, which provoked so many important agricultural improvements in the eighteenth and nineteenth centuries? COLIN CLARK

FOOD SCIENCE

Recent Advances in Food Science

Papers read at the Residential Summer Course, Glasgow, September 1960. Edited by Prof. John Hawthorn and Jas. Muil Leitch. Vol. 1: Commodities. Pp. xv+284+xvi. Vol. 2: Processing. Pp. xv+317+xvi. (London: Butterworth and Co. (Publishers), Ltd., 1962.) 70*s.* each volume.

THE acceptance of the term 'food science' effectively dates from the publication in 1951 of the papers presented to the short courses in food science held by the Low Temperature Research Station in Cambridge. The volumes under review result from a residential course on recent advances in food science held at the Royal College of Science and Technology in Glasgow in 1960.

The 54 papers are separated into two volumes entitled *Commodities* and *Processing* respectively. Volume 1 is divided into three sections, and the first introductory one contains an article on teaching and research on food science in the United States by Prof. E. M. Mrak of the University of California, who acknowledges the great contributions made by British scientists in this field. Three papers follow on food and micro-organisms where, after general considerations by Prof. J. Hawthorn and a review of the effects of environment on the growth of micro-organisms by Prof. E. O. Morris, there is a discussion by Prof. J. W. Howie on the hazards of handling unclean food, that is, food poisoning, where he shows that *Salmonella typhimurium* is, at least in Great Britain, by far the most important disease-producing organism.

Section 2, entitled "Animal Foods", contains 13 papers and deals with such topics as the anatomical distribution of edible tissues (R. W. Pomeroy), adipose tissue and animal lipids (C. H. Lea), the physiology of milk secretion and its relationship to milk composition (J. A. B. Smith), some physical, chemical and structural characteristics of egg shells (C. Tyler), the bacteriological and chemical changes in spoiling fish (J. M. Shewan) and autolytic changes in the lipids of fish flesh (J. A. Lovern).

The final section of Volume 1 is entitled "Vegetable Foods" and consists of papers on polyphenols (E. C. Bate-Smith), wheat proteins (J. Pace), wheat lipids (N. Fisher), malting (I. A. Preece), cereals other than wheat and barley (W. F. Geddes) and fresh fruit and vegetables (J. C. Fidler).

Volume 2 starts with a comprehensive paper on the accelerated freeze-drying process by J. C. Forrest, who lists some of the features worthy of further study which could not be completed owing to the closure of the experimental factory of the Ministry of Agriculture, Fisheries and Food at Aberdeen. The author of the second paper, E. G. B. Gooding, was also prominent in the research programme of the Aberdeen factory; he contributes a discussion on the effects of storage on the quality of dehydrated foods, especially vegetables. Deterioration of dehydrated meat is considered in two further papers; the first, by Dr. Matheson, demonstrates the presence of enzymes after freeze drying, but states that their importance in the rapid deterioration of products above certain moisture-levels has not yet been proved. The second is a consideration of non-enzymatic browning in dried meat, very clearly presented by Dr. J. G. Sharp.

In a section on sterilization and refrigeration, heat sterilization (T. G. Gillespy), sterilized milk (H. Burton and D. J. Jayne-Williams), modern cans (R. K. Sanders) and processing and canning machinery (T. W. Uthwatt) are considered. The preservation of fish at sea both by chilling and freezing is discussed by G. C. Eddie with considerable attention to its engineering and economic aspects. This is followed by a general consideration of quick freezing of fruits and vegetables (W. B. Adam) and an extremely interesting paper on pre-cooked frozen foods (J. H. Hulse) especially with regard to present practice in North America. This is a field in which the author predicted the great expansion that is now taking place in Britain.

The remainder of the volume contains a group of papers on such varied topics as cheese starters (R. J. M. Crawford), egg preservation (N. R. Knowles), new developments in milling processes (C. R. Jones), continuous baking processes (J. J. Devlin and N. Chamberlain), sugar preserves (Mamie Olliver) and the bacteriology of meat-curing brines (M. Ingram).

It is of interest that after all the great hopes expressed for radiation preservation of food only a few years ago there is not a single paper devoted to this subject; however, the subject is referred to in the text by three authors.

The volumes can be thoroughly recommended to all interested in recent developments in food science as being the most up-to-date collection of information available on a wide series of topics in this rapidly developing field. P. L. PELLETT

WILLIAM HENRY BRAGG AND THE NEW CRYSTALLOGRAPHY*

By Prof. P. P. EWALD, F.R.S.

President of the International Union of Crystallography

THE year 1962 has a double significance in crystallography. It is fifty years since Max von Laue's discovery of the diffraction of X-rays in crystals, the basic venture that detected the port of entry to the New Crystallography, and it is a hundred years since the birth of William Henry Bragg, the older of the two pioneers who together were the first to find the path to the riches of the untrodden land lying behind the coast. It follows by simple arithmetic that William Henry Bragg was fifty years of age when he began the work that makes his and his son's name famous as long as our present science will persist. This is, in itself, an unusually high age for discoveries of prime importance in physics, but even more remarkable is the continuous hold of leadership in the new research which W. H. as well as W. L. Bragg have maintained—Sir William up to the last months before his death at the age of eighty, and Sir Lawrence, fortunately, to this day, and, we hope, long beyond.

The title of the lecture makes mention only of William Henry Bragg, because he is distinguished, this year, by the centenary of his birthday. Yet, so far as the work on the new crystallography is concerned, the contributions of father and son are interlinked to such an extent, especially in the early years, that it would be an invidious attempt to single out one or the other.

Let us now go back a long stretch of time in order adequately to describe the setting of the early and most dramatic work of the Braggs in the tension-loaded years of 1912–14. It is hard for the present generation of scientists, be they advanced or still budding, to keep in mind how many facts which now belong to our well-established picture of the physical world were either quite unknown or very hypothetical in 1912, the year of Laue's experiment. The atom, far from being represented by the simple and well-established present model, was still a nebulous entity in 1912 about which nothing decisive was known. Rutherford's model, it is true, had been announced the year before as an alternative to J. J. Thomson's entirely different conception of the atom, but it had yet to be established on more evidence than that of the scattering effect on α-particles. No convincing quantitative connexion to the mysterious Rydberg number had yet been found—this amazing feat was to occur only in the next year, 1913. The quantum of action, represented by Planck's constant *h*, had made its first appearance in Planck's theory of black-body radiation in 1900, but up to 1912 it remained an *ad hoc* hypothesis which most of the physicists who were steeped in classical physics regarded as a temporary impasse which might soon be solved by the invention of a better model.

There existed, thanks to Clausius, Maxwell and Boltzmann, a fairly satisfactory model of matter in the gas state, but for the more individualistic aggregations, solid and liquid, scarcely anything was known. We can perhaps best visualize this vagueness of knowledge by considering how little detail is known even to-day of the liquid state: one is still at work trying to find out what are the fundamental features of the structure of a liquid which determine its physical properties. It was the same with crystals before 1912. An elaborate theory of crystal structure had grown from the very beginnings of quantitative measurement of crystals about 1781 and had, somewhat more than a hundred years later, led to a highly perfected, complete, but rather abstract theory of space groups which has now found its right place, but which at the time we are speaking of was void of any but the most formal connexion to the problems of solid matter. No physicist, no crystallographer was able to assign correctly to a given crystal one, or even a small selection, of the 230 possibilities of arrangement which this theory predicted, and it was left wide open for discussion what was the nature of the particles that were arranged in the three-dimensionally periodic fashions which the theory allowed: individual atoms, molecules, characteristic little crystal fragments?

The attempt to use the forerunner of the complete theory, the mere notion of a simple periodic arrangement of molecules exerting central forces on one another, had led the eminent mathematicians Cauchy, Navier, Poisson in the 1820–30's to results concerning the elastic behaviour of such solid matter which were not confirmed by experiment, and this failure discredited the entire concept of internal regularity of crystals. Besides, before the invention of the powder diffraction method by Debye and Scherrer and by Hull about 1916 crystals were regarded rather as oddities of Nature than as the typical state of true solid matter of everyday life. In the first few years of X-ray diffraction anyone who was interested in this new method combed the mineralogical collections for suitable crystals having faces of at least 5 × 5 mm. or thereabouts, and it was only in the mid-'twenties that the advantages of using much smaller crystals were found out.

If, then, in 1912 the nature of the solid state was still wide open to speculation, so was the entirely different problem of the nature of X-rays. These rays, discovered in 1895 by W. C. Röntgen in Würzburg, continued to retain much of the mystery that enshrouded them from their first beginnings. Rarely has the discovery of a physicist aroused such a storm of public reaction as that of Röntgen's unknown rays, the X-rays. Röntgen was a character who hated to part with any unfinished experiment by publishing it. He made the chance observation which led him to X-rays on November 8 and, working feverishly in the next six weeks, found nearly all the properties of X-rays that were to be known within the next ten years before he sent his *First Communication* to press on December 28, 1895. The news of these uncanny rays with which you could see the bones in the quick made headlines and gave rise to comments and cartoons in all countries Physicists and medicals alike tried in vain to find out more about the properties of these rays than Rontgen had indicated in his 10-page pamphlet which he sent out to his friends as a New Year's gift

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* Substance of the first Bragg Lecture delivered at the University of Leeds on June 7 and at the Royal Institution, London, on June 13.

Within a year of Rontgen's discovery three theoreticians gave the same explanation of the nature of X-rays Stokes in Manchester, Liénard in Paris and Wiechert in Konigsberg Their idea was that the electrons, while travelling from the cathode to the anode, form an element of current and are surrounded by a mixed electric and magnetic field On hitting the target they are suddenly brought to rest, and thenceforth produce only an electrostatic field. The sudden transition from one kind of field to the other produces a kink in the lines of force which travels away in all directions like any disturbance caused by the acceleration (or deceleration) of a charge According to this view, the single X-ray is a pulse in the ether similar to the pulse in air caused by a ball of shot landing on its target The emission of the X-ray field could be calculated by Maxwell's equations, and it could be seen that the field would be concentrated on or near to a cone having as axis the direction of deceleration

Among the physicists who carried out calculations along this line of thought was Arnold Sommerfeld, then professor of theoretical physics in the University of Munich He further discussed the faint indications of diffraction which first the Dutch Haga and Wind, and later, in a perfected manner, Walter and Pohl in Hamburg had obtained by using a wedge-shaped, very carefully worked and adjusted slit The intensity profiles on the photographic plates showed not the clear fringe systems obtainable with monochromatic light, but they could be interpreted as the diffraction of a pulse of width of the order of 0 4 Å This order of magnitude was the same as that predicted several years earlier by Willy Wien, who reversed the argument by which Einstein, in one of his four famous papers of 1905, had explained the photoelectric effect In Wien's argument the energy E of an electron falling through the voltage V applied between the cathode and the target of an X-ray tube is given as $E = e\mathrm{V}$, where e is the electron charge This energy is found again in the X-ray photon of frequency $\nu = c/\lambda$, according to the conversion formula between energy and frequency $E = h\nu$, where h is Planck's constant Looking back, Wien's argument was excellent, and we have by now become accustomed to the application of such formulæ in the production of radiation which are not backed by explanatory mechanism, but, at the time we are considering, Maxwell's theory still reigned supreme, and from that point of view these arguments were just black magic which could not be trusted This argument, which predicted an unchecked wave-length from a not understandable formalism, had little effect

On the other hand, there were substantial reasons for doubting the wave-nature of X-rays, and W H Bragg was one of those who felt them most strongly He came to this field, not from the classical Maxwellian theory, but from the study of radioactivity, which he had entered on in 1904, when it was quite a young and most disquieting branch of physics and chemistry Bragg was then professor of physics in the University of Adelaide, and this work was his first research work after seventeen years devoted nearly exclusively to teaching and organizing the University Important results appeared at once from the research The dependence of the ionizing power of α-rays on their velocity, their finite range and the statistical straggling of its length these were results of fundamental importance which quickly made W. H Bragg's name well known He was called to Leeds in 1908, organized there the physics teaching laboratory, and as soon as this was done continued the line of research started in Adelaide The fact that not the intensity but the 'hardness' of γ-rays, measured by their penetrating power, determined the velocity of the secondary β-rays created by them, struck Bragg as a fact of utmost importance In conjunction with H J Porter he investigated whether the same was true for the creation of secondary β-rays by X-rays that is, for the emission of fast photoelectrons by the absorption of X-rays He showed it to be the case, and explained the ionization produced by X-rays to be mainly an indirect one, the energy of the X-ray being used, in the absorption process, for the creation of the fast photoelectron which then, on colliding with the surrounding matter, knocks off further electrons and thereby ionizes the medium

In the primary process, the absorption of the X-ray, a large energy is suddenly transferred to the photoelectron, a fact impossible to understand if the X-ray, like the classical light-wave, conveyed a widely distributed energy flux Such a sudden, localized energy transfer is characteristic of collisions between particles, and therefore was a strong argument for attributing to X-rays a similar nature to that of cathode rays, namely, to consider them as a stream of fast particles. These, however, had to be neutral, since there is no deflexion by electric or magnetic fields This view, which accounted well for all observations implying the photelectric effect and absorption, was put forward by W H Bragg in his book *Studies in Radioactivity*, which appeared early in 1912

Then came, in this very year, Laue's discovery of the diffraction of X-rays, which was conceived out of the opposite assumption, namely, the wave-nature of X-rays It solved the old conundrum of X-rays in a seemingly irrefutable fashion, confirming the wave nature and rejecting the particle assumption The experimental evidence was so strong and simple that it could not be doubted Anyone who has formulated a theory of his own and assembled a large amount of evidence in its favour will appreciate the blow it must have been to Bragg to renounce his theory and to accept the opposing facts Only in one letter to *Nature*, the first of a whole chain of letters by the Braggs, is a statement made which can be interpreted as an attempt to salvage the corpuscular theory, even though this is not mentioned by name It is based on the remark which W L Bragg made to his father during the analysis of the famous diagram of zincblende which formed part of the Laue–Friedrich–Knipping publication, to the effect that all the directions of the secondary X-rays leaving the crystal are 'rational' directions, or 'avenues' between the crystal atoms It is clear that if this were a general property of the Laue diagrams it would lend itself to the interpretation that these 'avenues' are the paths of least resistance to the passage of a stream of corpuscles It was not clear at the time that the statement held only for particular conditions of incidence

Another great physicist, who also came from studies of ionization and was in search of an alternative explanation of the Laue diagrams, Johannes Stark, made a very similar attempt to account for the directions of diffraction by the assumption that 'photons' were channelled in the crystal along the avenues between the atoms or crystal pits (*Kristallschachte*), as he called them.

This letter to *Nature* is dated October 18, 1912. It came at the end of attempts made by W. L. Bragg during the preceding months to test the 'avenue' hypothesis experimentally. They came to nothing, and so were not published; but Sir Lawrence has recently described to me how he went about them. Imagine a crystal slab and a photographic plate fastened to the front and back side of a flat box respectively. The box was tilted in all directions during the exposure, so that X-rays fell on the crystal within a certain solid angle. If they were conducted preferentially along certain avenues of the crystal structure, then, since crystal and plate did not change their relative position during exposure, one would expect strong blackening on the plate where the directions of the avenues intersected it. This experiment was done—like that of Laue–Friedrich–Knipping—with 'white' X-rays, and it showed no effect. Had it been done with a strong monochromatic component in the incident X-ray spectrum W. L. Bragg should have obtained a 'wide-angle' diagram which, though disproving the point it was intended to prove, would have led to an entirely different experimental approach to the whole subject.

There is one last reference which shows that it was not an easy decision for both father and son fully to abandon the particle hypothesis. This is the title of W. L. Bragg's first publication, a paper read to the Cambridge Philosophical Society on November 11, 1912. It is the famous paper in which the spots of the Laue diagrams are explained as reflexions of the incident X-ray beam on the internal atomic planes of the crystal. X-rays are here treated as light waves, full use being made of their destructive interference so characteristic of wave-motion. But, as Sir Lawrence himself notes in 1942 in his address to the first Conference on X-ray Analysis in Industry: "I was careful to call my paper on the structure of zincblende 'The diffraction of short electromagnetic waves by a crystal', because I was still unwilling to relinquish my father's view that the X-rays were particles, I thought they might possibly be particles accompanied by waves".

Although no further mention of the corpuscular theory of X-rays appears in the Bragg publications of the next decades it would certainly be an error to assume that it was entirely eliminated from their minds. Laue, so far as I know, never did any work on ionization and photoelectric processes. He knew of them, of course, but he also knew that all attempts at explaining the photoemission of electrons on classical lines had ended in failure. He could see that right at his doorstep. The photoeffect was one of the major topics of the second Solvay Conference in Brussels in 1913, and Sommerfeld and Debye had prepared a report showing that it would take a resonator a long period of time to accumulate by resonance to an incident spectral line as much energy as is found in the kinetic energy of the ejected electron—whereas no delay in the emission was observed. On the other hand, Laue also knew, of course, of the successful application by Einstein of the quantum relation $h\nu$ = energy to the photoeffect, which, though not giving a satisfactory explanation, rendered the facts. So I presume that Laue was resigned to accepting the corpuscular side of X-rays as one of the many still mysterious quantum phenomena, for the understanding of which the time was not yet ripe. His personal attachment to this side of X-rays was less strong than that of Sir William Bragg.

It is obvious to-day that γ-rays and X-rays presented in the most evident way the general dilemma which later went under the name of particle-wave aspect and gave rise to much fundamental thought, including the principle of indeterminacy of Heisenberg, Bohr's principle of complementarity, and de Broglie–Schrodinger's wave-mechanics.

Such was the expansion of new territory that lay open to investigation along the lines of purely classical optics that even Sir William Bragg did not return to the quantum effects of X-rays. He left the discovery and exploration of Auger effects, Compton effect and atomic and crystalline energy-levels to others.

At the time of Laue's experiment the commercially available medical X-ray tubes usually had a platinum target. Tungsten was only just becoming available, through Langmuir's work, in malleable and workable form, and the Coolidge type fully evacuated X-ray tube, which depended on tungsten, dates only from 1913. Tubes with other targets had to be made to order, and often did not last very long because they could not be sufficiently outgassed. From the work of Barkla in Edinburgh it was known that under the condition of running tubes at rather low voltages there were homogeneous components in the emitted X-radiation, recognizable by the fact that this part of the radiation did not get harder by traversing a number of aluminium sheets, and that its hardness depended only on the target material and not on the applied voltage which affected the remaining part of the emission. There was a definite regular increase discernible in the hardness of these homogeneous components or 'characteristic rays' as the atomic weight of the targets was increased, and this led Barkla to distinguish between a K-series, observed on the lighter targets, and an L-series coming from the heavier ones. Barkla's discovery of the characteristic X-rays together with his second discovery, that of the polarization of X-rays, were the two, and only two, notable advances in the knowledge of X-rays since Rontgen's first nearly exhaustive investigation. It was assumed that the characteristic X-rays were comparable with the line spectra emitted by elements in the optical region, and that the non-characteristic part of the radiation represented the result of the sudden stoppage of the cathode ray electrons on the target, according to the Stokes–Liénard–Wiechert idea. But it must be remembered that the 'kind' of X-rays could at that time be characterized only by measuring the absorption coefficient, and that this means of discrimination had a very low resolving power. The comparison of the characteristic radiation to an optical line spectrum was therefore a conjecture rather than the actual demonstration of the existence of sharp lines.

Laue and Friedrich had planned their experiment so as to obtain a strong secondary radiation. It was known to Friedrich from Barkla's work that the radiation from a commercial tube with platinum target, run in the then usual way with an induction coil and a parallel sparkgap, would excite strongly the K-radiation of elements of atomic weight between 50 and 100. This accounts for Friedrich choosing a copper sulphate crystal in his first attempt, and then ordering the famous zincblende crystal from Steeg and Reuter with which the final photographs were taken that were reproduced in the original publication Laue formulated the theory of diffraction by a three-dimensional grating after the experimental proof of diffraction had been given In applying it to the zincblende diagram, he found that each spot was formed by a definite wave-length λ (or its overtones λ/2, λ/3), different for different spots Since at that time monochromatic X-rays were thought of only in connexion with characteristic radiation, Laue attributed the diffracted rays to a characteristic radiation of the crystal This was not at all in keeping with his own theory, but a misconception which lasted for quite some time, so that when the original publication was reprinted in *Annalen der Physik* this point was reiterated and enlarged on in March 1913 in one of three notes added to the reprinting that is, at a time when W L Bragg had fully explained the pass-filter action of the crystal

Let us, in fact, return to W L Bragg's first paper in the *Proceedings of the Cambridge Philosophical Society*, November 1912 The three important advances made in this paper are

(1) *Reflexion/Diffraction* The spots on the Laue diagram of zincblende are explained as reflexions of the incident beam on internal atomic net planes of the crystal This idea also explains the shapes of the spots—in fact, it was originally deduced from a consideration of these shapes

(2) *Continuous spectrum/Characteristic rays* The appearance of sharply defined wave-lengths in the spots is explained by the selective action of the crystal, which passes only certain wave-lengths out of an incident continuous spectrum

(3) *Face-centred/Simple cubic lattice* It is shown that certain reflexions, the absence of which had already been noted by Laue, should indeed not be observed if the lattice of zinc sulphide was a face-centred cubic lattice instead of the simple cubic one assumed in Laue's discussion

As to the first of these points, W L Bragg states that he sees no contradiction in the reflexion statement to Laue's statement that the spots are caused by diffraction—though his reasons are not elaborated on

Regarding point (2), Bragg assumed the incident radiation to be a 'pulse', that is, not to be of a periodic nature The periodicity in the secondary beam is created by the regularly repeated reflexions of the pulse on the successive net planes This creation of a monochromatic ray has nothing to do with the excitation of characteristic radiation, but it is intimately connected to the topics of the resolving power of a grating and to the concept of coherence of a ray Now Laue had started his career as assistant to Planck, with important papers touching closely on these very topics The notion of the creation of periodicity by the action of a grating should have been alive in his mind I believe it is first to be found in Lord Rayleigh's papers, many of which Laue was familiar with But it was most forcefully explained in Sir Arthur Schuster's *Optics*, a book that was probably unknown to Laue, whose knowledge of English was poor On the other hand, this book formed the base for the teaching of optics in Cambridge by C T R Wilson, and its influence as a background on W L Bragg's argument is patent

The third advance made in Bragg's paper, the determination of the face-centred cubic lattice for zincblende, was a brilliant discovery which could not have been made without the second one For the interpretation of absent reflexions on a diagram taken with a standing crystal presupposes that—within certain limits—all wave-lengths are contained in the incident radiation, that is, that the incident radiation consists of pulses That Bragg was familiar with Pope's arrangements of packing of spheres, including as it does the face-centred cubic lattice, was a help, but without the correct view of the pass-filter action of the lattice it might not have led anywhere It is interesting to note in this connexion that Laue, in one of the three notes added to the reprinting of his original paper (*Ann der Phys*, Leipzig, March 1913), develops the theory of the structure amplitude of a lattice of several atoms, the discussion of which would have given him the correct explanation for the missing reflexions had he not been mesmerized by the idea of "characteristic radiation"

After W L Bragg had presented this brilliant paper to the meeting of the Cambridge Philosophical Society, C T R Wilson suggested in the discussion that since internal crystal planes reflected X-rays, so might external faces do provided they were smooth enough This prompted Bragg to try reflecting the X-rays on a strip of split mica, with the gratifying result of obtaining a reflected image after a very short exposure, and at the expense of a burnt-out platinum contact on the hammer-break of the induction coil This experiment was reported in a letter to *Nature* dated December 8, 1912

It seems justified to regard W L Bragg's paper and the subsequent experimental verification of the reflexion idea as the starting point of a new development leading to the two main fields of application of Laue's discovery X-ray spectroscopy and X-ray crystal analysis In *Nature* of January 23, 1913, W H Bragg reports that the reflected rays which his son registered photographically could also be detected by their ionization This must have been the preliminary experiment on the result of which the construction of the X-ray spectrometer was based· this most powerful instrument with which practically all progress was achieved in 1913 and 1914 and the early post-War years It was an instrument which could be constructed rapidly by transforming an optical spectroscope, and its efficiency was due to the well-placed system of slits at the tube and at the entrance to the ionization chamber, to the sensitive ionization chamber, and the avoidance of variations of capacity by placing the electrometer by means of which the ionization current was measured under the axle of the spectrometer about which the chamber was being turned It embodies much of the experience W H Bragg had gained in the course of his previous work on ionization by α and other rays

The spectrometer revealed for the first time the correctness of the assumption that the radiation coming from the tube contains a mixture of 'white' X-rays or Bremsstrahlung pulses and of nearly monochromatic radiation The resolving power of the slit system was not at first good enough to separate sharply the spectral lines—but this was, perhaps, very fortunate, for Moseley and Darwin, who had started similar work independently and only a little later in Manchester, using fine slits, missed the characteristic lines altogether before they knew from Bragg's work where to look for them

While W H Bragg perfected the spectrometer, his son in Cambridge succeeded in making the first structure determinations by comparing the Laue photographs of various alkali halides These first structure determinations were of fundamental importance. They solved the mystery of what was the nature of the primary particles assumed but not defined in the structure theories. They laid down the absolute scale in which atomic distances and X-ray wave-lengths could be measured. They used, as a criterion of the correctness of the assessment of atomic sites, the idea of the completeness of the diagram, that is, the notion that, within certain limitation of angular and spectral range, the diffracted spots that could be expected were actually there, no more and no less.

Using this rock-salt structure and the reflexion angles measured on the spectrometer, W. H. Bragg determined the absolute values of the wave-lengths of the L-radiations of platinum, nickel and tungsten. He had to show that the peaks found on the spectrometer were indeed those of characteristic radiation. By measuring the absorption coefficient he identified the radiation according to Barkla as either K-radiation from an element of atomic weight 74, or L-radiation from atoms of weight 198. The latter is in good agreement with the platinum target (at. wt., 195). Again, according to Whiddington, the production of rays of the measured absorption coefficient requires electrons of a certain minimum energy, and this value fell close enough to that obtained from the wave-length by means of the equivalence $E = h\nu$. It is interesting to note this roundabout argument, the only one then available; its importance lies in the fact that by confirming the absolute value of the wave-length obtained via the sodium chloride structure, this structure itself, which was derived only with the help of λ/a ratios, was also confirmed. It thus became certain that the nodal points of the structure really carried atoms, and not sodium chloride molecules as might have been expected.

The further development of the work of Sir William Bragg and his son is better known than these quite early stages, and I need mention only a few points which appear to me to be of particular importance.

(1) The exploration of X-ray spectroscopy, although begun by W. H. Bragg, was soon taken over systematically by Moseley in Manchester and Oxford and Maurice de Broglie in Paris. Both found the photographic registration of spectra more convenient and more accurate for angular measurement than the ionization method. This work was of the greatest importance for the then developing atomic theory.

(2) The determination of the structure of diamond in 1913 was an important step forward because of the simplicity and beauty of the structure, and because it was the first structure which found the undivided approval of physicists as well as chemists. Prof. Bragg was to report on this structure at the Birmingham meeting of the British Association for the Advancement of Science of that year, and I had seen the announcement of the talk in *Nature* (I was then living in Munich and a few months married). The announcement prompted me to throw overboard all plans for a honeymoon on the Bay of Biscay and to follow the inner call to Birmingham. Neither my wife nor I has ever regretted this, for it was a fine meeting, with Lord and Lady Rayleigh receiving the guests at the reception and Oliver Lodge delivering the presidential address, and Marie Curie, the "silent woman, rushing off to France", as the disappointed reporters put it, getting an honorary degree. Bragg's talk, with the wonderful result and the beautiful directness and simplicity of the argument, made a deep impression on all. After the talk, a crowd of colleagues and friends gathered round the speaker congratulating him and asking questions. When they were gone I approached him—quite unknown to him and a young man who might have been his son—and after a few words of admiration told him that I disagreed with his calling the first reflexion observed on the octahedral face a first-order spectrum while also calling the first observed reflexion on the cube face a second-order spectrum. The latter should be considered as a fourth-order if referred to the same cubic axes. I am telling this because I think that Sir William's reaction was that characteristic of a truly great and liberal mind. Instead of being convinced of his better understanding, or being annoyed, he thought for a few seconds and simply said "Yes, you may be right. I see your point".

(3) In the third instance I would like to mention the 'normal intensity sequence' in the successive orders of reflexion obtained from a set of equal equidistant net planes. The theory of intensities was not well developed until well into the late 1920's, when W. L. Bragg and his co-workers in Manchester advanced it to a useful stage. On the other hand, as soon as any information is needed on the mutual arrangement of atoms in the basic cell of the crystal this can only be obtained by a discussion of the intensities of the reflected rays. It would have held up crystal structure analysis for 10–15 years if one had wanted to wait for an applicable theory of intensities—which in fact remains even to-day one of the major problems. W. H. Bragg circumvented this difficulty by introducing a normal intensity sequence of 100 : 20 : 7 : 3 : 1. Where this was not observed, it was an indication of unequal distances and unequal atomic populations of planes within the set of parallel planes of atoms. This approximate rule, gathered from the great wealth of measurements made, was most helpful for overcoming the formidable difficulties in interpreting the measurements in terms of structure.

Although W. H. Bragg started out as a mathematician—he was third wrangler in the Mathematical Tripos Part I in 1884 and prepared himself for his double role of professor of mathematics and physics in Adelaide only on his way out in 1886 by reading up on electricity and magnetism—he became fully convinced of the strength of simple reasoning in physical terms, and for his own use discarded as much as possible mathematical formalism. His determinations of crystal structures like those of diamond, quartz, or anthracene–naphthalene are based on the simplest of geometrical arguments, and this makes the reading of these papers or of their condensation in the classic volume *X-rays and Crystal Structure* so instructive and delightful. The method of deduction followed there trains the reader to geometrical perception in three dimensions, an aptitude that can be greatly improved by practice and which both Sir William and Sir Lawrence possessed or developed to a remarkable degree. It makes the results appear to be gained in a nearly effortless way, and when one listened to Sir William lecturing on his work he seemed to live through the joy of discovery and of the clinching argument every time afresh. He was, and will remain, one of the great masters of scientific exposition, and this art he may well have acquired during his first, non-productive period at the University of Adelaide.

This direct approach also explains Sir William's attitude towards the formal theory of space groups. As a trained mathematician he might have seen its general importance, but as one converted to direct physical argument he had no use for this formalism. Shoji Nishikawa, who was one of the earliest to appreciate the help to be obtained from space-group theory, on visiting Sir William in 1919, handed him a paper he had written on this subject asking him to publish it if he thought it was meaningful—but it never again saw the light of day. About 1923 Bernal had worked through space-group theory on his own—but this attempt was not encouraged. When, not much later, Astbury and Yardley had worked out their space-group tables it took "some persuasion" to obtain Sir William's backing for the publication.

In his Bakerian Lecture of 1915 Sir William spoke in very clear terms of the connexion between Fourier analysis and diffraction effects. But this remark lay dormant for more than ten years, and the present application of Fourier methods in all parts of diffraction theory sprang from Duane and Havighurst's work in the United States rather than from Bragg's Bakerian Lecture. Again I believe it was too formal an approach to fit into the simple line of thought Sir William meant to cultivate.

Sir William was, as his son is now, fully aware of the responsibility of leadership in scientific thought implied in his position as director of the Royal Institution. He felt strongly the honour and the obligation of working at the little mahogany desk which Faraday had used. Over his many and responsible duties as officer of the Royal Society, the International Union of Pure and Applied Physics and of a large number of special committees, he did not neglect the traditional duties towards the coming generation. His Christmas Lectures on "The World of Sound", "Old Trades and New Knowledge", "The Universe of Light", are wonderfully suited to arouse the interest of children or adolescents in science. I vividly remember the beautifully simple and impressive lecture Sir William gave in 1931 on the occasion of the centenary of Faraday's discovery of electromagnetic induction in the lecture room of the Royal Institution where Faraday himself so often lectured, and with Faraday's original coils and instruments for the demonstrations. I remember, too, the loving pains he gave to the edition of Faraday's diaries with all their little interspersed drawings, and how pleased he was to see this greatest treasure of the Royal Institution safely published. Sir William knew well what he owed to those who worked before him, and he served them in filial piety. It is up to us to render the same service to him, the great man, friend and teacher whose work opened the way for us.

TIME-RESOLVED INTERFEROMETRY OF RUBY LASER EMISSION

By T. P. HUGHES

Research Laboratory, Associated Electrical Industries, Ltd., Aldermaston Court, Aldermaston

IN connexion with the possible use of the ruby laser as a light source in plasma diagnostics[1], and in order to elucidate further the mechanism of laser action, it is necessary to know how the spectrum of the laser emission varies in time. The time-integrated spectrum has been examined by a number of workers[2-5]. This article describes what are believed to be the first observations of the time-resolved spectrum, obtained with a Fabry–Perot interferometer and a rotating mirror camera, and proposes a new explanation of the 'spikes' of laser light intensity.

A cylindrical pink ruby crystal, 5 cm long and 0·6 cm in diameter, cut with its crystal axis at 90° to the axis of the cylinder, was used in the experiments. Its ends, flat to $\lambda/10$ and parallel to 6 sec of arc, were coated with dielectric multi-layer reflectors, one being almost opaque and the other transmitting a few per cent at 6943 Å. The crystal was mounted inside an Associated Electrical Industries helical xenon flash tube (type *FA*26), energized by a condenser bank of capacity 1,200 μF charged to 2·5 kV. Under these conditions, which were kept constant throughout the experiments, laser action began about 400 μsec after the flash tube was triggered and lasted for about 1·4 msec, so the pumping energy was well above the laser threshold. The flash tube and ruby crystal were cooled by a rapid flow of air at room temperature.

The optical arrangements used for most of the work are shown in Fig. 1. The front surface of the laser crystal was 44 cm from the fore slit S_1. In the absence of the etalon E the rotating mirror camera swept an enlarged image of a narrow strip of the laser emission pattern across the photographic plate P. When the etalon was in place, the fringe pattern it produced was focused by the lens L_2 on to the slit S_2, with the vertical diameter of the pattern coincident with the slit centre. Each etalon place had a small wedge angle, disposed so that with the aid of S_1 and the lens L_1 both the principal ghost images were rejected by S_2. The etalon could be set up with the plates separated by 1·00, 3·16 or 10·15 mm, for which the changes in wave-length required to displace a fringe by one order are 2·4, 0·76 and 0·237 Å respectively in the region of 6943 Å.

Each pulse of laser emission consisted of a large number of the well-known 'spikes' of intense light. By rotating the mirror at a speed sufficient to resolve these in time, a series of snapshots of that part of the laser pattern which entered the slit S_1 was produced. The sweep speed required to do this made it impossible to record the whole duration of the laser emission, but with appropriate synchronization any part of it could be examined at will.

By removing S_2, replacing S_1 by a small circular aperture, reversing the order of the telescope lenses

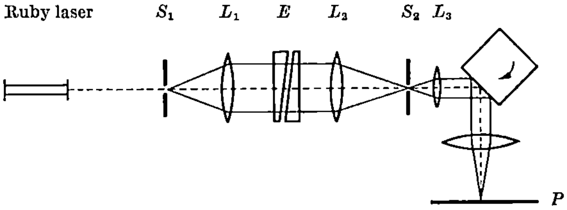

Fig. 1. Optical arrangements

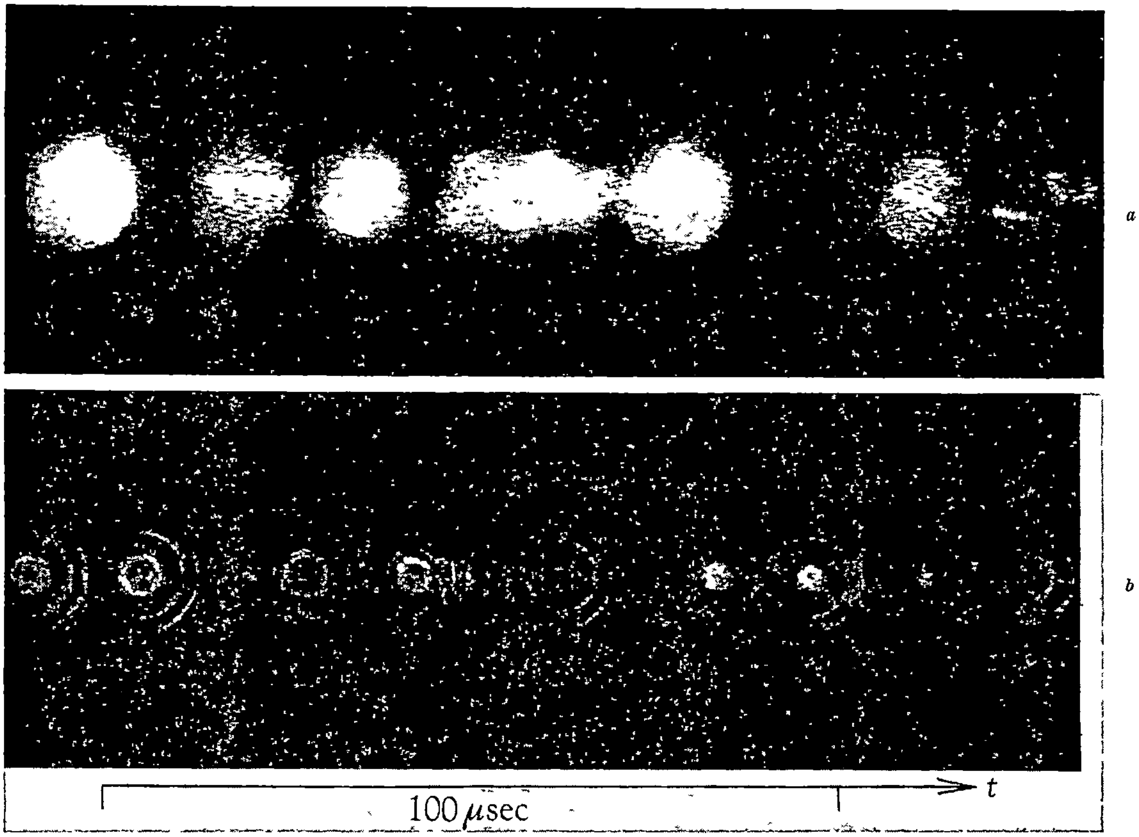

Fig. 2. Streak photographs of whole field, towards end of laser pulse. *a*, without etalon; *b*, with 3.16-mm etalon

L_2 and L_3 and moving the laser closer to the camera, a small image of almost the whole field of the laser emission could be recorded for a sequence of spikes. At a suitable mirror speed it then became possible, towards the end of the laser pulse when the spikes were well separated in time, to record the complete patterns due to successive spikes in considerable detail, often entirely without overlap. An exposure made in this way without the etalon is shown in Fig. 2*a*. It may be seen that, in agreement with the observations of Borie, Durand and Orszag[6] using a framing film camera and of Clark, Wuerker and York[7] using an image convertor camera, elements distributed over the whole of the far field contribute simultaneously to a spike, and the intensity pattern changes (often drastically) from one spike to the next. The very sharp images obtained from most spikes show that in general their duration is less than 0.3 μsec, but some of the patterns appear to persist for 2 μsec or longer. Fig. 2*b* was obtained similarly to Fig. 2*a*, but with the 3.16-mm etalon in position. The wave-length distribution of the emission appears to be the same over the whole pattern in a spike but varies considerably between spikes.

Fig. 3*a* is a streak photograph obtained using the optical arrangements of Fig. 1 without the etalon. The mirror was rotating sufficiently slowly for the whole laser pulse to be recorded; only a few spikes are resolved. Figs. 3*b* and *c* were obtained in that order from successive pulses separated by about 1 min, under the same conditions as Fig. 3*a* but with the 3.16-mm etalon in place. The mean radii of the fringes decreased by about half an order during each pulse, corresponding to an increase in the mean laser wave-length of about 0.4 Å. Taking the value quoted by Maiman[8] for the rate of change of the R_1 line wave-length with temperature (about 0.05 Å °C^{-1} at room temperature) this effect can be explained by a rise in the crystal temperature of about 8° C (observations quoted by Pringsheim[9] indicate that the wave-length increases as the temperature rises). The flash tube emission persists for some msec after the laser action ends, so a further rise in temperature must occur. Only part of the energy absorbed by the crystal will be dissipated in 1 min, so in the next pulse the crystal will be somewhat warmer at each stage and the fringe radii correspondingly smaller, as is observed.

The photographs shown in Figs. 4*a*, *b* and *c* were taken at eight times the speed used for Fig. 3, and only the first part of the laser pulse was recorded. From the streak photograph taken without the etalon (Fig. 4*a*) it may be seen that at the beginning of the laser action only the central region of the field is illuminated. Fig. 4*b* was taken under the same conditions as Fig. 4*a*, but with the 3.16-mm etalon in position. The variations in output wave-length are confined to a range ± 0.4 Å about the mean. This was confirmed by observations with a 1-mm etalon. With a 10.15-mm etalon Fig. 4*c* was obtained, which reveals the nature of the laser light in more detail. The wave-length distribution in a single spike consists of one, or a few, highly monochromatic components. The half-intensity breadth of each component must be $\leqslant$ 0.03 Å. However, the central wave-lengths of the components vary considerably between spikes, and these variations show remarkable regularities. The magnitudes of the wave-length

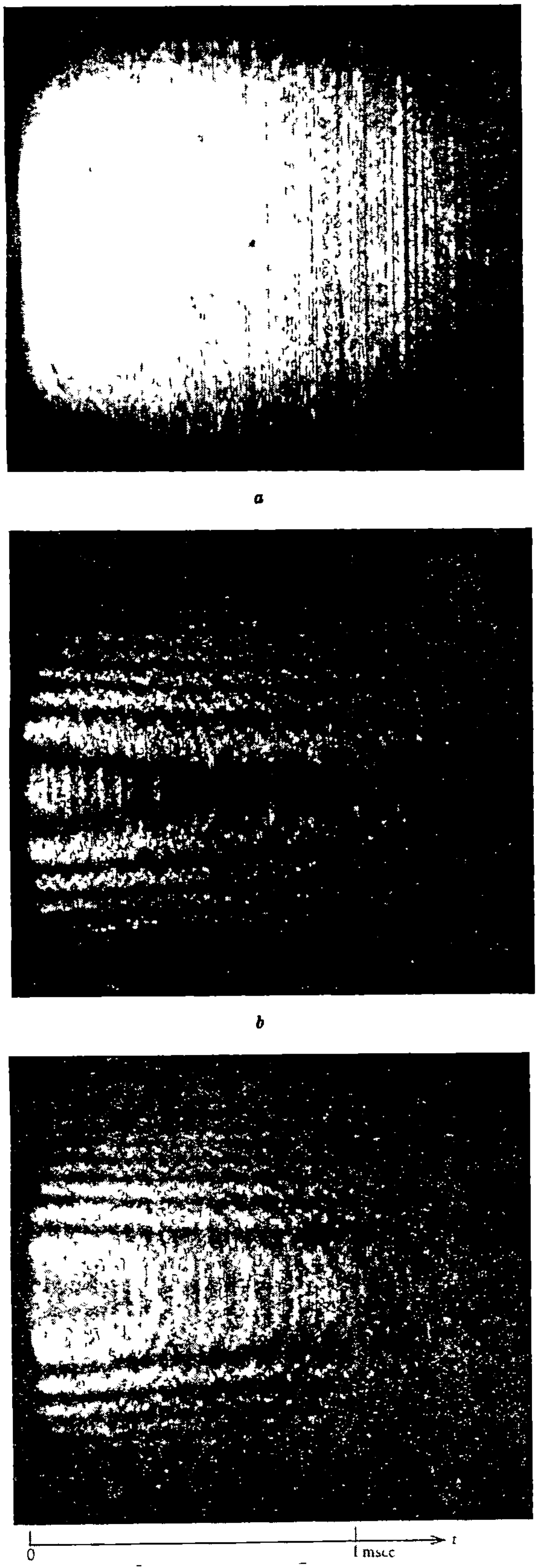

Fig 3 Slow streak photographs of complete laser pulses *a*, without etalon, *b*, *c*, with 3 16-mm etalon two successive pulses

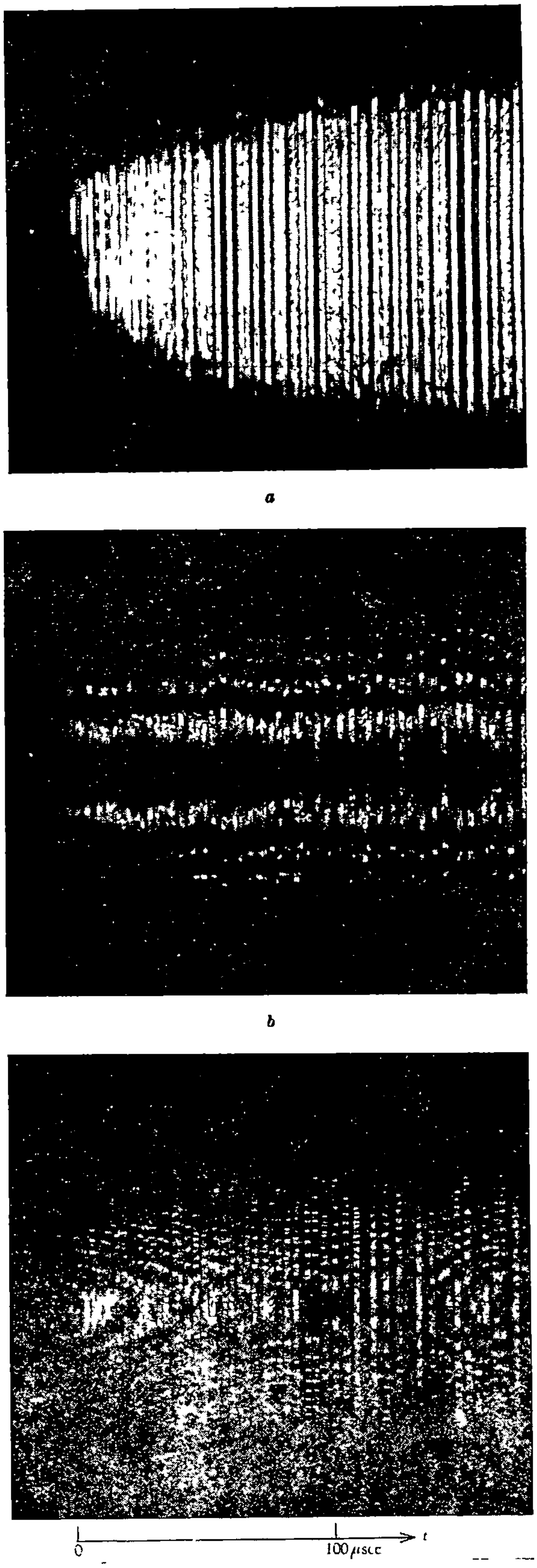

Fig 4 Streak photographs of beginning of laser pulse *a*, without etalon, *b*, with 3 16-mm etalon, *c*, with 10 15-mm etalon

steps very often appear to be 0·025 or 0·05 Å, which are approximately those required to change by one or two the number of wave-lengths in twice the optical length of the ruby crystal. This result is in agreement with the observations of Abella and Townes[4] and of Ciftan *et al.*[5], who deduced from their time-integrated interferograms and spectrograms that the laser resonates in the longitudinal mode at a number of eigenwave-lengths λ near the R_1 line centre, such that $n\lambda = 2D$, where D is the optical length of the crystal, and n is one of a series of adjacent integers. Under the conditions of the present experiment n takes about 30 values in the neighbourhood of 2.5×10^5.

In general, only one or two eigenwave-lengths are excited in any one spike, though occasionally a few more are present. The same eigenwave-length very rarely appears to occur in consecutive spikes. At the beginning of the laser pulse there is a steady step-wise progression towards longer wave-lengths which is reproduced in all exposures obtained under these conditions. If the interpretation given above of Figs. 3*b* and *c* is correct this effect cannot be explained by thermal changes, which are an order of magnitude too slow.

These results suggest the following explanation of the mechanism of spike emission. Although the natural lifetime of the excited state giving rise to the R_1 line is long, the R_1 line profile of the crystal as a whole is broad because different Cr^{+++} ions are subject to different perturbations. This broad profile may be divided into small elements surrounding each eigenwave-length of the laser. Then the condition for laser action to occur at one of these eigenwave-lengths, say λ_i, is that sufficient population inversion has been achieved between the excited state and the ground state in that group of ions for which the R_1 transition lies very close to λ_i. It follows that in the first spike of laser emission only those λ_i's closest to the centre of the R_1 line profile will be present, and only a fraction of the total population of excited ions will be de-excited. The next spike will occur a little later, when those ions associated with eigenwave-lengths slightly lower down the R_1 line profile have been pumped sufficiently, and so on. Supposing the R_1 line profile to be Gaussian for simplicity, with a half-intensity breadth of 2 Å, and neglecting losses, it may be shown that if at constant pumping power the first spike occurs after pumping for t sec, then the twenty most central eigenwave-lengths (with intervals of 0·025 Å) will be emitted within the next $t/30$ sec. Losses will make laser action impossible outside a certain wave-length range, which will be broader at higher pumping powers. However, before these distant eigenwave-lengths have been reached others nearer the R_1 line centre will be active again as a result of further pumping and of relaxation processes. It should be noted that, unless the R_1 line profile is asymmetrical, this model does not explain why early spikes progress towards longer wave-lengths preferentially.

Similar considerations presumably apply to other laser materials and geometries.

By cooling the crystal, and thereby narrowing the fluorescent line profile, the number of eigenwave-lengths and their overall spread will be reduced but their separation will be unchanged. Also, the separation of adjacent eigenwave-lengths is inversely proportional to the length of the crystal. Thus a short cool crystal may possibly operate in a single longitudinal mode, even well above the threshold pumping power. Further experiments designed to test these ideas are in preparation.

I thank Dr. J. Dyson and Dr. A. A. Ware of this Laboratory, and Dr. R. Dunsmuir of the Associated Electrical Industries (Rugby) Research Laboratory, for helpful discussions, and Mr. R. P. de Ste. Croix for assistance with the experiments. I also thank Dr. T. E. Allibone, director of this Laboratory, for permission to publish this article.

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OBITUARIES

Sir John Felton, O.B.E.

SIR JOHN ROBINSON FELTON died at Sutton, Surrey, on June 21 in his eighty-third year. He was sixty-seven when he retired in 1947 after having been for five years H.M. Chief Inspector of Mines and Quarries in the Ministry of Fuel and Power, now the Ministry of Power.

The son of George and Isabella Felton, he was born at Blyth, Northumberland, on February 28, 1880, and educated at Gateshead Higher Grade School, then at Armstrong (now King's) College, Newcastle upon Tyne.

His mining engineering apprenticeship was served at West Stanley Colliery, Stanley, Co. Durham, and Stobswood Colliery, Northumberland. After obtaining his Home Office certificate of qualification he was appointed manager of West Stanley Colliery in 1903. He left in 1908 to begin what was to prove a long and distinguished career in H.M. Inspectorate of Mines and Quarries, then under the Home Office.

Felton served in the South Staffordshire Mines Inspection District for fifteen years until 1923, the first seven as an assistant inspector and eight after being promoted to senior inspector. For a period in 1920–21 he was deputy director of production under the Coal Controller. He was appointed an officer of the Order of the British Empire in 1920.

In 1923 he was transferred as senior inspector to the Yorkshire and North Midland Division. This mines inspection division was split the following year and he was promoted to be the first divisional inspector of the newly formed East Midland Division.

He was promoted to H.M. Deputy Chief Inspector at London Headquarters in 1937, then appointed Chief Inspector in 1942. Knighted in 1946, he retired in 1947. For some time after his retirement he was a member of one of the Valuation Boards under the Coal Industry Nationalization Act.

Felton was an honorary member of the Midland Counties Institute of Mining Engineers. He had been president during 1936–37 and adopted as the subject of his presidential address the "Safety and Health of Mineworkers". He further contributed to the *Transactions of the Institution of Mining Engineers* "Some Notes on a Visit to Germany" and was the author of five special reports on colliery disasters at Bilsthorpe (1934), Markham (1937), Barnsley Main (1942), Manvers Main (1945), and Harrington (or Lowca) in 1947.

His recreations were reading and almost certainly writing, for his penmanship was outstandingly good. He had a deep love of music and of church music in particular. He was organist and choirmaster at Methodist churches at West Stanley and elsewhere, for as he moved about the country he remained until the end a devout and active church member.

Sir John was a man of wide and deep sympathies, greatly interested in the welfare of young people and for many years associated with the National Children's Homes. He was also very much concerned with the welfare of old people and took a leading part in the provision of houses for them in the Sutton district.

His wife died in 1936 and his only child, a daughter, in 1953. In recent years he had been greatly troubled by failing eyesight, but he bore all his misfortunes with fortitude and invariable cheerfulness.

T. A. Rogers

Prof. A. J. Grove

Alfred John Grove, professor emeritus in the University of London, who died on June 5 at the age of seventy-four, held from 1947 until his retirement the newly created chair of zoology at Queen Mary College. Indeed, this appointment was largely a tribute to his work in building up the Department from small beginnings during the years 1929 and onwards.

Grove took his first degree at Birmingham when he was only nineteen and was at once awarded a scholarship to work on entomological problems. A series of papers, mainly on the functional morphology of insect mouth parts, was recognized by the award of the D.Sc. degree of that University, and soon by the grant of an 1851 Exhibition which took him to Cambridge in 1910, where he continued his studies for a year as a member of Emmanuel College. From 1911 until soon after the outbreak of the First World War, Grove was employed as an entomologist in the Indian agricultural service, but he was then drawn back into university life as a lecturer at his old University. When it became apparent that there was a need for medical entomologists, Grove volunteered for service in the Royal Army Medical Corps and worked with teams controlling malaria in the Middle East, particularly in Palestine. He resumed his interrupted academic career by accepting a lectureship in the University of Sheffield shortly after the War ended and stayed there until 1929, when he joined G. P. Mudge in the small department at East London College (as Queen Mary College was then called). Up to that time the department had been solely concerned with preparing students for first medical examinations, but now it began to accept students for general and special degrees in zoology. Re-equipment and expansion were inevitable and in 1936 the department moved, with Grove as its head (Mudge having retired), into new quarters in the newly built west block, where it is still housed.

The Second World War put a brake on further material progress, for in 1939 a greatly diminished Queen Mary College was moved to Cambridge for the duration of the War, but there can be no doubt that the war years spent in the stimulating atmosphere of Sir James Gray's Department was of lasting benefit to Grove and his colleagues; it is a pleasure to place on record that this is still remembered with gratitude. The happy relations between the two Zoology Departments was in no small measure due to Grove's eagerness to co-operate and to take full advantage of the splendid resources put at his disposal.

Grove will be remembered by his colleagues as an authority on the reproduction of earthworms as well as for his contributions to insect morphology, while many generations of students are grateful for his text-books and laboratory manuals which bear witness to his ability as a meticulous teacher. Grove took a full share in examining for the University of London and never shirked any of the onerous and time-consuming administrative duties inevitable in a large university either within the College or at the Senate House and by the time he retired he had been a dean for many years and chairman of several important committees, posts which he filled with great success.

G. E. Newell

Prof. Franciszek Adamanis

Polish pharmacy suffered a great loss when Prof. Franciszek Adamanis, professor of pharmaceutical chemistry at the Medical Academy in Poznan, died of cancer on April 14. His intellectual qualities, combined with his open-hearted friendliness, earned him the respect and affection of many pharmacists abroad as well as in Poland. His own interests and affections were deeply connected with the profession of pharmacy. He was a champion of the improvement of the status of this profession, in opening the door for a pharmacist to the analytical and biochemical laboratories and to the pharmaceutical industry. The aim of his life was to gain recognition for pharmacists in the fields of analytical chemistry, biochemistry and microbiology.

Franciszek Adamanis was born in Libawa (Latvia) on October 4, 1900. He finished his secondary education in Homl (Russia) in 1918 and entered the Faculty of Law in the University of Poznan. Later, he became a member of the Faculty of Pharmacy, and in 1927 he gained his Master's degree for work on Heinz Aldol. For his first scientific work he was awarded the bronze medal by the Senate of the University.

In the same year he started to work as an analytical chemist in the chemical industry and in 1929 he became a director of the firm Akwawit in Poznań. In 1930 he started to work on his Ph.D. thesis under the guidance of Prof. Konstanty Hrynakowski. He studied the additive combinations of the molecular compounds of urea by the method of thermal and optical analysis. He determined the forces of co-ordination existing between components of the molecular compounds and the effect of these forces on the biological action. He also pointed out the relation between the additive properties of tri-valent nitrogen and its pharmacological action. The very promising results of his research made possible his graduation for Ph.D. in 1932 and his habilitation for docent in 1937.

After the death of Prof. Hrynakowski in 1938, he became the head of the Department of Pharmaceutical Chemistry at the University of Poznan

After the outbreak of the Second World War he was taken prisoner and sent to the concentration camp in Gusen, Austria, from 1940 until 1945 The deep honesty of his character helped him to maintain an inflexible position In trying to save the lives of many people, he organized the production of drugs in the simple and primitive conditions of the camp's laboratory He distributed more than 40,000 ampoules of injections among people, running the risk of losing his own life every day.

After the War he returned to Poland and started to rebuild from the ruins of the University of Poznań the Department of Pharmaceutical Chemistry His post-war scientific work extended into two main efforts First, he pioneered the teaching of pharmaceutical chemistry and the change of the old traditional programme which was based on the division of drugs according to their chemical structure In his book *Chemia Lekow*, which appeared in three editions, he presented a modern approach in chemistry of drugs, giving their division from the pharmacological point of view The post-war research mainly concerned the adaptation of modern analytical methods, for example, chromatography to the analysis of active substances of plants, especially alkaloids and glycosides Another line of research which resulted from his analytical school was that on the stability of drugs, which will be continued by his disciples He was the author of 75 original research papers and several theoretical reviews He guided Ph D theses for 19 pharmacists

In 1946 Adamanis was nominated professor of pharmaceutical chemistry, and in 1958 he became full professor In addition, he was director of the State Institute of Medicinal Plants in Poznań until 1954, and dean of the Faculty of Pharmacy during the academic year 1951–52, for the past four years he was pro-rector of the Medical Academy in Poznan He took part in several international conventions of pharmacists and worked as an active member of scientific and pharmaceutical organizations He was a member of the Committee of Pharmaceutical Sciences at the Polish Academy of Science, president of the Section of Pharmacy at the Poznan Society of Friends of Science, president of the Polish Pharmaceutical Association from 1960, member of the Polish Pharmacopœia Commission, vice-president of the Section of Medicine at the Main Council of the Ministry of Education, and member of the American Pharmaceutical Association In addition to this, he took part in work of social organizations His great endeavours were rewarded by the City of Poznan and by the Government

Adamanis was a man with an unusual background who had a variety of interests his knowledge of Russian literature was exceptional, and he was an expert in architecture and sacral art His main 'hobby' was photography, and he was also a strong supporter of the cultural background of his students Now death has taken this great man, whose deep honour and honesty will remain the ideal for all future pharmacists

DANUTA MALEJKA

NEWS and VIEWS

California Institute of Technology

ON June 30, four distinguished scientists retired from their chairs in the California Institute of Technology, each having reached the full retiring age of seventy the honour of professor emeritus has been awarded to them

Prof A H Sturtevant

PROF A H STURTEVANT was Thomas Hunt professor of genetics He has been at the Institute since 1928. He was a member of Morgan's original '*Drosophila* group' that pioneered the new science of genetics, largely through the study of the *Drosophila* fruit fly. Among many major contributions, Prof Sturtevant demonstrated that the genes lie in linear order and he helped to develop chromosome theory Prof Sturtevant is the author or co-author of 137 scientific publications, including several books Among the books are *An Introduction to Genetics*, written with Dr George W Beadle, formerly of the Institute and now president of the University of Chicago, and *Genetics and Evolution*, a collection of Prof Sturtevant's scientific papers selected by Dr E B Lewis, professor of biology in the Institute

Prof C. C Lauritsen

PROF C C LAURITSEN was professor of physics, and pioneer in nuclear physics and rocket research He has been on the faculty of the Institute since 1929 He was one of the pioneers in research in the field of nuclear physics, and under his leadership the Kellogg Radiation Laboratory has, for three decades, been one of the world's leading centres of nuclear studies Prof Lauritsen was also scientific director of the very large and active rocket-development group at the Institute during the Second World War, and in subsequent years became one of the leading advisers to the Government on military and scientific matters He received the Medal of Merit for his war work In addition to serving as technical consultant to the Army, Navy and Air Force on nuclear weapons and rockets, he spent a year at Los Alamos as special consultant on the atomic bomb project He also helped formulate plans which led to the establishment of the Office of Naval Research

Prof E C. Watson

PROF EARNEST C WATSON, professor of physics, was formerly dean of the faculty, and one of the builders of the California Institute of Technology Since 1960 he has been on leave as scientific attaché to the U S Embassy in India He supervised the planning and development of the well-known Norman Bridge Physics Laboratory at the Institute His wife, Jane Werner Watson, is the author of nearly two hundred children's books

Prof F J Converse

PROF F J CONVERSE is an authority on soil mechanics He is president of the Converse Foundation Engineering Co and has been consulted as a foundation engineer in the construction of many dams, tunnels, factories, military installations, shipyards, airports, power plants and sub-stations throughout the United States, and has done such specialized jobs as investigating the ground settling at Terminal Island

Explosives Research and Development Establishment, Waltham Abbey

Dr. G H S Young

Dr G H S Young, of the Ministry of Aviation's Explosives Research and Development Establishment, Waltham Abbey, has been promoted to deputy chief scientific officer and given general charge of all development work and chemical engineering in the Establishment After graduating from the Honours School of Chemistry at the Imperial College of Science and Technology, London, in 1937, he took the diploma in chemical technology in 1938 and continued thereafter his research under the late Sir Alfred Egerton on the slow combustion of hydrocarbons which gained him a Ph D in 1939 At the outbreak of war, Young was drawn into technical work for the Services, initially at Imperial College and later at Woolwich and Swansea During 1941–45 he was attached to the Proof and Experimental Establishment at Inchterf in Dumbartonshire, where he learned the hard way about practical matters connected with the performance of cordite in guns, and concurrently about the related problems of propellent manufacture at the nearby Ordnance Factories

Young became a foundation member of the staff at Waltham Abbey when the Research and Development Establishment was set up there in 1945 by the Ministry of Supply From then onwards until 1953 he was in L A Wiseman's group studying the combustion of liquids and gases In particular, he made some of the earliest measurements in Great Britain of the attenuation and modulation of micro-wave signals by rocket exhausts, and looked into the controlling factors During this period Young served as secretary of the Spectrochemical Committee of the Scientific Advisory Council In 1953 he was promoted to superintendent rank, and although this necessitated a switch into predominantly development work he has nevertheless found time to build up the team responsible for rheological research on solid propellents and broaden its scientific basis

Botany in Kashmir

Dr T N. Khoshoo

Dr T N Khoshoo, who has recently been appointed head of the Department of Botany at the University of Jammu and Kashmir, was born on April 7, 1927, at Srinagar, Kashmir, He gained an M Sc (Hons) in botany at the Panjab University, Lahore, in 1946 and was the recipient of several prizes, including the "Prince of Wales Gold Medal" for high performance in biology in general and botany in particular Soon after the partition of India, he joined the Panjab University, Chandigarh, where he gained his doctorate and became a senior lecturer In May 1962 he joined the University of Jammu and Kashmir as reader and head of its Postgraduate Department of Botany Dr Khoshoo has been actively engaged in working on the cytology of gymnosperms and the experimental evolution of some polytypic angiosperms of north-western India On these topics he has published about fifty-five research papers, including his work on polyploidy in gymnosperms, cytogenetical evolution in gymnosperms, the karyotype in *Welwitschia mirabilis*, etc Of special interest are his investigations of the *Sisymbrium irio* complex, which is still being continued by him Several other plant species have now been included in this project With his wide experience in field botany of the north-western Himalayas, it is hoped that he will be able to enlarge these investigations and soon build up an active school in experimental evolution and ecogeography of the plants of the Kashmir region Dr Khoshoo is a Fellow of the Indian Academy of Sciences, Bangalore, and joint editor of *Silvae Genetica Journal of the Forest Genetics and Forest Tree Breeding*, published from Schmalenbeck

Government Support for the British Academy

In a written answer in the House of Lords on July 10, the Minister for Science, Lord Hailsham, said that in the light of discussions between the British Academy, and the Government, and at the suggestion of the Government, the British Academy prepared an outline scheme under which the Academy itself would administer an additional grant for the support of research in the humanities The scheme excludes the social sciences which present a different problem involving a wider field of Government interest The Government proposed an additional grant of £25,000 to the British Academy in 1963–65 for this scheme, and it was envisaged that in two or three years the figure might rise to a steady level of £50,000 a year Any extra administrative costs incurred by the Academy in administering the scheme would be found out of the grant The British Academy warmly welcomed an arrangement on these lines and the Government appreciated the efforts of the Academy both in presenting the problem constructively to them and in devising the scheme

Grant for University of London Nuclear Reactor

In a debate on science and technology in the House of Commons on July 12 it was announced that a grant of about £250,000 is being offered to the University of London by the Department of Scientific and Industrial Research to provide a low-power nuclear reactor for research and training The reactor, a 100-kW *Consort* made by the General Electric Co , Ltd , will be operated by Imperial College on behalf of the University and will be built at Imperial College's Field Station at Silwood Park, near Virginia Water, Surrey While intended primarily for the University of London, it will be available for use by other research organizations in the London area In May 1961, the Government announced that it had agreed in principle to the award of three grants for low-energy nuclear reactors for British universities one to the four Scottish Universities and the Royal College of Science and Technology, Glasgow, jointly, one to the University of London , and one to the Universities of Liverpool and Manchester jointly Final approval of these projects had to be postponed in July because "the scientific expansion in all fields seemed likely to exceed planned financial resources" In December the Council was able to go forward with one of these awards—£450,000 to the Scottish Universities Work on this project is going forward to schedule, and the Scottish consortium has been joined by the Queen's University of Belfast At that time the Department of Scientific and Industrial Research stated that it hoped to make an announcement later about the remaining two projects

Research Group in Epigenetics at Edinburgh

The Medical Research Council has recently agreed to the formation at the University of Edinburgh of a Research Group in Epigenetics The Group is to be under the direction of Prof C H Waddington, and will be attached to the Department of Animal Genetics It is expected that the support will be made available by the Research Council for a period of at least ten years, the total sum involved over this period being of the order of £200,000 The Wellcome Trust have provided a capital sum, up to £50,000, to make possible the erection of a new laboratory to house the work The field of the Research Group covers all those processes by which a newly fertilized egg develops into the differentiated cells of the adult organism As medical science finds ever more powerful methods for the control of infectious disease and the repair of accidental damage, disorders of developmental processes are bound to acquire increasing importance among the medical problems of the future If such degenerative diseases as rheumatism, arthritis and senescence in general, disorders of growth (as in cancer) and the developmental mishaps which produce congenital abnormalities are to be dealt with satisfactorily a much more profound understanding will be required of the processes by which the simple cells of the embryo acquire, and maintain, the specific characters of the different tissues of the adult animal In the past few years, several particular problems within this general field have been under investigation at the Edinburgh Institute of Animal Genetics, both by members of the University staff and by workers financed by the Nuffield Foundation, the British Empire Cancer Campaign, the Agricultural Research Council and the Damon Runyon Trust for Cancer of New York The resources now provided by the Medical Research Council will make it possible to build up a group of workers who can bring to bear most of the numerous modern relevant techniques in an organized attack on the very complex problems of fundamental biology which underlie the many diverse manifestations of developmental disorder with which mankind is afflicted

The Forestry Commission

The forty-second annual report of the Forestry Commission for the year ended September 30, 1961, provides not only an account of the Commission's work but also shows the progress in both State and private forestry (Pp 70+8 plates London H M S O, 1962 5*s* 6*d* net) At the same time, there is the disturbing note that although 100,000 acres were planted with trees during 1960–61 (64 per cent by the Commission), insufficient land was acquired by the Commission to support the planting programme adopted in 1958 There is still a certain reserve of land suitable for planting, but with this reserve diminishing instead of being maintained until nearer the end of the planting programme it may cause some difficulties with planning nursery work where provision for plants must be made perhaps some four years before they are required The Forestry Commission's effective estate, including land to be planted, was 1,671,081 acres on September 30, 1961 On the production side, 23 million hoppus feet of timber were cut from the Commission's forests There were reduced pitwood purchases by the National Coal Board, particularly in Scotland, but developments in forest utilization in Britain included the opening of a chipboard factory at Inverness, the addition of a small hardwood pulp mill to a paper mill in Kent, plans for an integrated saw mill and chipboard factory at Thetford and the possibility of a large-scale pulp mill in the Scottish Highlands Two of the significant points which arise out of this report are the utilization developments just mentioned and the help given to private forest owners by the State in the form of planting and management grants (now £1,183,000 a year) and by the Timber Growers' Organization and the Scottish Woodland Owners' Association These two bodies are materially helping to bring more and more management into private forestry and thus increase the productivity Other activities of the Forestry Commission included research, education and the provision for the public of facilities of recreation and amenity

The Royal College of Science and Technology, Glasgow

The annual report for the session 1960–61 of the Royal College of Science and Technology, Glasgow, records the approval by the University Grants Committee of proposals for a new faculty for industrial and social studies (Pp 75 Glasgow The Royal College of Science and Technology, 1962) Such a faculty would include industrial economics, economic history, political theory and institutions and administration, and provide degree courses and facilities for postgraduate study and research, within which the Department of Industrial Administration and General Studies would be integrated The report lists the titles of theses approved for the award of the Diploma of Membership of the College and the Associateship and recent publications by members of the College There are the usual departmental reports, but a brief record of research work in progress, with departmental lists of publications and the titles of theses approved for higher degrees, is issued in a separate *Research Report*

Cost of Books

In the article on "Gas Chromatography" (*Nature*, 194. 822, 1962), Dr. D H Desty, commenting on the book *Gas Chromatography* Second International Symposium held under the Auspices of the Analysis Instrumentation Division of the Instrument Society of America, June 1959 (Academic Press, Inc 16 dollars), wrote "The increase of nearly 50 per cent over the latter [First Symposium] is also somewhat surprising" Mr Kurt Jacoby, vice-president of Academic Press, Inc, New York, writes giving some interesting bibliographical data "First Symposium, edited by Coates *et al*, 1958, 323 pages, 12 dollars, or 0 037 dollars a page, Second Symposium, edited by Noebels *et al*, 1961, 463 pages, 16 dollars, or 0 034 dollars a page Since there was a general price increase in the manufacture of books between 1958 and 1961, the price is both proportionally and actually lower for the Second Symposium" We are pleased to publish this statement from Academic Press, for it is certainly true that publishers have been facing, and are continuing to face, increases in costs of printing, paper, block-making, etc, not all of which are passed on to the book-buyer

Space Bibliography

The diverse literature of space flight is listed and described by F I Ordway III in his *Annotated Bibliography of Space Science and Technology* (Pp x+77 Washington, D C Arfor Publications, P O Box 6285, 1962 2 95 dollars) The bibliography covers the years 1931–61, and aims at including all significant books on the subject in the English language The books are listed according to the year of publication each entry consists of the author, title, full bibliographical details of the American edition and about fifty words of summary After the main list comes an "Astronomical Supplement", which gives, without summaries, books on 'fringe' subjects, the Sun, the Moon, geophysics, etc Finally, there are indexes of authors and titles Mr Ordway's claim that he has listed "all significant English-language titles since 1931" is very nearly true, and his summaries are fair and informative Altogether, this is a most useful reference book, both for librarians and for working scientists

The European Common Market

A THIRD revised edition of the bibliography compiled by J E Wild on *The European Common Market and the European Free Trade Association* has been issued as *Special Subject List No 35* (Pp 64 London The Library Association, 1962 9*s* , L A Members, 6*s* 9*d*) It is in two parts, the first covering books, pamphlets and reports, and the second periodical articles There is also a short list of bibliographies, reading lists, etc There are some 750 entries arranged alphabetically by authors, and also a name and subject index The entries are selected to cover as many aspects of European economic integration as possible, including, for example, scientific manpower, and giving prominence to such controversial matters as agriculture, the Commonwealth problem, competition and sovereignty

The International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in the *Bulletin of Zoological Nomenclature* (19, Part 4, published on July 16, 1962) (1) Validation and interpretation of the generic name *Endothyra* Phillips, [1846] (Foraminifera) *Z N* (*S*) 768 (2) Designation of a type-species for *Boa* Linnaeus, 1758 (Reptilia) *Z N* (*S*) 1188 (3) Validation and interpretation of the specific name *Culex aegypti* Linnaeus, 1762 (Insecta, Diptera) *Z N* (*S*) 1216 (4) Suppression of the specific name *Pachyodon nucleus* Brown, 1843 (Pelecypoda) *Z N* (*S*) 1470 (5) Validation of the emendations *Dorhynchus* (of *Dorynchus* Thomson, 1873), and *stirhynchus* (of *stirynchus* (*Axius*) Leach, 1815), designation of a type-species for *Callinectes* Stimpson, 1860, validation of the family name Potamonidae Ortmann, 1896, validation of the specific names *Callinectes sapidus* Rathbun, 1896, and *Goneplax sexdentatus* Risso, 1827 (Crustacea, Decapoda) *Z N* (*S*) 1499 (6) Validation of Morch, 1853–1858, *Catalogus Conchyliorum* and designation of a type-species for *Pseudamussium* Morch, 1853 (Pelecypoda) *Z N* (*S*) 1501 Any zoologist who wishes to comment on any of the above cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S W 7, before January 16, 1963

The Institute of Physics and the Physical Society

AT the second annual general meeting of the amalgamated Institute of Physics and the Physical Society, held in London on July 10, the following were elected to office *President*, Sir Alan Wilson, *Vice-President*, Prof B H Flowers, *Honorary Treasurer*, Dr J Taylor, *Honorary Secretary*, Dr C G Wynne The three new Ordinary Members of the Council elected were Dr D H Follett, Prof M H L Pryce and Prof A M Taylor Prof P M S Blackett, Sir John Cockcroft, Prof W E Lamb, jun , and Prof C F Powell were elected Honorary Fellows in recognition of their eminent work and valuable contributions to knowledge in the field of physics

Natural History Certificate Course

THE University of London awards a certificate of proficiency in natural history This certificate is available to all persons interested in the study of living things in their natural habitats The work involves a directed course of private reading at home, attendance at a practical course of four-weeks duration in the spring and summer, an approved plan of field-work suited to the student's locality to be written up in the form of an essay, and examinations Students wishing to begin their directed course of reading can apply at any time before November 15 for registration with the view of attending next year's practical course which (subject to sufficient entries) will be held at suitable field centres for one week in April and for three weeks in August The regulations and application form can be obtained from the Secretary, Natural History Certificate Course, Department of Extra-Mural Studies, University of London, Senate House, London, W C 1

Grants for Humanities and Social Sciences in Israel

THE Edmond James de Rothschild Memorial Group is offering several fellowships to enable leading authorities in the humanities and the social sciences to spend a period of six months to a year at the Technion–Israel Institute of Technology, Haifa Candidates must be men of recognized academic standing at a university or other institution outside Israel, of sufficient calibre and experience to enable them to assist in the broadening of the basis of the humanities and the social sciences at the Technion They will be expected to deliver a course of lectures in their field of specialization and generally stimulate (and receive stimulation from) the student body and faculty of the Institute Each fellowship-holder will receive a grant of 7,000 dollars per year (or 3,500 dollars for six months), and tourist-class round-trip fare between his home country and Haifa Technion will undertake to provide him with suitable housing for the duration of his stay Application should be made in writing to the Registrar, Technion–Israel Institute of Technology, P O B 4910, Haifa, Israel, before September 1 for the academic year 1962–63, and February 1 for the academic year 1963–64

Symposium on Antarctic Biology

A SYMPOSIUM on Antarctic Biology will be held in Paris during September 2–8 It is being organized by the Scientific Committee on Antarctic Research (S C A R) with the co-operation of the French Academy of Sciences at the Faculté de Médecine, 45 Rue des Saints-Pères, Paris Its aims are to review progress in Antarctic biological research, to discuss the most profitable lines of future research, to integrate Antarctic work with biological research elsewhere, and to discuss the special problems involved in research in the Antarctic and Sub-antarctic, including international co-operation The opening address will be given by Prof P P Grassé, chairman of the Organizing Committee

The following sessions will be held the life sciences in the Antarctic, medical research, microbiology, biogeography and systematics—botany, biogeography and systematics—marine zoology; biogeography and systematics—terrestrial zoology, marine productivity, ecology of invertebrates, ecology of vertebrates, ethology of vertebrates, conservation, animal physiology, future research programmes. About sixty papers will be read. Delegates will attend from Argentina, Australia, Belgium, France, New Zealand, Norway, South Africa, U.S.S.R., the United Kingdom and United States. Further information can be obtained from the Secretary of the British National Committee on Antarctic Research, c/o Royal Society, Burlington House, Piccadilly, London, W.1.

University and College News:

Belfast

THE following appointments have been made at the Queen's University of Belfast: *Lectureships*, F. W. Boal (geography), M. F. Moore (psychology), Dr. A. E. Henderson (zoology), Dr. K. A. Jones (geology).

Melbourne College of Pharmacy

MR. NIGEL MANNING, immediate past-president of the Pharmacy Board of Victoria, has been appointed dean of the College of Pharmacy at Melbourne. He takes up his new post in February 1963. For many years Mr. Manning was lecturer in pharmaceutics at the College. He is at present a partner in a retail pharmacy in Melbourne and is joint editor of the *Australian Pharmaceutical Formulary*. Although the College has been established a number of years, it is now housed in a new building which was opened in March 1960, and has accommodation for more than 600 students.

Sheffield

DR. ALICE GARNETT, reader in geography, has been appointed professor of physical geography, as from October 1. S. A. F. Murrell has been appointed lecturer in metallurgy, and J. A. Whiteman has been appointed to be C. H. Desch Research Fellow.

Southampton

DR. G. J. HILLS, lecturer in physical chemistry in the Imperial College of Science and Technology, has been appointed to the chair of physical chemistry. The following appointments have recently been made to lectureships: L. C. J. Johnson (chemistry), R. Butterfield and A. Coull (civil engineering), A. G. Howsen (pure mathematics), D. Ramsden (physics), A. M. P. Lockwood (zoology). R. M. Laird has been appointed to a research fellowship in chemistry and J. W. Powell to be Vokes Research Fellow in mechanical engineering. Dr. R. E. Parker, lecturer in chemistry, has been appointed secretary and registrar of the Royal Institute of Chemistry.

Announcements

PROF. J. YUDKIN, of Queen Elizabeth College, University of London, has been awarded the William Julius Mickle Fellowship for 1961–62 by the Senate of the University of London for his work on nutrition.

THE Dr. W. S. Bruce Memorial Prize for 1962 has been awarded to Mr. K. V. Blaiklock, in recognition of his contributions by way of navigation to the exploration of the Antarctic continent and for his mapping of lands bordering the Weddell Sea. The Joint Committee of Award for this Prize is nominated by the Royal Physical Society, the Royal Scottish Geographical Society and the Royal Society of Edinburgh, and the Prize is in commemoration of Dr. W. S. Bruce, the Scottish explorer and scientific investigator of polar regions.

AN international symposium on "Chromatography", organized by the Société Belge des Sciences Pharmaceutiques, will be held in Brussels during September 14–15. Further information can be obtained from the Secretary, Société Belge des Sciences Pharmaceutiques, 11 rue Archimède, Brussels 4.

A GENERAL discussion on "High Resolution Nuclear Magnetic Resonance", arranged by the Faraday Society, will be held in the University of Oxford during September 17–19. Subjects under discussion will include: nuclear magnetic resonance in diamagnetic materials, nuclear magnetic resonance in paramagnetic materials, applications to the determination of molecular structure, applications to the study of kinetic processes. Further information can be obtained from the Secretary, the Faraday Society, 6 Gray's Inn Square, Gray's Inn, London, W.C.1.

THE annual summer conference of the Universities Federation for Animal Welfare will be held at Knuston Hall, near Wellingborough, Northamptonshire, during September 14–21. The programme will include talks and discussions on: the humane treatment of laboratory animals, the control of deer, the trapping of fur-bearing animals. Further information can be obtained from Universities Federation for Animal Welfare (Summer Congress), 7a Lamb's Conduit Passage, London, W.C.1.

THE eighteenth International Congress on the History of Medicine will be held in Warsaw and Cracow during September 17–24. Topics under discussion will include: medicine and humanism in past centuries, educational relations between Polish and foreign medical world, medical men who were famous as writers and artists. Further information can be obtained from the Organizing Committee of the Eighteenth International Congress on the History of Medicine, Warszawa, ul. Chocimska 22.

THE eighth Latin-American Congress of Chemistry will be held in Buenos Aires during September 16–22. The Congress will be divided into the following sections: inorganic and radiochemistry, physical chemistry, organic chemistry, biological chemistry, analytical chemistry, food chemistry, pharmaceutical chemistry and pharmaceutical industries, industrial chemistry and chemical engineering, history of chemistry and chemical education. Further information can be obtained from the Secretary, Octavo Congreso Latinoamericano de Quimica, Casilla de Correo 2153, Buenos Aires.

A NATIONAL astronomical convention, arranged by the Rank Taylor Hobson Astronomical Society in collaboration with the Ratcliffe College Astronomical Society, will be held in Leicester during September 7–9. The programme will include: contemporary astronomy (Dr. R. v. d. R. Woolley), some highlights of the 1961 International Astronomical Union meeting (Dr. D. Dewhirst), the design and construction of astronomical instruments (Dr. E. H. Linfoot), the design of radio telescopes (Mrs. P. R. Foster), contributions of radio astronomy to cosmological inquiry (Dr. R. D. Davies), aspects of the history of astronomy (Dr. H. C. King). Further information can be obtained from Mr. V. W. Littlejohn, 19 Southdown Drive, Thurmaston, Leicester.

THE FOREST PRODUCTS RESEARCH LABORATORY

THE Forest Products Research Laboratory of the Department of Scientific and Industrial Research held, during May 29–31, its first open days since the War This Laboratory, which was set up at Princes Risborough in 1927, has the function of carrying out research on all aspects of timber utilization, its interests range from detailed chemical studies of some of the extractives found in timber to engineering work on the production of laminated wood beams About thirteen hundred people visited the Laboratory during the three days, the visitors representing university departments, other research institutions and many industrial and commercial organizations connected with the timber trade or interested in some aspect of timber utilization

Although in the past much of the work of the Laboratory was concerned with determining the properties of imported timbers, about 30 per cent of the present effort is devoted to work on home-grown timbers in collaboration with the Forestry Commission Many exhibits illustrated work in this category Examples of investigations which are specifically linked to home-grown timber are experiments to find a means of preventing the growth of staining fungi in freshly felled softwoods by the development of a suitable spray treatment, the development of methods for preventing attack by ambrosia beetles on softwood logs in the forests of Argyllshire, a trial of the diffusion process for the preservative treatment of impermeable softwoods such as spruce, and the development of techniques for making an assessment of the basic properties of wood by examining small borings 11 mm in diameter taken from the standing tree

This last project is being used to examine the wood in trees which have been provisionally selected, because of certain outstanding features such as stem shape and vigour, to serve as breeding stock for future generations, and the Laboratory is engaged on an examination of the borings from these trees in order to ensure that the basic properties of the wood are satisfactory, and to select those with outstanding properties for a breeding programme The measurements made on the borings include fibre dimensions and density variations, new equipment which enables many measurements of density and variations of density within the tree to be carried out rapidly was on show The method is based on the absorption of β-particles from radioactive carbon-14 in carefully microtomed sections of the borings 100μ thick

Thinnings form a substantial proportion of the yield from any forest plantation and are now in excess of what is required for pit props, fence posts, etc The Laboratory has therefore embarked on a programme of evaluating the quality and yield of paper pulp obtainable from home grown softwoods Equipment for pulping and for measuring basic paper properties was on show together with samples of paper obtained during this work.

The suitability of home-grown timbers for structural purposes was demonstrated by means of a model of the wooden bungalow built by the Forestry Commission at Alice Holt Lodge, Farnham, from Sitka spruce and Scots pine The design was based on working stresses for these timbers obtained during the general programme of assessing home-grown timbers in progress at the Laboratory Conventionally, wood is quality graded by visual assessment of the number and distribution of knots, and working stresses are derived from experimental data obtained on defect-free material suitably adjusted by empirical factors to allow for the effects of natural variability and of knots A prototype machine which measures the working stresses of timber at the rate of 80 ft per min was on show When fully developed and in use this should result in much more rapid and accurate grading and more economic use of timber for structural purposes

Throughout the life of the Laboratory, kiln schedules have been developed which permit commercial timbers to be dried without serious splitting or twisting A new aid to good seasoning practice was demonstrated in the form of an experimental kiln trolley which uses the weight of the load to clamp the timber stack, thus reducing the amount of twisting which can take place during seasoning This will be specially useful with certain difficult timbers

In view of the great amount of energy consumed in cutting wood and the large proportion of a log that is wasted during woodworking operations, the potential value of research in this subject is considerable Equipment designed to measure the forces involved in cutting, and the temperature of a circular-saw tooth during operation were on show

There was a number of exhibits demonstrating work on new methods of manufacturing glued laminated structures These include the use of low voltage, infra-red and radiofrequency heating to set the edges of the glue lines This is achieved after a short period of time and the laminates can then be removed from the jig and the rest of the glue line allowed to set during storage The growing importance of chipboard was evident in research which is being supported by the British Plastics Federation The Laboratory is undertaking a study to evaluate the importance of various factors in the manufacture of chipboard and pilot-scale equipment for investigating various stages of the manufacturing process was on show

A new and patented process for the manufacture of '*Y–D*' spade handles was demonstrated during the open days This development stemmed from the discovery that steamed and compressed wood can be satisfactorily bent after it has been allowed to cool This process, which results in a substantial reduction in losses due to broken bends, is being taken up by several industrial concerns

One of the more important functions of the Laboratory is to ensure that, where appropriate, timber is effectively treated with preservatives to prevent attack by fungi or insects, and methods for assessing the initial toxicity of preservative formulations were illustrated New and improved methods are under investigation, and the Laboratory is co-operating with many European scientists to develop standard methods of test Radiography is being used to assess the rate of development of wood-boring larvæ in treated blocks of wood and X-radiographs on show demonstrated the usefulness of this technique in facilitating work on preservatives

With some timbers it is extremely difficult to obtain penetration of preservative liquid deeply into the wood and a variety of exhibits illustrated work on this problem The rate of flow of an inert gas under a fixed pressure is being used to determine the permeability of wood specimens in the three directions of the grain, and a technique has been developed which enables the process of impregnation under pressure to be studied photographically or by direct observation A short film strikingly demonstrated the variation in the rate of penetration of liquids in different regions of a wood specimen

While the common furniture beetle and the death-watch beetle are widely distributed throughout Britain, the house longhorn beetle (*Hylotrupes bajulus*) causes serious damage only in and around Surrey, and in this area a large increase in damage has been noticed during the past twenty-five years It was illustrated by means of maps that this area coincides with that of the highest mean temperatures during the breeding months of this insect (June–September) It is of some interest that an experiment is being planned in collaboration with scientists in the four Scandinavian countries to explore in a controlled manner the effect of temperature and humidity on the activity of this insect

Wood is used by a few industries on a very large scale and by very many smaller industries in small quantities but in a highly specialized manner The range of problems still to be solved was well illustrated by the exhibits on show on this occasion

E J GIBSON

INTERNATIONAL HYDROGRAPHIC RESEARCH

THE eighth International Hydrographic Conference opened in Monaco on May 8, under the presidency of Prince Rainier of Monaco The opening address was delivered by Rear-Admiral Knox, president of the Directing Committee of the International Hydrographic Bureau, who welcomed delegates from forty-one countries, as well as observers from non-member countries and from international scientific and maritime organizations He expressed gratitude for the generous contributions of data to the Bureau, especially those from the Academy of Sciences of the U S S R and the Woods Hole Oceanographic Institute of the United States Another welcome gift came from Portugal—a complete set of the *Cartographic Monument of Portugal* from an Executive Commission appointed by the Portuguese Government commemorating the five-hundredth anniversary of the death of Prince Henry the Navigator.

The General Bathymetric Chart of the Oceans, which can be seen in the Musée Océanographique in Monaco, was the life-work of the late Prince Albert of Monaco, and therefore is one of the reasons for the Headquarters being in Monaco It is now proposed to complete and print the Chart in such a way as to satisfy the demands of hydrographic officers, oceanographers and others. In national hydrographic offices the bathymetric data is plotted to 1/1,000,000, however, the General Bathymetric Chart of the Oceans is plotted to 1/10,000,000 This has become a financial responsibility of the International Hydrographic Bureau, and it was decided that with co-operation of all State members and other interested agencies, the Chart would be compiled under the auspices of the Bureau

It has become necessary to revise the statutes of the International Hydrographic Bureau and bring them into line with more forward-thinking policies However, before this can be carried out, a decision must be taken concerning the Convention on Juridical Personality At the conference held in 1957 action was started to gain juridical personality recognized, this being necessary before diplomatic status can be requested, as with other international bodies After submission to Foreign Office Ministers, only fifteen signatures of State members were obtained, which was insufficient, twenty-four signatures being required It has now been decided to prepare a convention with a new text, and this will be drafted in Paris, preferably before the end of the present year One interesting and useful matter discussed was the setting up of working committees between the five-year conferences, when work could be set in motion in preparation for discussion at conference-level. Agreement was reached on the importance of studying, together with other bodies, a comprehensive system of classification for documentation covering, principally, hydrography, oceanography, geodesy and allied subjects, with the view of establishing a documentation service

The question of agreement on the velocity to be adopted for all oceanic soundings using echo-sounding apparatus was thoroughly explored, as some standardization is desirable It was agreed that machines be used 'preferably' calibrated for a sounding velocity 820 fathoms (1,500 metres) per second

Progress towards the international adoption of the metric system is very slow, principally on account of the confusion it would cause to navigators of many countries, whose instruments are calibrated in fathoms, and soundings on charts are given in fathoms and feet The International Hydrographic Bureau felt that as yet it is unable to take any effective action since hydrographic offices tend to conform to their national unit of measurement, and until general standardization at an international level has been reached little can be done In the meantime, all countries will endeavour to work towards that goal and report progress every five years

The importance of mean sea-level in the choice of the geoid was the subject of a proposal that the Bureau should be responsible for determining the norms to be followed in the determination of mean sea-level with the ultimate object of standardization for future work of wider scope As this does not fall within the field of hydrography, the Bureau felt that it was not competent to carry out the work It was decided that this study should be transferred to the Permanent Service for Mean Sea Level for consideration and that State members be advised of their reply

A proposal was carried which recommended that, in the terminology of lights in their national language, hydrographic offices adhere as closely as possible to the document entitled *Nomenclature of Lights Used for Marine Navigation*, drawn up by the International Association of Lighthouse Authorities in June 1960 and distributed to hydrographic offices by the Bureau

An exhibition of recent instruments and equipment used in hydrography was prepared for the Conference, and revealed the great advances made in the field of electronics H M S *Vidal* arrived to assist in the demonstration of equipment, and Rear-Admiral Irving, hydrographer of the Navy, headed the British delegation at the Conference A 6-hr cruise was made along the coast when the delegates were given a practical demonstration of a 'Tellurometer' (Vickers) microwave system for use in hydrographic survey, where the master installation is on the ship with known base remote stations ashore It is intended for round-the-clock operations and to save manpower The automatic plotting table provides an automatic plot of the ship's position on a 24-in × 24-in. chart with an accuracy approaching 1 part in 10,000 An enterprising exhibitor chartered a launch for the duration of the Conference to demonstrate a highly accurate radio survey-accuracy position-fixing system The 'Track Plotter' (Decca) provides a pictorial display of the vessel's movements on a special chart; the position-line numbers can at the same time be read from easily defined sequential counters The control operates regardless of visibility and the absence of conventional facilities

A new development also demonstrated was a 'Towed Surveying Asdic' (Kelvin Hughes) This apparatus contains a transducer facing sideways, giving a fan-shaped beam—1½° horizontal plane, 19° vertical plane—of supersonic energy which scans as it is drawn along in the sea The returning echoes make their imprint on an 18-in wide chart, thus providing details of the sea bottom over a swathe 800 yd wide when the depth may be only 30 yd

As with all conferences, there is often a gay beam threading its way between the scientific intricacies. This was admirably demonstrated by one of the closing social events—a dinner given by the Monagasque Government The president of the conference, Ingenieur Général Guggenheim, expressed on behalf of the participants of the conference appreciation of the warm welcome extended to them by the Principality of Monaco, where forty years ago the Bureau was founded He pointed out the work of hydrographers is of an international character and not solely for hydrographic services This aspect was foreseen even before the First World War, but did not materialize until the birth of the International Hydrographic Bureau ALICE KENNEDY

THE INTERNATIONAL BIOLOGICAL PROGRAMME

DURING May 21–22, at Morges, Switzerland, a programme of co-operative research in basic biology on an international scale was outlined by a Planning Committee organized for this purpose by the International Council of Scientific Unions Two years of preliminary discussions by members of the International Council and three of its affiliated Unions—the International Union of Biological Sciences, the International Union of Biochemistry and the International Union of Physiological Sciences—had convinced these scientists that there is a genuine need for such a programme These discussions had further emphasized the fact that, although biologists might derive inspiration and encouragement from the success of the International Geophysical Year, a large-scale programme of intensive research, highly concentrated in a small period of time, is not practicable in the biological sciences The scope of biology is so vast, and affects so many facets of human life, that it could not possibly be covered in a single co-ordinated effort Consequently, the Committee appointed by the International Council of Scientific Unions decided not to attempt to organize a single International Biological Year comparable with the International Geophysical Year

On the other hand, the biologists meeting at Morges, both during preliminary discussions and at the meeting itself, became increasingly aware of the need for an International Biological Programme of a somewhat different nature This need arises from the fact that many problems of vital importance to man's future welfare can be solved only by means of new scientific information Such knowledge can best be acquired through co-ordinated research in basic biological science on an international scale Problems requiring such an international approach have arisen chiefly through the sudden changes in his environment, which man has produced in recent years, as well as the spectacular growth of human populations and the advance of civilization They include man's catastrophic transformation of natural communities of living organisms which often has greatly reduced their productivity, the need for increasing greatly the productivity of living communities all over the world to keep up with the world's growing population, the physiological stresses to which people are exposed because of their rapidly changing ways of life, and the changes in genetic constitution of human populations caused partly by the rapid advance of civilization

As an aid to solving these problems, the Planning Committee outlined a series of interrelated projects, for each of which a detailed programme of research will be prepared by a small sub-committee of scientists selected by the Planning Committee Under the general title of "The Biological Basis of Productivity and Human Welfare", the following specific projects will be organized Three projects will deal with the subject "Biological Productivity of Terrestrial Communities" The first will devote its efforts to a general survey of terrestrial communities The second will study the metabolism of terrestrial communities, particularly photosynthesis, the nitrogen-cycle, and the physiological interrelationships between organisms A third project on terrestrial communities will seek methods of conserving them and increasing their productivity

In addition to the projects concentrating on the land, one project will be devoted to fresh-water communities, and another to the biological resources of marine communities

A group of human biologists, consisting of physiologists and geneticists working in collaboration with each other, will investigate the biological basis of human adaptability, both physiological and genetical

The size and scope of these projects, as well as their duration, will be worked out by the sub-committees, which are now being appointed by the Planning Committee

Prof G Montalenti (University of Rome), convenor of the Planning Committee, expressed great satisfaction with the outcome of the conference He announced that detailed plans for each of the proposed projects should be ready for consideration by the Central Planning Committee early in 1963, after which they will be submitted to biologists in all the nations which are members of the International Council of Scientific Unions for further revision, financial support and for co-operation in organizing the actual teams of research workers

Similar optimism was expressed by other members of the Planning Committee These included the following the International Council of Scientific Unions was represented by its vice-president, S Horstadius (Uppsala, Sweden), and F W G Baker, who acted as secretary of the conference Representing the International Union of Biological Sciences were its president, C H Waddington (Edinburgh), general secretary, G L Stebbins (Davis, California), Jean Baer (Neuchâtel), of its Division of Zoology, and D Steinberg (Leningrad) The International Union of Biochemistry was represented by its president, M Florkin (Liège), the International Union of Physiological Sciences by J S Weiner (Oxford), and the International Union of Geography by H Boesch (Zurich)

Although the research to be carried out under the International Biological Programme will be entirely in fields of basic biology, it will be oriented toward solving practical problems of human welfare For this reason the conference was attended by the following consultants S J Holt, representing the Food and Agriculture Organization of the United Nations, R L Dobson, of the World Health Organization, and H Seligman, representing the International Atomic Energy Association The advice of consultants from these organizations will be frequently sought by sub-committees for each project, while making their plans

Interest in the Programme has been expressed by the officials of Unesco, whose representative, A Kepes, attended the conference The co-operation of the Organization is assured, particularly for those aspects of the programme which involve the training of additional research workers to carry on the work of the projects

Because of the importance of the Programme for the conservation of natural communities throughout the world, the International Union for the Conservation of Nature acted as host for the conference, which was held at its headquarters, Les Uttins, at Morges In addition to its president, Jean Baer, a member of the Planning Committee, the International Union for the Conservation of Nature was represented at the conference by two consultants, E M Nicholson (London) and E H Graham (Washington, D C)

At the conclusion of the session, General G Laclavère, treasurer of the International Council of Scientific Unions, discussed with the conference members his experiences with the planning stages of the International Geophysical Year, and gave many helpful comments on procedure He expressed great satisfaction with the work accomplished by the Committee

THE CARNEGIE UNITED KINGDOM TRUST

THE forty-eighth annual report of the Carnegie United Kingdom Trust for 1961* covers the first year of a new quinquennium in which new policies included schemes for the distribution of allocations for pioneer projects in new communities and for the benefit of youth projects Few applications, however, have survived the assessment of innovation, but a good start has been made with the award of grants for youth club improvements, for which 44 grants, totalling £6,216, were offered Payments during the year amounted to £152,533, and at the end of the year £541,315 was committed to projects maturing during the quinquennium £45,000 has been allocated for continuance during the quinquennium of the policy of aiding the development of the museum and art gallery services During the year expert reports were commissioned on 25 museums and development grants offered to 13 on which reports had been earlier approved

In a final statement on the distribution during 1957–61 of £194,123 in improvements grants to nearly 1,500 village halls, for which purpose the Trust has now spent some £371,520 in grants for 2,156 schemes, the report refers particularly to the special difficulties of tiny communities on small Scottish islands During 1956–61 the Trust also provided £3,856 to finance the installation of film projection equipment in 16 remote communities where it was difficult or impossible for the Highlands and Islands Film Guild to provide a mobile service

Dr J H Nicholson's study of the social problems of new communities was issued in a report published in August 1961 under the title *New Communities in Britain Achievements and Problems*, and Dr Nicholson's recommendations are detailed in the Trust's report Although no grants were made during the year for pioneer projects to provide social amenities in new communities, some *ad hoc* grants made to new communities are now producing results At Cumbernauld encouraging progress is reported in dealing with problems that face families transported into developing new communities The Bristol Social Project was finally terminated in 1961 and a report on the work and a pamphlet containing a personal interpretation of the results by Prof R Wilson, chairman of the Projects Committee, are expected to appear in 1962 The year also saw the end of the Trust's responsibility for the Liverpool Personal Service Society's experiment in rehousing selected homeless families in rehabilitation houses under case-work supervision, a report on the inquiry into the needs of families with handicapped children is also in the press The Trust is spending £45,000 on two projects, one for establishing a centre at Shrewsbury providing a training and educational group for children aged two and upwards, a centre for continuing assessment, focus for a counselling assessment, and an information centre and meeting ground for parents, and the other for a centre in Glasgow where a child-welfare clinic, a handicapped children assessment centre and a day nursery for handicapped children will be placed under one roof

Although grants for education totalled only £19,670 during the year, they cover some interesting projects The David Marshall Lodge, Aberfoyle, in the Queen Elizabeth Forest Park, is now out of the responsibility of the Trust, but the Trust is hopeful of achieving an attractive scheme in the Brecon Beacons National Park for a sheltered viewpoint and picnic rendezvous, with facilities for the national park's warden and information services The decline in applications for field-study bursaries continued and only 79 people received help at a cost of £237 Ten grants for field work in archæology by amateurs working under skilled direction were promised to a total of £562, and four applications for assistance in field work by local groups of amateurs in natural history were approved, with a total commitment of £279 A grant of £500 a year for the years 1962–65 was offered to the Royal Meteorological Society to maintain its programme of courses for amateurs at the level achieved in 1961, the Conservation Corps founded by the Council for Nature in 1959 is to receive grant assistance from the Trust, totalling £14,500 over five years until 1963

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* The Carnegie United Kingdom Trust 48th Annual Report, 1961 Pp viii – 76 (Dunfermline, Fife The Carnegie United Kingdom Trust, 1962)

HEALTH IN THE AMERICAS

HEALTH conditions in the Americas, especially in Central and South America, have recently been reviewed (*WHO Chronicle*, 1, No 50, 1962) Latin America has a rapidly growing population By 1980 that of South America may reach 234 million and that of Central America 115 million, the combined population of 349 million exceeding by nearly 80 million the estimated population of the United States and Canada for that date The population of Latin America is young, approximately 40 per cent being less than 15 years of age and only 3 per cent 65 years and more By comparison, only 27 per cent of the population of the United States is less than 15 years of age, while 8 per cent is 65 years and more

Birth-rates in Latin America are high—more than 40 per 1,000 population in many countries, and in general exceeding 30 per 1,000 Death-rates in infancy and early childhood are also high Whereas, in the United States, the death-rate for this period of life is 1 1 per 1,000 population, in all except two of the Latin American countries with accurate data available, it was at least eight times as high in 1956, and in one it was nearly forty times as high These high death-rates are closely correlated with high rates of illness In Guatemala City, for example, children less than 5 years of age accounted for 28 per cent of patient-days in hospital, their average length of stay being more than twice as long as that of other patients

Among the communicable diseases which exact their toll in the Americas, malaria, smallpox, yellow fever, tuberculosis, leprosy, the venereal diseases and the zoonoses are all important

Malaria The original malarious area of the Americas, covering 16,083,000 km, contained a population of 142,702,000 in mid-1960 Of this population, 38 per cent lived in areas from which it was claimed that malaria had been eradicated and 7 per cent in areas where the consolidation phase had been reached Some 37 per cent lived in areas where eradication was in the attack phase, and 14 per cent in areas where the eradication campaign had not yet begun Four thousand, seven hundred and ninety-two cases of smallpox were reported from seven countries

Aedes aegypti, the vector of urban yellow fever, has been eradicated in Bolivia, Brazil, British Honduras, the Canal Zone, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Honduras, Nicaragua, Panama, Paraguay, Peru and Uruguay, and in other countries such as Mexico and Argentina the programme is making progress The main problem at present is the continued existence of the mosquito in the United States and in some of the Caribbean Islands Fifty cases of jungle yellow fever were reported in 1960 from Bolivia, Brazil, Colombia, Peru and Venezuela, 30 being from Bolivia Although this disease is not amenable to the measures used in the eradication of urban yellow fever, elimination of the urban vector removes the risk of its spread

Although diagnostic facilities and reporting of cases of tuberculosis are less advanced in Latin America than in North America, relatively more new cases are reported in the former, and death-rates there in 1959 were at least three times greater Reporting has improved, but, despite this, the number of cases reported is declining everywhere Tuberculosis still remains one of the principal causes of death in Latin America, however

In 1958, there were nearly 10,000 new cases of leprosy distributed over 13 countries and 13 territories Approximately 200,000 persons are known to be suffering from leprosy in the Americas, and, if all cases were registered, this number would be higher In Latin America at present, more than 30,000 beds or 2½ per cent of existing hospital beds, are in leprosaria or leprosy units

Both syphilis and gonorrhœa are serious health problems in most Latin American countries Death-rates from syphilis and its sequelæ, including cardiovascular syphilis and syphilitic psychoses, are higher in more than half the Latin American countries than in the United States In one country the rate is more than four times as high

The zoonoses are widespread in the Americas Rabies is to be found in all countries of the region, except Uruguay, the total number of cases reported in man in 1958 being 176, in animals more than 7,000 Brucellosis also occurs widely in man and animals Testing of sample herds in Mexico showed that up to 68 per cent of the animals were reactors, and similar testing in Brazil showed a proportion of 57 per cent A Mexican authority estimates that there are 20,000 human cases due to *Brucella melitensis* in Mexico every year

In the twenty countries of Latin America, 33 million city dwellers, or 39 per cent of the urban population, in 1961, were without water from a community water supply Smaller cities and rural areas are even worse off It is estimated that 100 million persons in Latin America need proper water supplies This situation results in unfavourable living conditions, a high incidence of gastro-intestinal disease, and hampers industrial development

In Latin America most cities have no adequate sewage disposal facilities, and in rural areas many houses lack any kind of facilities for the disposal of excreta Little attention has hitherto been paid to water- or air-pollution or to the public health aspects of housing or municipal planning Measures to control industrial accidents and occupational diseases are unknown or just beginning to be introduced, and yet, as investigations in 13 Latin American republics have shown, there is a high incidence of sickness in industry and large sums have to be paid out in compensation. On the average, the cost of disability among the gainfully employed amounts to approximately 15 per cent of the national income.

Nutritional deficiencies are common in Latin America. In 10 countries, the average calorie consumption ranged from 1,960 to 3,100 calories daily in 1957. In some areas the animal protein available is less than 10 gm. per person per day. Cereals often provide up to 78 per cent of the total protein. As a result of the low consumption of good-quality protein, specific deficiency diseases, lack of resistance to infection, and poor physical development are often found. Inadequate consumption of necessary foodstuffs is to be attributed largely to lack of purchasing power and lack of understanding of the value of certain foods.

Dental caries affects more than 90 per cent of the adult population and there is no hope of increasing the number of dentists rapidly enough to carry out adequate treatment. The emphasis has thus been on prevention. Fluoridized water supplies are not found in 43 cities in Latin America.

In no country of the Americas are funds now available to build or to staff and maintain hospitals for the institutional care of all the mentally ill needing such care. In many countries, such as the United States, nearly half the number of hospital beds are in mental institutions. In Latin America, approximately 20 per cent are in mental institutions, the number varying from 0.1 to 1.5 per 1,000 population.

A METHYL ANALOGUE OF COBAMIDE COENZYME IN RELATION TO METHIONINE SYNTHESIS BY BACTERIA

By Dr. J. R. GUEST, Dr. S. FRIEDMAN* and Prof. D. D. WOODS, F.R.S.
Microbiology Unit, Department of Biochemistry, Oxford

AND

Dr. E. LESTER SMITH, F.R.S.
Glaxo Research, Ltd., Greenford, Middlesex

PREVIOUS investigations at Oxford into the biosynthesis of methionine by cell-free extracts of several strains of *Escherichia coli* have given strong evidence that there are two mechanisms for the final methylation of homocysteine[1–3]. One of these operates in many strains of the organism (for example, *PA*15, a serine/glycine auxotroph), but is absent from auxotrophs with an alternative growth requirement for vitamin B_{12} or methionine; it is independent of vitamin B_{12}, but has a strict requirement for a conjugated form of folic acid (for example, tetrahydropteroyltriglutamate, H_4PtG_3) as coenzyme for the transfer of the one-carbon unit from serine or formaldehyde, the ultimate source of the carbon of the methyl group. The second mechanism depends on the presence of a vitamin B_{12}-containing enzyme (B_{12}-enzyme) and can utilize either H_4PtG_3 or tetrahydropteroylmonoglutamate (H_4PtG) as co-factor; it is the obligatory pathway in vitamin B_{12}/methionine auxotrophs (for example, strain 121/176) and can be evoked in other strains by the addition of vitamin B_{12} either to the growth medium or to ultrasonic extracts.

The B_{12}-enzyme takes part in the overall enzymatic transfer of a methyl group from a methylated tetrahydrofolate (probably N^5-methylH_4PtG (refs. 4–6) to homocysteine[3]; $NADH_2$, FAD, ATP, Mg^{2+} and other enzymes (present in several other strains of *E. coli* tested) are also required. The reaction (a specific transmethylation) clearly differs from the isomerase reactions of the type catalysed by the known coenzyme forms of vitamin B_{12} (the cobamide coenzymes). The discovery[7] of a method for the chemical synthesis of these coenzymes and their analogues made possible metabolic investigations with the methyl analogue of dimethylbenzimidazolylcobamide coenzyme, that is, the analogue with a methyl group replacing the 5′-deoxyadenosyl group linked to the cobalt. The analogue was prepared[7] by the action of dimethyl sulphate or methyl iodide on fully reduced hydroxocobalamin and was purified by passage through DEAE- and CM-cellulose columns in series and crystallized from aqueous acetone. For brevity this analogue will be referred to as 'methylcobalamin'.

When homocysteine was incubated with 'methylcobalamin' there was a slow spontaneous reaction yielding methionine. The rate of this reaction was increased at least ten times by a purified (hundred-fold) preparation[8] of the B_{12}-enzyme (Table 1); the differential between spontaneous and enzyme-catalysed reactions could presumably have been increased by increased enzyme concentration since the rate of the reaction was directly proportional, over the range tested, to the concentration of enzyme (Fig. 1). Continuing spontaneous formation of methionine after incubation with the enzyme had ended was prevented by oxidizing the residual homocysteine. Reaction mixtures were heated at 100° for 10 min. and cooled; after adding 0.1 ml. of 6 per cent (w/v) hydrogen peroxide the mixture was stood for 15 min. at room temperature. Residual hydrogen peroxide was removed by treatment with catalase (0.1 ml. of 8 μM) for 10 min. and the enzyme then destroyed by heating at 100° for 10 min. This procedure did not reduce the recovery of added methionine; the test organism in the microbiological assay used[9] responds equally to methionine and its sulphoxide.

Maximum enzymatic formation of methionine occurred over the range *p*H 7.4–8.7, and no substrates or co-factors were required other than homocysteine and 'methylcobalamin' (Table 1). The reaction proceeded linearly with time up to the limit tested

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* Fellow of the U.S. National Foundation.

Table 1 FORMATION OF METHIONINE FROM HOMOCYSTEINE AND 'METHYLCOBALAMIN'

Addition or modification	L-Methionine formed (mμmoles)
None	9*
B_{12}-enzyme	188
B_{12}-enzyme + 'co-factors'	113
B_{12}-enzyme (aerobic incubation)	100
B_{12}-enzyme (heated 3 min at 100°)	14
Albumin (0 5 mg)	17

* Zero if 'methylcobalamin' was omitted, indicating 9 mμmoles were formed by the non-enzymatic reaction

The reaction mixture contained 'methylcobalamin' (0 7 μmoles), DL-homocysteine (7 5 μmoles), mercaptoethanol (10 μmoles), potassium phosphate buffer, *p*H 7 8 (160 μmoles), B_{12}-enzyme (12 units, ref 8) in a volume of 1 ml The 'co-factor' mixture contained alcohol dehydrogenase (0 15 mg) and (μmoles) FAD, 0 1, ATP, 1 0, $MgSO_4$, 1 0, DPN, 2 0, ethanol, 45 Incubated 1 5 h at 37° under hydrogen

(3 h), but the rate was almost halved by aerobic incubation (Table 1) Quantitative yields of methionine in terms of the methyl group of 'methylcobalamin' were obtained in the presence of excess homocysteine, and the colour of the reaction mixture changed from red to the brown characteristic of vitamin B_{12r} (ref 10) It is not, however, possible to designate the latter as a product of the reaction because it could be formed from hydroxocobalamin and the sulphydryl compounds present in the reaction mixture

The fact that hydroxocobalamin was inactive in the reaction described above provides indirect evidence that the methyl group of 'methylcobalamin' transferred to homocysteine is that linked to the cobalt and not one of those present as side groups on the corrin ring Direct confirmation was obtained by the use of [Me-^{14}C] 'methylcobalamin', which was prepared from $^{14}CH_3I$ (4 7 μc /μmole)[7] After incubating this with B_{12}-enzyme and L-homocysteine the methionine formed was isolated free from B_{12}-derivatives by developing descending chromatograms on paper with *n*-butanol/acetic acid/water (4 1 5) The eluted methionine was diluted with inactive carrier methionine and recrystallized to constant specific activity Degradation by the technique given by Sakami[11] showed that carbon-14 was present principally in the methyl group (Table 2)

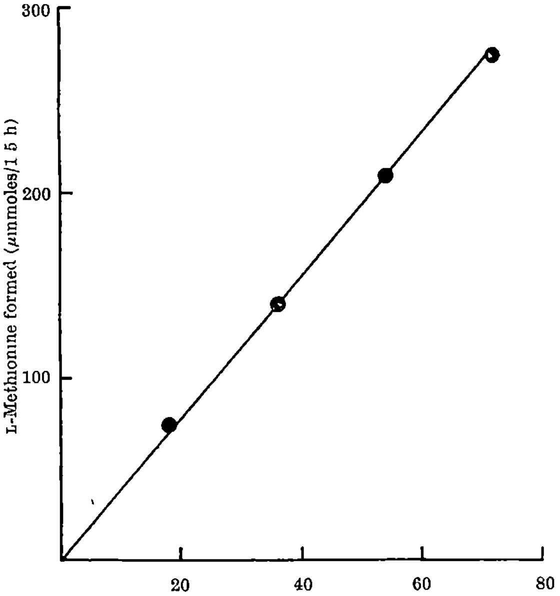

Fig 1 Effect of enzyme concentration on the formation of methionine

Conditions as in Table 1, except that the amount of B_{12}-enzyme was varied The amount of methionine formed non-enzymatically during the period of incubation (10 μmmoles) has been deducted

Table 2 INCORPORATION OF [Me-^{14}C] 'METHYLCOBALAMIN' INTO METHIONINE

	Specific activity (counts/min/μmmole)
'Methylcobalamin'	2,660
Methionine isolated	2,450
Methyl group of methionine (as tetramethylammonium iodide)	2,220

The specificity of the enzymatic reaction was examined qualitatively by chromatography 'Ethylcobalamin' (prepared by an analogous method to that used for the methyl derivative) and dimethylbenzimidazolylcobamide coenzyme ('5′-deoxyadenosylcobalamin' in the present terminology) were each incubated with homocysteine and crude or purified preparations of the B_{12}-enzyme, there was no significant formation of ethionine or *S*-adenosylhomocysteine, respectively 'Ethylcobalamin', however, caused a 65 per cent inhibition of methionine formation from 'methylcobalamin' when present at three times the concentration of the latter No *S*-methylcysteine was detected when 'methylcobalamin' was incubated with the enzyme and cysteine instead of homocysteine, and only traces of *S*-adenosylmethionine when '5′-deoxyadenosylcobalamin' was incubated with methionine The enzyme is thus relatively specific for both donor and acceptor substrate

'Methylcobalamin' was four times more effective than any other cobalamin derivative tested in promoting the *in vitro* formation of B_{12}-enzyme from a crude source of apo-enzyme in the absence of a reducing system, when the latter was present 'methylcobalamin' and hydroxocobalamin were equally active and superior to cyanocobalamin (Table 3)

Table 3 FORMATION *in vitro* OF B_{12}-ENZYME

Cobalamin derivative added	B_{12}-enzyme formed (units) with reducing system Absent	Present
Hydroxocobalamin	6	22
Cyanocobalamin	2	12
'Methylcobalamin'	22	24
'5′-Deoxyadenosylcobalamin'*	4	4
Sulphitocobalamin	4	5
None	1	1

* Dimethylbenzimidazolylcobamide coenzyme

The cobalamin derivative (1 2 μmmoles) was incubated for 30 min at 37° under hydrogen with an ultrasonic extract of *E coli PA*15, which had been dialysed and treated with 'Dowex-1' resin The total volume of the reaction mixture, which also contained 200 μmoles of potassium phosphate buffer, *p*H 7 8, was 2 7 ml The reducing system contained alcohol dehydrogenase (0 3 mg) and (μmoles) FAD, 0 2, DPN, 3, ethanol, 90 Samples (0 6 ml) of the reaction mixture were assayed (ref 12) for B_{12}-enzyme in the presence of the anilide analogue of the vitamin

One possible interpretation of these results is that 'methylcobalamin' exists in a transient state as the prosthetic group of the B_{12}-enzyme in the B_{12}-dependent pathway for the methylation of homocysteine, and that oxidized, reduced and methylated forms of cobalamin occur successively during the action of the enzyme The fact that reduced cobalamin is labile to oxygen (and 'methylcobalamin' to light) perhaps explains why the form in which cobalamin has so far been isolated by us from purified B_{12}-enzyme is mainly hydroxocobalamin, sometimes with traces of a more cationic form[13]

An alternative possible mechanism for the new reaction demonstrated in this communication is that the apo-enzyme of the B_{12}-enzyme combines with 'methylcobalamin' to yield a complex that transfers the methyl group to homocysteine and at the same time dissociates liberating the de-methylated cobalamin (which may undergo secondary changes), and the apo-enzyme which then combines with another molecule of 'methylcobalamin' Since, however, vitamin B_{12} derivatives are in general strongly bound to protein, it is also possible that 'methylcobalamin' transfers its methyl group to whatever form of vitamin B_{12} is the prosthetic group of the B_{12}-enzyme, with resultant methylation of either the cobalt atom or of one of the methene bridge carbons of the corrin ring[14], the methyl group is finally envisaged as passing to homocysteine

'Methylcobalamin' could only act catalytically (either as the prosthetic group of B_{12}-enzyme or as a free intermediate) in the overall methylation of homocysteine by serine if there were a mechanism for its reconstitution from the de-methylated cobalamin derivative and *N*-methyltetrahydrofolate Further work on this aspect is in progress In this connexion it may be noted that both methionine synthesis mediated by the B_{12}-enzyme and the synthesis of cobamide coenzyme by extracts of *Propionibacterium shermanii*[15] and *Clostridium tetanomorphum*[16] require the presence of a reducing system containing a flavin derivative, this suggests that in all these processes reduction of cobalamin must precede substitution, as it does also in the chemical synthesis of the coenzyme and its methyl analogue[7]

Finally, it should perhaps be recalled that, in strains of *E coli* that are not vitamin B_{12} auxotrophs and when H_4PtG_3 is the folic acid coenzyme (and its N^5-methyl derivative the immediate methyl donor) the methyl group is transferred to homocysteine without the necessity for the presence of a B_{12}-enzyme, of reducing conditions or of ATP[3]

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CONDITIONS FOR ESTABLISHING IMMUNOLOGICAL TOLERANCE TO A TUMOUR VIRUS

By Prof H RUBIN

Department of Virology, University of California, Berkeley

THE viruses that are responsible for the avian leukosis complex of neoplasms can be transmitted from hen to progeny through the egg[1,2] When a virus of this group is thus congenitally transmitted, it multiplies extensively throughout the embryonic and post-embryonic life of the affected chicken[3,4] The congenitally infected chicken gives no sign of immunological responsiveness to the virus either by the production of neutralizing antibody or elimination of the virus from the blood Since it is fully competent to produce antibody to other antigens, such as influenza virus, it is considered to be specifically tolerant to the congenitally transmitted virus

Uninfected chicks placed in contact with congenitally infected chicks develop a transient viræmia which is almost invariably followed by antibody production[4] The sharp distinction between the consequences of congenital and of contact infection suggested that deliberate infections of embryos and young chicks at closely spaced intervals of time could be used to define more precisely the factors involved in producing the alternative states of immunological tolerance or responsiveness to the virus The experiments to be described show that tolerant infection with an avian leukosis virus can be produced in chickens by deliberate infection of the embryo The chief requirement for eliciting immunological tolerance rather than responsiveness in a chicken is that the virus multiply to a high level during the first few weeks of life after hatching

The leukosis virus used in the work described here is known as 'resistance-inducing factor' (RIF), because it is assayed by interference with the growth of Rous sarcoma virus (RSV)[2] The RIF stock was obtained from the plasmas of congenitally infected chickens and had 1–2 × 10^6 infectious units per ml The birds used were of the Kimber Farms strain 813 in which the incidence of leukosis is low My experience with this strain indicates that only about 1 in 50 embryos is naturally infected with RIF Up to the sixth day of embryonic incubation infection was carried out by direct inoculation of the embryo or into the yolk sac From the ninth day onward, inoculation was either intra-embryonic or intravenous After hatching, inoculation was either intraperitoneal or intracardiac Unless otherwise stated, the inoculum was 0 1 ml of undiluted RIF stock and therefore contained 1–2 × 10^5 infectious units

Heparinized blood was obtained from each chick one week after hatching and every other week afterwards The plasma was tested for the presence of RIF by the induction of interference with RSV in cultures of chick embryo cells When cultures were infected with plasmas containing more than 10^5 infectious units of RIF per ml , and challenged with RSV on transfer 4–5 days later, there were less than one-tenth as many RSV foci as in control cultures uninfected with RIF Plasmas with approximately 10^3–10^5 and 10^1–10^3 infectious units of RIF induced comparable reductions in RSV foci in chick embryo cultures challenged after 2 and 3 transfers, respectively

The presence of antibody to RIF was detected by neutralization of RSV The validity of this procedure is based on the fact that RIF and RSV have cross-reacting antigens, and that the neutralizing activity of a serum against one of these viruses is roughly proportional to its activity against the other[3,4]

The testing of repetitive bleedings showed that the chickens could be divided into three categories according to their immune response to infection The first category consisted of birds which had become immunologically tolerant to RIF These birds had a high-level viræmia (1–2 × 10^6 infectious units of RIF per ml plasma) which showed no signs of subsiding by the final bleeding made at 16 weeks of age At no time was there any evidence of antibody to RIF or RSV

The second category, designated as partially tolerant, consisted of only a few birds They had levels of RIF which ultimately reached a fairly constant level between 10^3 and 10^5 per ml of plasma (that is, interference with RSV did not become evident until infected cultures had been transferred twice) Birds in this group occasionally had low levels of antibody to RSV concurrently with low levels of RIF

The third category consisted of birds which had a transient viræmia followed by the appearance of antibody This group was fully competent to react immunologically to the viral antigens

Age at Inoculation and Induction of Tolerance An experiment was carried out to determine the effect of age and route of infection on the induction of tolerance to the virus Inoculations were made by various routes into embryos and chickens up to 43 days after hatching In Table 1, the outcome of infection of the various groups is represented It can be seen that the yolk sac was an inefficient route for inducing tolerance In fact when the yolks of six freshly laid eggs were inoculated with RIF no evidence of virus growth was obtained, that is, neither tolerance nor immunity ensued when the birds became immunologically mature

Table 1 EFFECT OF THE ROUTE AND AGE OF INOCULATION ON THE IMMUNE RESPONSE

Route of inoculation	Day of infection	No inoculated	Tolerant	Partially tolerant	Immune
Yolk sac	0 day embryo	6*	0	0	0
	3 ,, ,,	4	2	0	2
	6 ,, ,,	6	1	2	3
Intra-embryo	4 ,, ,,	7	7	0	0
	10 ,, ,,	5	4	0	1
	17 ,, ,,	2	1	0	1
Intravenous	9 ,, ,,	4	3	0	1
	13 ,, ,,	3	2	1	0
Intracardiac	1 day after hatching	3	2	1	0
Intraperitoneal	,, ,, ,, ,,	8	0	1	7
	15 days ,, ,,	7	0	0	7
	43 days ,, ,,	7	0	0	7
Totals		62	22	5	29

In each case, 0 1 ml of undiluted RIF stock containing 1–2 × 10^6 infectious units was inoculated Blood samples were obtained every other week beginning at one week after hatching and continuing until the 16th (or, in a few cases, the ninth) week after hatching All plasmas were tested for RIF and antibody to RSV

*There was no evidence of successful virus multiplication in any of the 6 embryos inoculated by the yolk sac route at 0 days, that is neither tolerance nor immunity developed

Out of 10 chickens infected in the yolk sac at 3 and 6 days only three developed tolerance to RIF while two became partially tolerant and five developed immunity The inefficiency of the yolk sac route in producing tolerant infection was probably due to a combination of the high viscosity of yolk and the presence in yolk of maternally transmitted antibodies The high viscosity prevents free movement of virus particles to sites at which they can multiply and allows ample opportunity for inactivation by antibody and heat

Direct inoculation of the embryo with RIF at 4 and 10 days of incubation resulted in tolerant infection in almost all (11 of 12) chickens Infection of 17-day-old embryos by this route seemed to be less efficient in establishing tolerance than earlier infection, but the small number of birds in this group was insufficient for a secure evaluation

Intravenous infection of embryos at 9 and 13 days of incubation led to tolerance in most (5 of 7) of the chickens, but partial tolerance and immunity followed in a few

Attempts to induce tolerance by infection after hatching were generally unsuccessful Only intracardiac infection at one day resulted in some tolerant infections (2 of 3) Intraperitoneal infection at one day resulted in immunity in most (7 of 8) birds and only a partial tolerance in one bird All infections at 15 and 43 days after hatching resulted in immunity These results showed that early infection was required to produce tolerance to RIF

Level of Virus Multiplication and Induction of Tolerance The failure to induce tolerance by the yolk sac route, despite early infection, indicated that the age of the chickens at the time of inoculation was not the sole factor in determining the type of immunological reaction in the host Inspection of the levels of viræmia and of antibody during the first 3 weeks after hatching indicated that the virus had to multiply to high concentrations in the young chick in order to establish a tolerant infection This requirement is evident from the results illustrated in Fig 1, where representative birds from the groups inoculated at different ages have been regrouped according to the outcome of infection These selected case- and group-histories are representative of the various levels and times of virus multiplication which culminate in tolerance or immunity

It can be seen from Fig 1 that the attainment of a high-level viræmia by the first week after hatching (class *A*) assured a tolerant infection A low-level viræmia in the first week (classes *B*, *C*, *D*, *F*, *G*) assured a high level of virus multiplication by the third week, but occasionally resulted in a partial rather than full tolerance (classes *F*, *G*)

Birds, the sera of which were free of detectable virus at one week but which had developed heavy viræmia by 3 weeks (classes *E*, *H*, *I*, *J*, *K*), had about an equal chance for developing tolerant or immune infection Some birds in this group which developed an immune response did so despite persistence of the high-level viræmia through the fifth week (classes *I*, *J*, *K*)

All birds which failed to reach high levels of viræmia by the third week developed immune infections

Table 2 EFFECT OF THE TIME AND LEVEL OF VIRUS MULTIPLICATION DURING THE FIRST 3 WEEKS OF LIFE ON THE IMMUNE RESPONSE

Conditions leading to	Viræmic level after hatching		Immunological outcome		
	1 week	3 weeks	Tolerant	Partially tolerant	Immune
Tolerance	+++	+++	16	0	0
	++	+++	7	2	0
	++	++	1	0	0
	+	+++	1	0	0
Tolerance or immunity	—	+++	4	1	3
Immunity	—	++	0	0	6

+++ = >10^5 infective units RIF per ml plasma , ++ = 10^3–10^5 infective units RIF per ml plasma , + = 10–10^3 infective units RIF per ml plasma , — = < 10 infective units RIF per ml plasma

The results are compiled from several experiments Birds which failed to develop detectable viræmia by the third week after hatching are not included , all such birds developed an immune response

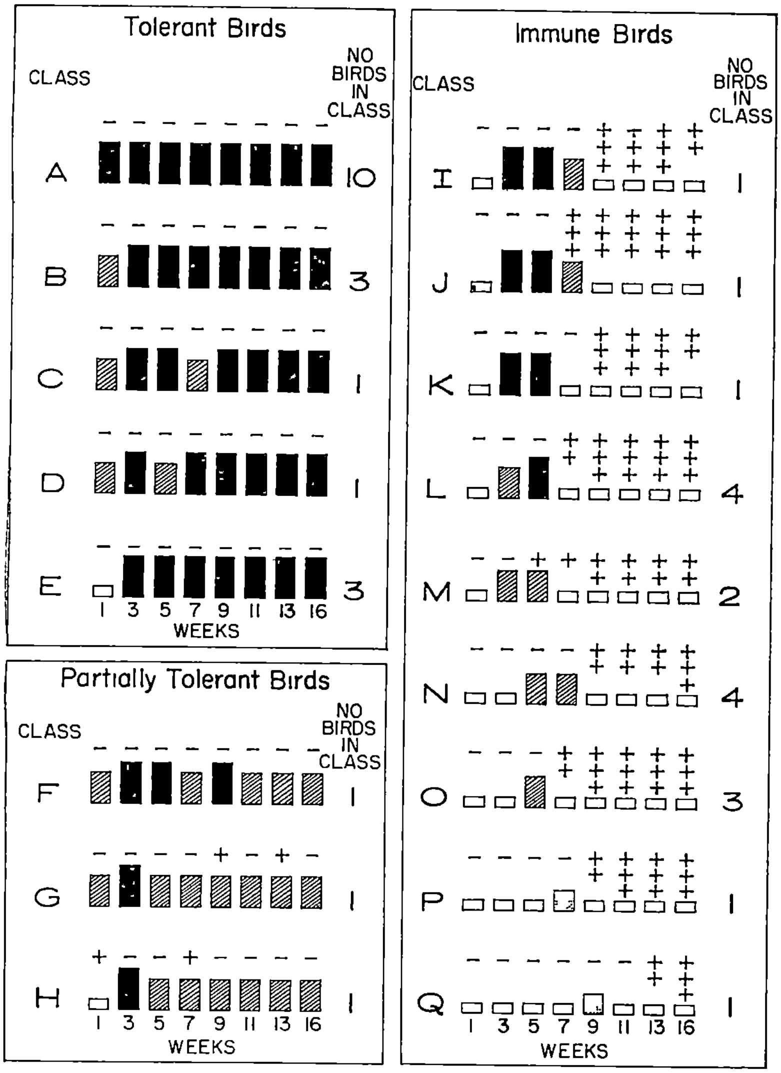


Fig 1 Relation between course of virus multiplication and immune response Representative birds have been selected from the experiment of Table 1 to illustrate in detail the range of situations with regard to viræmia and antibody which leads to tolerance or immunity Only those birds infected as embryos or on the first day after hatching are included

Antibody-levels

Symbol	RSV survivors after exposure to 1 10 dilution of plasma	Interpretation
–	0 5–1	No antibody
+	0 2–0 49	Weak antibody
+ +	0 1–0 19	Moderate antibody
+ + +	< 0 1	Strong antibody

Viræmic-levels

Symbol	Transfer at which plasma-infected culture became resistant to RSV	Interpretation
White	Remained sensitive through three transfers	Negative for RIF (< 10^1 infectious units/ml)
Stippled	Third transfer	Low RIF concentration (10^1–10^3 infectious units/ml)
Oblique shading	Second transfer	Moderate RIF concentration (10^3–10^5 infectious units/ml)
Black	First transfer	High RIF concentration (> 10^5 infectious units/ml)

(classes *L–Q*) Viræmia was never detectable in more than two successive bleedings in such birds, and was of a low or moderate level Antibody became detectable 2–4 weeks after the viræmia was first detected, and persisted indefinitely

Relevant data from several experiments is condensed in Table 2 Here again, it can be seen that a bird with viræmia at one week was almost certain to become tolerant to RIF Birds negative at one week but heavily viræmic at three weeks had an even chance for developing tolerance or immunity Birds negative at one week and only moderately viræmic by the third week became immune All birds which developed viræmia later than three weeks became immune

Inoculation Dose and the Induction of Tolerance The results described in the preceding section demonstrate that high concentrations of virus must be produced within the first few weeks after hatching to establish a tolerant infection with RIF Since it has been demonstrated that the initial growth-rate of RIF is a function of the infecting dose[2], it was anticipated that a reduction in the dose inoculated into the embryo would delay the appearance of a high-level viræmia and thereby lower the frequency of tolerant infections That this is indeed the case was demonstrated in the following experiment

Eleven-day-old embryos were infected intravenously with ten- and one hundred-fold dilutions of the RIF stock The inocula represented approximately 10^4 and 10^3 infectious units of RIF, respectively Beginning at one week after hatching, the birds were bled every other week until the ninth week This termination-time was chosen because the previous experiment had shown that a constant level of viræmia characteristic of a bird for its life-time was reached by nine weeks after hatching in those individuals which had been infected as embryos

The results of this experiment are presented in Fig 2. It can be seen that all five birds which received the 1 10 dilution of virus developed a viræmia at one week which led to tolerant infection, while this occurred in only one of the four receiving a 1 100 dilution of the virus In the remaining three birds receiving the 1 . 100 dilution, viræmia did not become detectable until the third week or remained undetected When the viræmia occurred in these birds it was of a low level and transient, and was followed by appearance of antibody Thus, it is clear that reducing the size of the virus inoculum delayed development of viræmia and resulted in immune rather than tolerant infection

The results of this investigation show that high levels of virus multiplication must be reached during the first few weeks after hatching if a tolerant infection with RIF is to be established in chickens Because of the slow multiplication of RIF, it is

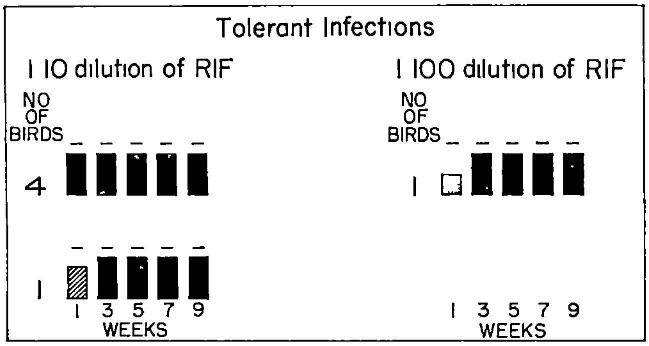
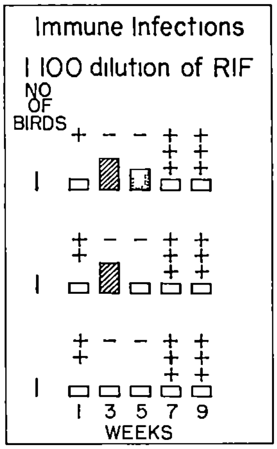


Fig. 2. Eleven-day embryos were infected intravenously with 0.1 ml. of a 1 : 10 or 1 : 100 dilution of RIF stock, and their viræmic and antibody status determined in bimonthly bleedings beginning at 1 week. Antibody detectable in the first bleeding was passively transferred from the hen. Symbols have the same meaning as in Fig. 1.



necessary to infect the embryo with high concentrations of virus to ensure tolerant infection. This finding is consistent with the observation that congenital infection, which results in high levels of virus multiplication in the embryo, results in tolerance to RIF, while contact infection, which occurs after hatching, generally elicits antibody production[4].

There are several alternative explanations for the efficacy of early exposure to RIF in establishing a tolerant infection. One possible explanation derives from Burnet's clonal selection hypothesis[5]. On this hypothesis, cells potentially capable of producing antibodies to RIF are eliminated in an unexplained way when the immature animal is exposed to high concentrations of the virus. This would imply that cells with immunological potential in the immature animal interact with antigen in a different way than they do in the mature animal.

Another, and perhaps more plausible, explanation is that cells with immunological potential in animals of any age can be diverted from expressing this potential, if they are exposed to high enough concentrations of antigen for a long enough time. This situation would be similar to the state of immunological paralysis induced in older animals by the inoculation of large amounts of polysaccharides[6] or soluble proteins[7]. Under this hypothesis the failure to establish tolerant RIF infection in older animals could be ascribed to the presence of antibody-producing cells which limit the concentration and persistence of the virus by restricting its spread and speeding its elimination. The ease of establishing tolerant infection in young birds might therefore be due merely to the absence of immunologically mature cells.

Selected data in this article can be used to support or refute either hypothesis, but the second hypothesis seems more attractive at present because it requires fewer assumptions. In any event, the RIF system should be a useful tool in elucidating the mechanism by which tolerance is induced since the concentration of the tolerance-inducing antigen can be accurately determined at all times by virus infectivity measurements, and the antibody response gauged with a high degree of sensitivity and specificity by the virus neutralization test.

Tolerance has been considered here largely as an all-or-none phenomenon. The inadequacy of such treatment is evident from the occurrence of birds classified as partially tolerant. Such birds develop low-level, but persisting viræmias, and sometimes have low concentrations of antibody. The segregation of these birds into a distinct category is probably artificial. There is no assurance that all the birds classified as fully tolerant are equally unreactive to the viral antigen. It can only be said that within a factor of 2 or 3 the level of persisting viræmia is about the same in all 'tolerant' birds and antibody can be detected in none. It is quite possible that there is a continuous spectrum of immunological behaviour running from complete to partial tolerance which escapes our present techniques of measurement.

This investigation was supported by grant No. 4774 from the U.S. Public Health Service and grant No. *E*-82 from the American Cancer Society.

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KININASES IN LEUCOCYTES AND OTHER TISSUES

By DR. J. SCHWAB

Lister Institute of Preventive Medicine, London, S.W.1

PLASMA[1], lymph[1] and kidney extracts[2] inactivate bradykinin, but the site of origin and the availability of the inactivator in cells and intercellular fluids, and its relation to inflammatory events, in which both it and bradykinin may play a part, are unknown[3]. Since the onset of the major phase of increased vascular permeability in the early stages of bacterial and other acute inflammation coincides with the onset of frank tissue leucocytosis[4], and the decline of this phase some hours later with massive

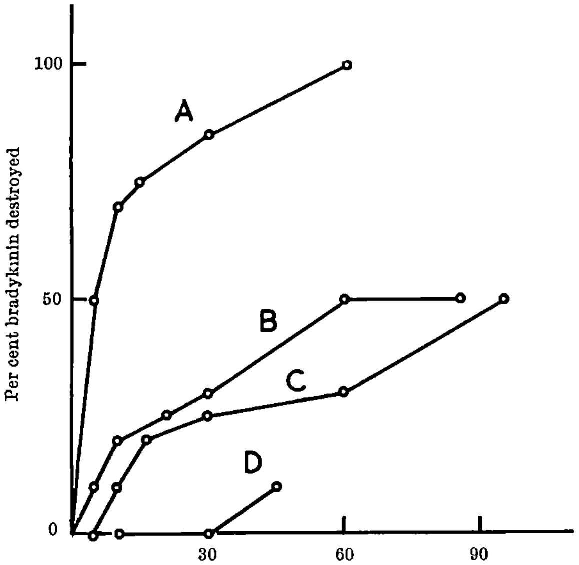

Fig 1

leucocytosis, it was of interest to find whether leucocytes produced either a bradykinin-like permeability factor or an inhibitor of such kinins

Leucocytes were collected from the peritoneal cavity of the guinea pig 4 hr after the injection of 25 mgm mussel glycogen in 25 ml 0 85 per cent saline, washed three times with 0 1 *M* phosphate-buffered saline or Tyrode solution at *p*H 7 4 They were disrupted either by 2½ min sonic vibration or by thrice freezing to the temperatures of carbon dioxide snow in ethanol and thawing The permeability effect of extracts and of bradykinin were measured in terms of the diameter of blue lesions in the dorsal skin of the trunk after intracutaneous injection of the material into depilated guinea pigs with pontamine blue in the circulation[5] As with most permeability-increasing substances, bradykinin induced in the skin lesions the diameter of which was proportional to log concentration, thus potency and change in potency could be estimated from three-point dosage–response lines, using concentrations producing lesions from 4 5–9 0 mm in diameter Crude bradykinin was prepared by tryptic digestion of ox serum globulin, and its kinin effect measured on the isolated atropinized guinea pig ileum in Mg-free Tyrode solution in a 10 ml bath at 34°

Permeability effect of leucocytes Three times washed leucocytes up to 15 × 10^6/ml , and disintegrated leucocytes from suspensions containing up to 15 × 10^6/ml had no effect either on vascular permeability or on the guinea pig ileum There is thus no readily available kinin in the cells

Kinin inhibitors in leucocytes Incubation of bradykinin for 1 hr at 34° with intact leucocytes slightly decreased its permeability-increasing potency incubation with extract, both sonic and freeze-thaw, decreased it four-fold An extract of 10 × 10^5 leucocytes/ml is required significantly to decrease the potency of three effective 'blueing' doses of bradykinin Equipotent amounts of histamine, the synthetic amine compound 48/80 or the protease permeability factors of human and guinea pig serum were unaffected by the extract Inhibition is dependent on the time and temperature of incubation, the concentration of bradykinin, and the medium (extracts in phosphate-buffer saline were three times as potent as in Tyrode at the same *p*H)

The ileum-contracting potency of bradykinin decreases in parallel with its permeability effect Fig 1 illustrates the dependence of inhibition of the contractor effect on time (*A*), the partial inactivation of the extract at 56° for 30 min (*B*), the feeble activity of intact leucocytes (*C*), and the very slight activity of the first washings of intact leucocytes after removal from the peritoneal cavity (*D*) The last observation excludes contamination with humoral substances in the exudate—particularly the powerful inhibitor present in plasma—as a source of the inhibitor The extract loses half its potency after 24 hr at − 10° Intact leucocytes held for 2 hr at 37° in Tyrode in siliconized tubes yield on extraction about half the amount of inhibitor obtainable from unheated cells

The extract was unaffected by immediately prior incubation for 60 min at 20° in Tyrode at *p*H 7 4 with 5 × 10^{-4} *M* di-isopropyl-fluorophosphonate, 0 25 mgm /ml soya bean trypsin inhibitor, 5 × 10^{-3} *M* ascorbic acid, 5 × 10^{-3} *M* ethylenediaminetetra-acetate, 20 × 10^{-3} *M* D-L-leucyl-glycyl-glycine, 20 × 10^{-3} *M* D-L-leucyl-glycine, 5 × 10^{-3} *M* iodoacetate, 5 × 10^{-3} *M* indole, and 5 × 10^{-3} *M* reduced glutathione The inhibitor appears to be an enzyme, distinct from trypsin, chymotrypsin, cathepsin *C* and leucine aminopeptidase

Differential counts of cells in guinea pig peritoneal exudates induced in various ways and calculation of the number of cells yielding extract enough to inhibit half a fixed amount of bradykinin (ID_{50} = 50 per cent inhibiting dose) suggest (Table, *a*–*c*) that cells other than granulocytes were the source of the inhibitor, though the suggestion is weakened by the relatively feeble yield from non-granular leucocytes induced by paraffin (*c*) Exudates induced by glycogen in the rabbit (*d*) were similar to those in the guinea pig (*a*)

Table 1

Exudate-inducing agent and period of induction (hr)		No of cells per ml (× 10^{-5}) yielding one ID_{50}		
			Non-granulocytes	
		Total	Per cent	No
(*a*) Glycogen	4	69	6	4 2
	4	52	7	3 5
	96	6 8	89	6 1
(*b*) Bovine albumin (allergic)	4	30	24	7 2
	72	8 2	70	5 7
	72	15	83	12 4
(*c*) Liquid paraffin	23	48	80	38
	96	25	90	22 5
(*d*) Glycogen (in rabbit)	18	61	10	6 1

The absence of any peculiar association of inhibitor with cells of the lymphoid-macrophage system is also evident from the activity of fresh guinea pig tissues with varying content of lymphoid tissue Extracts were made of guinea pig organs which had been perfused *in situ* with human-albumin-Tyrode solution before excision, rinsed in Tyrode, lightly blotted, weighed emulsified in Tyrode in a blender for 30 sec , thrice frozen and thawed, and centrifuged at 2,000 r p m for 12 min The wet weight in milligrams yielding one ID_{50} were kidney, 0 3, spleen, 0 8, liver, 1 0, thymus, 1 8, lymph nodes, 2 3, and heart muscle 2 5

Inhibitor in plasma and tissue fluids Guinea-pig plasma is particularly rich in inhibitor The ID_{50} of fresh citrated plasma in five guinea pigs averaged 5 8 × 10^{-4} ml Inhibitor also appears to be available in the intercellular fluid On intercellular perfusion[6], a strip of skin 30 × 15 mm yields most of its inhibitor in the first 3 ml. of perfusate, and in each of the five guinea pigs, the ID_{50} of this 3 ml. sample was consistently about one-fiftieth of that in the plasma—the average ID_{50} being $2{\cdot}7 \times 10^{-2}$ ml.—suggesting that the intercellular inhibitor originates in the blood.

Inhibitor in infected animals. Plasma and intercellular inhibitor are affected by bacterial infection. In guinea pigs with a single *Staph. aureus* lesion in the skin, 8 mm. in diameter and 24 hr. old, the titre of inhibitor in plasma and in perfusates of unaffected skin of the opposite flank was twice that of uninfected animals. This infection is local and regressive; in guinea pigs with a progressive infection, on the fifth day of a febrile, Group C streptococcal septicæmia, the titre in both plasma and skin perfusate was only half that of uninfected animals.

In summary, leucocytes contain a bradykininase-like substance which could be available for the destruction of kinins that might be formed in inflamed tissues. There is as yet little evidence that vaso-active kinins of this type are so formed; but the observation that kininases of plasma and intercellular fluids are increased in a non-progressive, and decreased in a progressive, bacterial infection is compatible with, though by no means indicative of, changes in a kinin-kininase system during infective inflammation.

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ROLE OF THE THYMUS IN NEONATAL LIFE

By Dr. DELPHINE M. V. PARROTT and Dr. JUNE EAST

Imperial Cancer Research Fund, London, N.W.7

PRESENT work indicates that the thymus plays an important part in the development of immune response in embryonic and neonatal life. For example, Kalmutz[1] found that the embryo opossum was able to react against foreign antigens as soon as the thymus was functional and before the spleen or lymph nodes became active. It has also been shown that thymectomy performed within 24 hr. of birth impairs the immune response of mice and rats to skin homografts[2-4] and to grafts of foreign cells[5]. In addition, Parrott[5] reported that mice thymectomized at birth died prematurely with wasting symptoms, and it is this aspect of the problem that we have now studied in greater detail.

Mortality of thymectomized mice (Table 1). Most of the *C3H/Bi* and *AKR* mice thymectomized during the first week of life using anæsthesia produced by cooling[6] grew normally until they were 4 weeks old, but those thymectomized within 24 hr. of birth died before the age of 3 months with severe wasting symptoms. The timing of the operation is obviously important because fewer animals died when thymectomy was delayed for 24–36 or 48–72 hr. and these deaths occurred over a long period of time. None of the mice thymectomized at 3–7 days has yet died, and they are now 8–9 months old and 56 animals sham-operated within 48 hr. of birth lived their normal life-span.

C57BL mice thymectomized on the day of birth died precipitately and at an early age. However, with one exception, outbred *TO* mice survived the operation without any apparent ill-effects. This may be attributable to the greater maturity of the thymus at birth and in fact 2/6 *TO* mice thymectomized immediately after delivery by Cæsarean section on the eighteenth day of gestation have already died with wasting symptoms at 8 and 11 weeks of age.

Table 1. MORTALITY OF MICE THYMECTOMIZED WITHIN 3 DAYS OF BIRTH

Strain	Age at thymectomy (hr.)	Mortality	Mean age at death and range (days)
C3H/Bi	< 24	17/17	45 (31–55)
	24–36	10/17	66 (34–169)
	48–72	5/15	141 (99–252)
AKR	< 24	12/12	38 (21–83)
	24–36	7/15	43 (25–75)
	48–72	5/16	83 (51–123)
C57BL	< 24	13/13	34 (30–39)
TO	12–24	1/9	52

Symptoms of wasting disease. Death in thymectomized mice was preceded by a progressive deterioration in their general appearance with stunting or loss of body-weight. Coat condition was poor, the mice were hunched, walked with a curious high-stepping gait and often had diarrhœa.

The most obvious post-mortem features were the thinness of skin and bone and the lack of subcutaneous fat. Liver necroses and hæmorrhage into the gut were frequently seen and the gut wall itself was often thin and flabby. The spleen and lymph nodes varied in size, but the Peyer's patches were always small or atrophic and histological examination showed that the lymphoid follicles of all these tissues were either disorganized or completely absent. The follicles of the lymph nodes were replaced by histiocytes and proliferating reticular tissue and there were sometimes accumulations of pyroninophilic cells in the medullary region. The red pulp of the spleen was increased and its sinuses were packed with polymorphs. Lymphoid depletion was already present in mice killed between 20 and 30 days of age and therefore preceded the onset of physical deterioration.

Changes in the leucocyte population of the circulating blood (Table 2) reflected the histological picture of the lymphoid organs. Lymphopenia was constantly found whether symptoms of wasting were present or not and was frequently accompanied by granulocytosis. Thus, the lymphocyte : polymorph ratio was often completely reversed although the total leucocyte count was unaltered. The association of neutrophilia with lymphopenia is known to occur in other experimental situations[7] but its significance is not understood.

Blood erythrocyte counts were within the normal range[5], although varying degrees of microcytic anæmia

Table 2. Leucocyte Counts of Mice thymectomized at Birth or after Delivery by Cæsarean Section

Strain	No. of mice	Age at thymectomy (hr.)	Age at examination (days)	Leucocyte counts (cells/mm.^{3} ± *S.D.*) Total	Lymphocytes	Polymorphs
C3H/Bi	3	< 24	31	1,533 ± 463	691 ± 364	761 ± 409
	4	Intact	31	3,225 ± 722	2,312 ± 261	692 ± 303
	9	< 24	39–60†	3,650 ± 1,839	466 ± 328	3,108 ± 1,680
	8	Intact	39–60	3,256 ± 647	2,222 ± 514	1,033 ± 424
AKR	4	< 24	32–50†	5,758 ± 1,006	1,240 ± 269	4,415 ± 927
	4	Intact	32–50	3,375 ± 201	2,357 ± 426	964 ± 370
C57BL	5	< 24	34–39†	4,980 ± 1,494	2,621 ± 762	2,357 ± 1,196
	4	Intact	34–39	7,500 ± 2,995	6,520 ± 2,650	902 ± 300
TO	2	C.S.*	42	4,950	1,178	3,579
	2	C.S.*	56–77†	4,200	735	3,226
	2	C.S. intact	56–77	5,550	4,400	1,070

* Mice thymectomized after delivery by Cæsarean section (C.S.) on the 18th day of gestation. † Symptoms of wasting present.

with anisocytosis and slight hypochromia were seen in smears from mice with wasting symptoms. Preliminary studies of the bone marrow showed an increase in the granulocytic, and sometimes a decrease in the erythropoietic, series of cells.

The possibility that thymectomized mice died from generalized bacterial or viral infections was examined despite the fact that the histological appearance of the lymphoid organs was not consistent with the presence of an infectious agent. Preliminary bacteriological tests and passage experiments were all negative, but it would be unwise to rule out the complication of incidental infection in such debilitated hosts.

Effect of foreign cells on mortality-rate (Table 3). *C3H* mice thymectomized within 24 hr. of birth died at an earlier age and with more acute symptoms of wasting if they were injected intraperitoneally with mature, incompatible, allogeneic cells when 12 days old. Moreover, such treatment increased the mortality in animals thymectomized later than 24 hr. after birth and advanced the time of death. A striking increase in mortality, from 1/9 to 16/16, was also shown by the outbred *TO* mice.

These results confirm the previous report[5] that the immune response of thymectomized mice to foreign cells is impaired. According to Simonsen[8], splenic enlargement can be regarded as an early and reliable index of a host's inability to defend itself against immunologically competent cells and a marked splenomegaly was seen in thymectomized *C3H* mice killed 8 days after the injection of foreign cells (Table 4).

Since the thymus is the main lymphoid tissue present in the fœtal and new-born mouse and its removal within strict time limits at birth causes lymphopenia and lymphoid depletion of the spleen and lymph nodes, we conclude that it is the most important and probably the only, source of lymphocytes during late embryonic and early neonatal life. It may also be responsible for initiating the production of lymphocytes in other lymphoid organs and this initiation could be brought about by a cellular 'seeding-out' process[9,10] or by some humoral mechanism as indicated by Metcalf[11].

The clinical and pathological effects of neonatal thymectomy are remarkably similar to those of runt disease[12] and F_1-hybrid disease[13] in laboratory animals. That is, the symptoms generally associated with a graft-versus-host reaction can apparently be produced by the simple expedient of neonatal thymectomy. It is tempting to assume, therefore, that in runt disease and F_1-hybrid disease the graft is simply the instrument which depletes the host's lymphoid tissue, and that it is this depletion and not the primary immune reaction which is responsible for the associated stunting, cachexia, granulocytosis and possibly the anæmia. This assumption is supported by the fact that foreign cells injected into thymectomized mice accelerated wasting and increased mortality but did not produce any additional or specific symptoms. Presumably such treatment merely exacerbated the already existing lymphoid depletion.

Table 3. Effect of Foreign Cells* on Thymectomized and Intact Mice—Mortality

Strain	Age at thymectomy (hr.)	Mortality	Mean age at death and range (days)
C3H/Bi	< 24	9/9	28 (26–31)
	24–36	10/10	34 (24–43)
	48–72	9/9	44 (31–70)
	Intact	1/25	34
TO	< 24	16/16	37 (23–70)
	Intact	0/24	—

* 2 × 10^7 spleen cells from 2-month-old *C57BL* donors. Recipients injected intraperitoneally at 12–13 days of age.

Table 4. Effect of Foreign Cells* on *C3H* Mice thymectomized within 24 Hr. of Birth—Spleen Weight

Treatment	No. of mice	Wt. spleen (mgm. ± *S.D.*) Absolute	Relative/10 gm. body-wt.
Intact + cells	5	26.4 ± 10.1	37.5 ± 11.9
Thymectomy	6	25.0 ± 10.1	38.0 ± 10.0
Thymectomy + cells	5	80.7 ± 23.5	130.9 ± 22.5

* 2 × 10^7 spleen cells from 2-month-old *C57BL* donors. Recipients injected intraperitoneally at 12–13 days of age and killed 8 days later.

We have shown that thymectomy of mice at birth not only impairs their normal immunological development but also drastically curtails their life span. What is the relation between the immunological role of the thymus and the fatal consequences of its removal? We would suggest that thymocytes or lymphocytes carry out vital functions in addition to those concerned with immune reactions and that the impaired immune response of thymectomized mice is but one symptom of a wider metabolic dysfunction.

We thank Dr. J. Craigie for advice concerning Tyzzer's disease and Dr. S. Gray Anderson of the National Institute for Medical Research for performing bacteriological tests.

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EVIDENCE FOR A FUNCTIONAL DISULPHIDE IN PHOTOPHOSPHORYLATION

By Dr J W. NEWTON

Northern Utilization Research and Development Division, Agricultural Research Service, U S Department of Agriculture, Peoria, Illinois

THE investigation reported here was prompted by results of immunochemical studies on chromatophores of photosynthetic bacteria and by the finding that the photochemical apparatus of *Rhodospirillum rubrum* yielded serologically univalent fragments after disulphide scission[1] The presence in these cell particles of a repeating antigenic sub-structure with individual determinant groups spaced between each pair of disulphides was therefore indicated. Production of the univalent sub-units of chromatophores can be brought about by treatment with sulphite, heavy metals under alkaline conditions, mercaptans, or sonic oscillation[2] Since the antigens under consideration are placed in the chromatophore during photosynthetic growth[3], and since the photophosphorylation system appears in the chromatophore under these conditions[4], the possibility of a functional involvement of these structural disulphides in photophosphorylation has been examined

Disulphides are known to undergo interchange reactions as follows

$$R'—S—SR' + R^2—S—SR^2 \rightleftharpoons 2R'—S—SR^2$$

Such a reaction was studied by Ryle and Sanger with model disulphides[5] They showed that the equilibrium under physiological conditions could be forced to the right either by raising the *p*H or by adding a catalytic amount of a thiol At neutral *p*H and with no thiol added, this interchange is slow

If we designate the photochemical apparatus as *ch*—S—S—*ch*, where *ch* stands for a chromatophore sub-unit and *R*—S—S—*R* is an added disulphide, a reaction of the following type can be envisaged

$$ch—S—S—ch + R—S—S—R \overset{(OH)^-(SH)^-}{\rightleftharpoons} 2ch—S—S—R$$

Such a reaction could lead to the insertion of any chosen half disulphide into the chromatophore substructure The physiological consequences of such a situation have recently been examined

Chromatophores were prepared from *Rhodospirillum rubrum* and assayed for photosynthetic phosphorylation by the spectrophotometric method of Frenkel[6] The water-soluble, aromatic disulphide 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was prepared by the method of Ellman[7] Chromatophore preparations having an optical density of about 10 at 800 mμ were pre-incubated for 24 hr in glycyl–glycine buffers at 2° C with 10^{-4} *M* DTNB and other additions Aliquots of the pre-incubated solutions were then added to the phosphorylation test mixture and incubated in light at 25° C for 10–15 min along with dark controls, and the rate of formation of adenosine triphosphate was measured Table 1 shows the results of pre-incubation of chromatophores with 10^{-4} *M* DTNB in the fully reduced and oxidized form, as well as in the presence of a catalytic amount of a thiol at *p*H 8. Although there is inhibition of photophosphorylation either by oxidized DTNB or by excess reducing agents, the most potent inhibition occurs when DTNB is combined with a catalytic amount of thiol, as expected if the inhibition were due to disulphide exchange Either cysteine or sodium monosulphide can be used as catalyst Photophosphorylation was also inhibited by sulphite pre-incubation at relatively high *p*H and by arsenite Inhibition by sulphite was greatest under conditions that produce univalent fragments from chromatophores[2]

Table 1 EFFECT OF DTNB-THIOL PRE-INCUBATION ON PHOTOPHOSPHORYLATION

Pre-incubation mixture	ATP in light mμmoles 15 min
DTNB 10^{-4} *M*	13
DTNB 10^{-4} *M*, Na_2S 10^{-3} *M*	18
DTNB 10^{-4} *M*, Na_2S 10^{-4} *M*	24
DTNB 10^{-4} *M*, Na_2S 10^{-5} *M*	0
H_2S 10^{-3} *M*	15
H_2S 10^{-4} *M*	15
H_2S 10^{-5} *M*	28
No addition	32

Rhodospirillum rubrum chromatophores Pre-incubated at *p*H 8, 2° C , 24 hr at a concentration D_{880} = 10 7 (5 × 10^{-5} *M* chromatophore disulphide) and assayed after 1 10 dilution of pre-incubation mixture in 0 1 *M* glycylglycine *p*H 8 0 containing phosphorylation test mixture

Table 2 EFFECT OF DTNB-THIOL PRE-INCUBATION ON ENZYMES OF CHROMATOPHORES

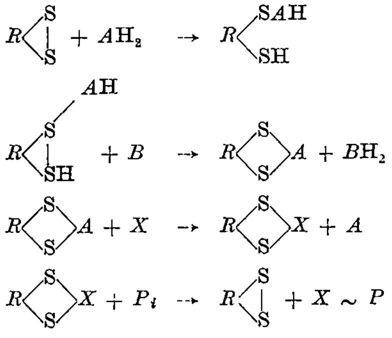
Pre-incubation mixture	Succinic dehydrogenase	Cytochrome photo-oxidase	DPNH-diaphorase
DTNB 10^{-4} *M*	7 0	10 4	6 0
DTNB 10^{-4} *M*, cysteine 10^{-5}	6 9	8 5	5 0
Cysteine, 10^{-5} *M*	10 0	9 8	6 5
No addition	7 0	8 9	6 5

All data are mμmoles acceptor reduced in 5 min by chromatophores (D_{880} = 0 87) after 24 hr pre-incubation at 2° C

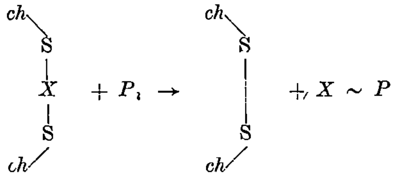
DTNB at 10^{-4} *M* inhibited crystalline rabbit-muscle lactic dehydrogenase and yeast alcohol dehydrogenase approximately 50 per cent, but inhibition was decreased by catalytic amounts of thiols, as expected if the inhibition were a result of oxidation of the thiol groups known to be functional on these enzymes Inhibition of these enzymes was immediate and did not require the prolonged alkaline pre-incubation necessary with chromatophores, which also suggested that the mechanism of inhibition was different

There is a number of reasons for believing that chromatophore inhibition by this means is analogous to uncoupling The DTNB-thiol combination has little or no effect on other enzymatic electron transport reactions of chromatophores, succinic dehydrogenase, cytochrome photo-oxidase or the chromatophore diaphorases (Table 2) The stimulation of mitochondrial ATPase, related to the uncoupling process of mammalian oxidative phosphorylation, is brought about in mitochondrial preparations by precisely the same methods, for example, treatment with heavy metals, sonic oscillation, sulphite or thiols, used to produce univalent chromatophore fragments[8,9] Furthermore, Petrack and Lipmann have recently found that a light-induced ATPase can be unmasked in photophosphorylating preparations treated with very high concentrations of thiols[10]

Fluharty and Sanadi have proposed a mechanism for oxidative phosphorylation that involves interconversion of a vicinal dithiol-disulphide as a primary energy trapping system[11]



Their proposal was based on the uncoupling action of a mixture of 2,3-dimercaptopropanol and arsenite on oxidative phosphorylation Fluharty and Sanadi have left open the question of whether R in their equations is either a small-molecular-weight disulphide or a protein, or whether it is two closely juxtaposed protein chains Any of these possibilities would be consistent with their data The present work supports the Fluharty–Sanadi mechanism and extends it to include photophosphorylation and to the possibility that, since the chromatophore fragments involved are serologically univalent, R in the equations is indeed different protein chains separated by interchain disulphides



It is proposed that separation of these univalent chains constitutes the physical basis of uncoupling, both in chromatophores and in mitochondria

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DISCRETE DISTRIBUTIONS OF GENERATION-RATE

By Dr HERBERT E. KUBITSCHEK

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

GENERATION-rate (the reciprocal of generation time) appears to be distributed normally for widely different kinds of cells in balanced growth[1] This interpretation is based on measurements of generation times of a bacterium[1], and on data taken from the literature for yeast[2], a protozoan[3], and a strain of human tissue culture[4] When the coefficient of variation of generation-rate is calculated for each of these distributions, a striking relationship emerges these values fall into two rather narrow classes Thus it appears that the coefficient of variation, $C V$, for cell cultures in balanced growth might be limited to certain discrete values

The conditions necessary to obtain valid distributions of generation-rate and generation time have been described[1,5] Several investigations reported in the literature were discarded because they failed to satisfy one or more of these conditions (1) cultures were not in balanced growth, (2) results were presented as an integration of data from sub-cultures with different mean rates of division, whether or not in balanced growth, (3) the data are biased by the recording method, with selection against large values of generation time This kind of selection, first described by Powell[5], occurs when divisions are recorded during a definite interval and all these are used to determine the distribution As the end of the interval is approached, newly divided cells must have shorter and shorter generation times for their division to be recorded in turn Thus the distribution is biased toward shorter generation times This bias may be removed, as Powell pointed out, by measuring the same number of generations in every line of descent from the original ancestor

The same kind of selection also occurs even when the generation times of sister pairs are recorded for a definite interval Some pairs must be discarded because one or both cells fail to divide by the end of the record Thus distributions based on data of this sort again exclude an unduly large fraction of cells with long generation times Since generation time distributions are skewed to the right, this bias can make the distribution appear falsely normal This bias can be made negligible by discarding divisions of all daughter cells for parents born within two mean generation times ($2G$) of the end of the recording interval Alternatively, one can decide to wait for all daughter divisions up to a time at least as long as $2G$ If distributions are found with greater values of $C V$ than those reported here, then the period $2G$ must be appropriately extended

Table 1 COEFFICIENTS OF VARIATION OF CELL GENERATION-RATE

Cell	N	$C V$ (per cent)	$S E$	Ref
Tetrahymena geleii	766	9 2	0 24	(3)
Diploid *Saccharomyces cerevisiae*				
20°	86	10 0	0 8	
30°	186	10 2	0 5	(2)
Group I	1,038	9 4	0 2	
Diploid *Saccharomyces cerevisiae*				
38°, mother cells	84	17 1	1 3	(2)
38°, daughter cells	84	21 4	1 6	
Haploid *Saccharomyces cerevisiae*				
30°, mother cells	25	21 8	3 1	(2)
HeLa	91	18 3	1 4	(4)
Escherichia coli, B/r	142	18 1	1 1	(1)
Group II	426	18 8	0 6	

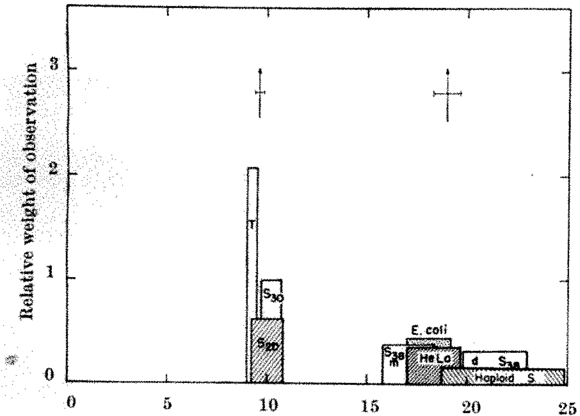

Fig. 1. The grouping of values of *CV* for cultures in balanced growth

Abbreviations: *T*, *Tetrahymena*; *S*, diploid *Saccharomyces*; *Haploid S*, haploid *Saccharomyces*; *m*, mother cells; *d*, daughter cells; a numerical subscript indicates temperature. The rectangles are centred on the value for *CV*, are of width 2 (*S.E.*), and have unit area. The arrows indicate the mean of each group, and the cross-bars indicate the standard error of the mean

The grouping of the values of *CV* for extremely different kinds of cells is evident from Table 1. This table also gives the number of measurements *N* of cell generation times and the standard error, *S.E.*, estimated from $S.E. \simeq CV/\sqrt{2N}$. Within each group the values of *CV* do not differ significantly from one another. This result is shown graphically in Fig. 1. It is quite possible that only two values of *CV* were observed.

These results were unexpected, and their meaning is still uncertain. Nevertheless, if they continue to be supported they must bear significantly on the nature of the control of cell division. They cannot be due simply to a common increase in the value of *CV* at temperatures of 37° C. and above, since the larger value of *CV* was also observed with haploid yeast at 30° C. However, on the basis of the response of diploid yeast, for which division is more variable at 38° C. than at 30° C. or 20° C., increased variability might be ascribed to a temperature-dependent loss or impairment of a control mechanism of cell division. Cytological evidence for the loss of control at 38° C., given by Burns[2], is the failure of mother and daughter cells to bud simultaneously at this temperature.

These findings suggest that very similar, perhaps identical, primary mechanisms control division in widely different kinds of cells. This similarity implies control at the molecular level. Furthermore, the close agreement between the distributions for haploid yeast at 30° C. and diploid yeast at 38° C. shows that although this control might be genetic, it does not depend on the number of chromosomes, and therefore, on gene number or the total content of deoxyribonucleic acid within the cell.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

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OSSAL LESIONS INDUCED BY ENCEPHALITIS VIRUSES IN MICE

By Dr. JANINA MARKOWA and Dr. ALFRED MAREK

Second and Third Surgical Clinics of the Cracow Academy of Medicine, and Rickettsia and Virus Department of the Vaccine and Serum Laboratory, Cracow, Poland

A CASE of non-bacterial osteitis of the entire femur in the course of a neuro-infection[1] prompted us to examine the viral lesions of bones.

Viruses of tick-borne encephalitis (strains S_{47}, K_{24} and L_{61})[2-4], and Western equine encephalitis were used in the experiments. Young, genetically pure-line white mice weighing 15 gm. were inoculated intra-ossally. Virus suspensions were prepared according to classical methods after controlling the virulence of the strains. Before each series of inoculations the purity of the virus strain was checked by biological methods. Mice injected intra-ossally with suspensions of healthy mouse brains without viruses served as controls. Bacterial sterility of the suspensions was checked on various culture media. Groups of mice infected with viruses, as well as controls, were anæsthetized at various intervals after inoculation and studied roentgenologically and histologically. (The material was decalcified with 6 per cent nitric acid, fixed in paraffin, and stained with hæmatoxylin–eosin and trichrome.) Table 1 shows the numbers of inoculated mice and the dosage of the different virus strains.

The suspensions used for the inoculations were free from bacterial contamination. Mice of the control groups remained healthy and did not develop any roentgenological or histological changes.

In all the mice infected with viruses histological lesions were found, and in some cases, depending on the duration of the disease, also morbid symptoms and roentgenographic changes (Fig. 1).

An evaluation of the histological lesions showed that two types of pathological process could be distinguished, which were found to be related to the infecting viral strain: (1) lesions beginning as œdema of the bone marrow (encountered in mice infected with the L_{61} and Western equine encephalitis virus, and in part of the mice infected with the S_{47} strain); (2) lesions beginning with extravasation of blood into the bone marrow (in mice infected with the K_{24}, and part of the mice infected with the S_{47} virus).

In the course of changes beginning with œdema of the bone marrow, already after 24 hr. a large amount of amorphous fluid was found in the marrow

Table 1

Kind of strains	General No. of infected mice	No. of intra-ossally infected mice	Intra-ossal doses (ml.)	Time of death (days)
S_{47}	98	38	0·03	46
K_{24}	57	35	0·015	23
L_{61}	62	35	0·015	40
W.E.E.	65	30	0·015	12
Control suspensions	100	65	0·015 0·03	—
Together	382	203		

W.E.E., Western equine encephalitis.

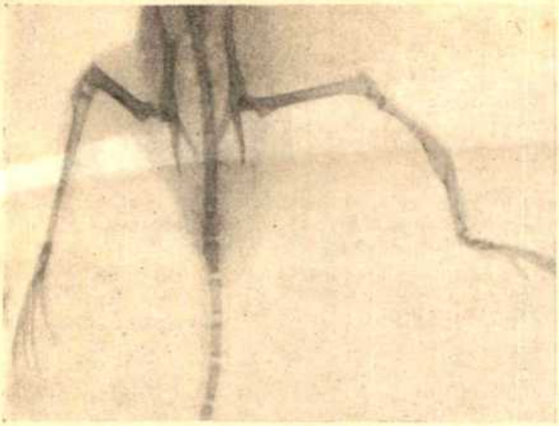

Fig. 1. Dilation of the upper half of the shaft of the tibia on the tenth day of illness

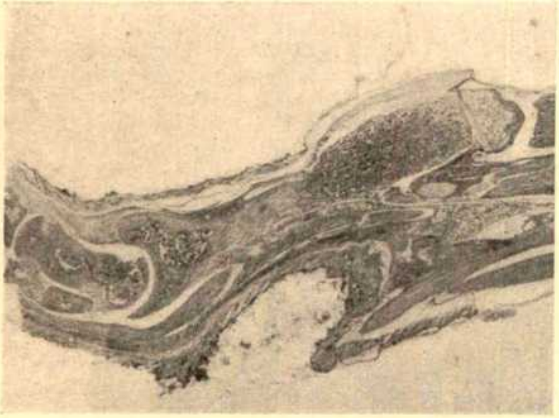

Fig. 2. Shaft of tibia dilated in the proximal half. The œdematous medullary tissue begins to proliferate and push out the rarefied walls of compact bony tissue

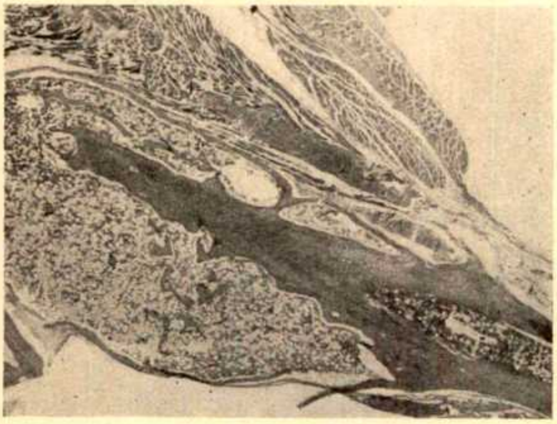

Fig. 3. Longitudinally detached outer lamellæ from the tibial shaft close the proliferating marrow. Lamellæ lying loose here and there in the region of the extra marrow cavity proliferating medullary tissue

cavity, increasing during 3 days and resulting in filling the nutrient and Volkmann canals. The bony tissue displayed necrotic alterations with disintegration. The compact bones disintegrated longitudinally, undergoing a kind of destratification, while the spongy bones showed chaotic decomposition. At the same time œdematous fluid as well as marrow cells were observed outside the marrow cavity, and sometimes even in the interfascial spaces in muscles. After 6–8 days the bone marrow showed signs of proliferation, undergoing partial or complete fatty degeneration (Fig. 2). Proliferating marrow tissue was often seen lying outside the marrow cavity or markedly distending the necrotically altered bone of the shafts (Fig. 3). At this time we also observed defects in the directional columns in the growth cartilages and degenerative changes of the chondroblasts. The articular cartilages showed gradual desquamation, and the bony covering of the epiphyses fissures with marrow cells lying in them. The latter were also present in the articular cavities, which were filled with more or less proliferative connective or fatty tissue.

Osteogenesis was observed after 9–10 days, both in the necrotically altered bones and surrounding them, and also on a substrate of muscle tissue in places containing large accumulations of small mononuclear and plasma cells. The osteogenetic processes took the form of cartilaginous metaplasia (Fig. 4). Small islands of cartilage were found in the destroyed bone, and the bone was surrounded by a cartilaginous and bony sleeve, usually enclosing about one-third of the length of the shaft (Fig. 5).

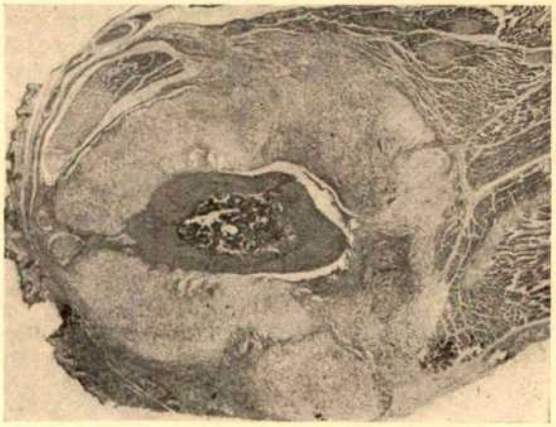

Fig. 4. Transverse section through the shaft of the tibia. A dense small-cell infiltration surrounded by the extended detached external periosteum. Chondromatous metaplasia has already begun

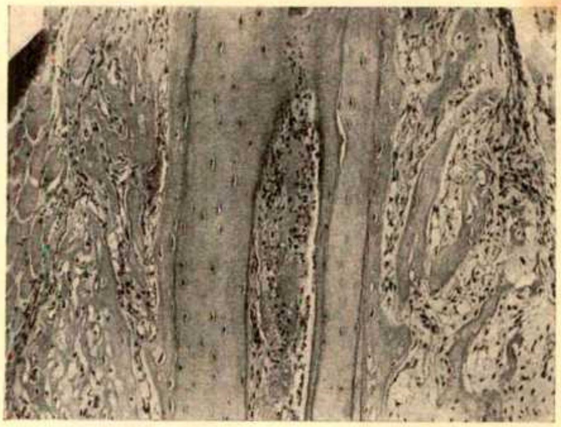

Fig. 5. Near the shaft, typical oval formations of small cells, of considerable dimensions and with well-marked boundaries, undergoing chondromatous metaphasia. There are already freshly formed paraskeletal trabeculæ. × c. 23)

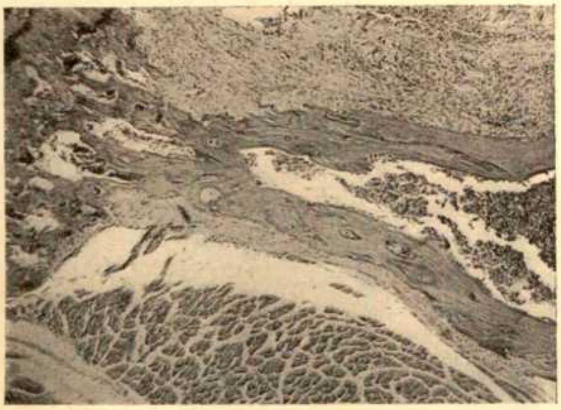

Fig. 6. Marked defects in the bony structure in the upper tibial paraepiphysis. Connective tissue with infiltrations has penetrated into the interior of the necrotically changed bone. The outer edges of the bone are irregular and ragged. (× *c.* 23)

A hæmorrhagic type of extravasation of blood in the bone marrow was noted, presented as massive hæmorrhage. At the same time the external surface of the shaft displayed small penetrating erosions filled with mononuclear cells. After 6–8 days the erosions narrowed the shaft to a quarter of its width, and lymphoid connective tissue was found growing into them (Fig. 6). Proliferation of connective tissue was also observed in the intertrabecular spaces near the epiphyses. In the remaining part of the shafts cavitary cribriform degeneration of the bone tissue took place, giving rise to a moth-eaten appearance. In mice infected with the K_{24} strain the bone marrow also showed proliferation developing similarly to the œdematous form. On the other hand, in mice infected with the S_{47} strain proliferative changes in the bone marrow or lesions of the growth cartilage were not observed. Osteogenetic processes were manifested by cartilaginous metaplasia, always internal or paraskeletal, but never outside the skeleton. Paraskeletal osteogenesis was characterized by formation of bony trabeculæ hanging downward from the bones in conformity with the weight of the extremity, in the form of stalactites.

On the basis of biological investigations carried out during the experiments, living Western equine encephalitis and S_{47} viruses were found in lethal doses in the bones as well as in the brains of the mice.

This summary of results points to the need for comprehensive investigations of the viral pathology of bones and joints. It may be supposed that the still unknown ætiology of many diseases of the skeletal system in man may be cleared up in this way.

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OCCURRENCE OF TWO PLASMA ALBUMINS IN THE DOMESTIC FOWL

By Dr. W. M. McINDOE

Agricultural Research Council, Poultry Research Centre, Edinburgh, 9

DURING an investigation of the water-soluble proteins of hen egg yolk by starch-gel electrophoresis, involving a comparison of these with the plasma proteins of laying hens, it became evident that the plasma of some hens contained what appeared to be two albumins present in approximately equal concentrations. Evidence has been obtained that both protein components are albumins, that they consistently occur singly or together in the plasma of different individuals, and that their distribution in a population is under genetical control.

Smithies's[1] method of starch-gel electrophoresis was utilized. Using the continuous and discontinuous buffer systems (pH 8·6) of Smithies[1] and of Poulik[2] heterogeneity of the plasma albumin of the fowl could not be detected. However, at acid pH values heterogeneity of the plasma albumin was indicated. Most of the electrophoresis was carried out at pH 5·4 using a discontinuous buffer system (gel; 0·005 *M* sodium hydroxide, 0·00365 *M* succinic acid: electrode compartments; 0·112 *M* *tris* 0·050 *M* citric acid). A continuous system was also used, the gel buffer then being 0·0112 *M* *tris*, 0·0050 *M* citric acid. Gels were stained in 0·05 per cent nigrosine for 30–50 min. Improved resolution of the presumed albumin bands was obtained if the plasma was diluted with water to give dilutions of 1 in 3 to 1 in 6.

It soon became evident that the hen plasmas were of three types (Fig. 1). In two of the types only one albumin band was apparent, accompanied by a minor component travelling the same distance ahead of the

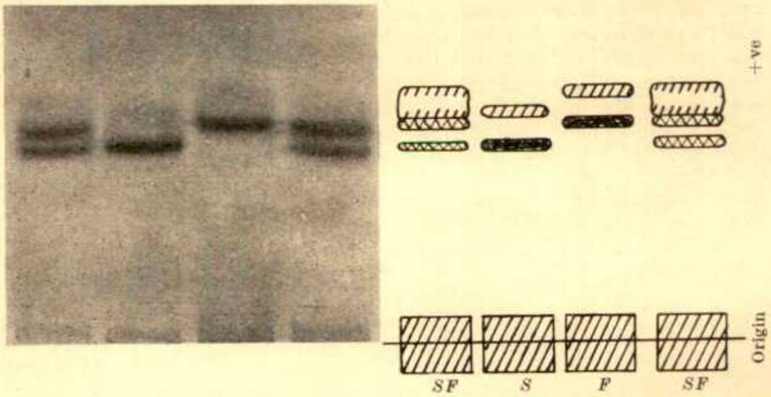

Fig. 1. Starch-gel electrophoretogram of plasma albumin types. Plasma diluted 1 in 4 in each case

albumin in each case. These two types could be distinguished by the rates at which the albumins and minor components migrated, and are designated *S* (slow) and *F* (fast). The third type of plasma contains both albumins, the *F*, and probably also the *S*, minor component, and is designated *SF*. In the discontinuous buffer system, the *S* minor component migrated between the *F* albumin and *F* minor component, but it was not well resolved in the *SF* type plasma. In the continuous buffer the *S* minor component travelled at the same speed as the *F* albumin and could not be detected in the *SF* type in this system. The albumins present in *S* and *F* plasmas occurred in approximately twice the concentration that they did in the *SF* plasma. If the *S* and *F* plasmas were diluted 1 in 6 dilution of the *SF* plasma 1 in 3 gave comparable band intensities. A mixture of *S* and *F* plasmas behaved in the same way as the *SF* plasma on electrophoresis.

Electrophoresis of diluted plasma of each type was carried out in continuous *tris* : citric acid buffer systems at selected *p*H's between 8·6 and 3·5. The single main albumin band evident at *p*H 8·6 gave rise to the components which migrated at different rates at *p*H 5·4. They began to migrate at significantly different rates when the *p*H was less than 8·0 and still had a different mobility at *p*H 4·8, when they were just moving towards the anode. At *p*H's more acid than this, when all the proteins moved towards the cathode, the electrophoresis was not satisfactory.

Both the major and minor *S* and *F* proteins had the solubility characteristics of plasma albumins. All four were soluble in 50 per cent saturated ammonium sulphate and so were present in the conventional albumin fraction of plasma. Mammalian plasma albumins have been shown to be soluble in ethanol after the plasma proteins have been precipitated with trichloroacetic acid[3-5]. Hen plasma was treated similarly and the acid ethanol supernatant examined electrophoretically at *p*H 5·4 after dialysis. Both *S* and *F* major and minor components were soluble in the ethanol and migrated with unaltered mobility.

These results, though based on limited criteria, leave little doubt that the major *S* and *F* components at least are plasma albumins. Evidence is presented below that they are genetic variants.

Genetical relationship of the albumins. Individual hens from five of the Poultry Research Centre's inbred lines of Brown Leghorns and from two of the Reaseheath highly inbred lines of White Leghorns and Rhode Island Reds were examined and typed *S*, *F* or *SF*. Most of the birds were sampled at least twice and two were examined six times, and a consistent pattern was obtained in each case. The results are summarized in Table 1. Although the number of birds examined from each line was small it is evident that two of the lines (*B* and *R*) are markedly heterogeneous, and the *L* and *S* lines are possibly homogeneous. The highly inbred White Leghorns and Rhode Island Reds also seem to be homogeneous. These results point to a genetical basis for the distribution of the plasma albumins in the individuals examined.

Table 1. DISTRIBUTION OF PLASMA ALBUMINS IN HENS OF DIFFERENT BREEDS AND LINES

Breed	Coefficient of inbreeding (per cent)	Type and No. of hens			No. of parents		No. of mating pairs
		S	*SF*	*F*	Dams	Sires	
Brown Leghorn							
1. *B* line	31	2	11	8	16	9	16
2. *I* ,,	46	13	1	0	11	4	11
3. *L* ,,	64	11	0	0	5	2	6
4. *R* ,,	74	4	8	4	7	2	7
5. *S* ,,	79	0	0	6	5	4	6
White Leghorn							
CH line	99·62	17	0	0	10	3	10
Rhode Island Red							
Q line	92·55	7	0	0	4	3	4

It was not possible to type the parents of the birds cited in Table 1, and in order to investigate the possible genetical relationship between the plasma albumins, a number of offspring from four types of matings was examined after the determination of the plasma albumin type of the parents. The types of mating involved were *SF* × *SF*, *S* × *F*, *SF* × *S* and *S* × *S*. The results are summarized in Table 2, and are consistent with a simple Mendelian inheritance of the two albumins and the two minor components. In none of the groups examined was the frequency of the three types significantly different from that to be expected.

Table 2. DISTRIBUTION OF PLASMA ALBUMINS IN FAMILIES OF BROWN LEGHORNS

Dams		Sire	Type and No. of offspring		
No.	Type	Type	*S*	*SF*	*F*
3	*SF*	*SF*	1	12	3
3	*S*	*F*	0	13	0
3	*SF*	*S*	7	3	0
2	*S*	*S*	13	0	0

This genetical relationship between the two plasma albumins implies a probable chemical similarity. Although an investigation of the structure of these proteins has not been carried out, the fact that they migrate at different rates at *p*H's below 8·0 and at the same rate at *p*H's above 8·0 suggests that a histidine residue may be involved in the difference between them. This also applies to the minor components and further indicates a genetical, and possibly a structural, relationship between the albumins and their minor partners.

In several other instances more than one protein in a presumed homozygote appear to be under the same genetical control, that is, the β-globulins of cattle[6-9], sheep[7,10] and pigs[11], the transferrins of mice[12,13] and the ovalbumins of hen egg-white[14]. Genetical control in these systems has been ascribed to one allele. However, since information on the structure of these proteins is lacking and our knowledge of the biosynthesis of polypeptide chains and complex proteins is limited, the precise nature of the genetical mechanism involved remains in doubt.

The only other plasma albumin difference recorded in the literature, so far as is known, is the occurrence in man of two plasma albumins in some of the members of a few families in Europe and the United States[15-17]. A simple Mendelian inheritance is suggested to explain this, but one of the homozygotes has not been observed.

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HÆMOGLOBIN MINORS AS POSSIBLE INTERMEDIATES IN HÆMOGLOBIN SYNTHESIS

By JERRY B LINGREL and HENRY BORSOOK

Division of Biology, California Institute of Technology, Pasadena

THE work reported here began as a search for intermediates in the terminal stages of biosynthesis of hæmoglobin The criterion for an intermediate was that it should have higher initial specific activity than the final product and lose specific activity in a chase experiment Such material has been found among the hæmoglobin minors of rabbit reticulocytes

Reticulocytes prepared from rabbits treated with phenylhydrazine were washed and incubated according to the procedures of Borsook *et al* [1] After incubation the cells were again washed The packed cells were lysed according to the method of Schweet *et al* [2] The 105,000*g* supernatant of the cell lysate was dialysed against the appropriate buffer and chromatographed on either DEAE-cellulose or 'Amberlite *CG*-50'

When washed reticulocytes were incubated with L(1-^{14}C)-leucine for 10 min and the resulting labelled hæmoglobins chromatographed on DEAE-cellulose, the elution pattern and specific activities shown in Fig 1 were obtained The hæmoglobin, as measured at 420 mμ separated into two fractions, designated here as *A* and *C* Fraction *A* (the major component) exhibited a specific activity of approximately 30 c p m /420, while that of fraction *C* (the minor component) was 190 c p m /420

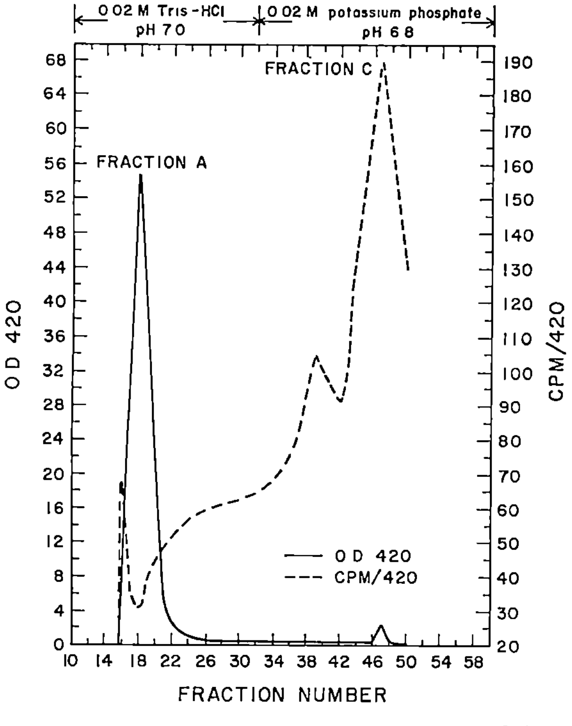

Fig 1 Rabbit reticulocyte hæmoglobin 10 mc incubation with leucine-1-^{14}C Chromatography on DEAE-cellulose

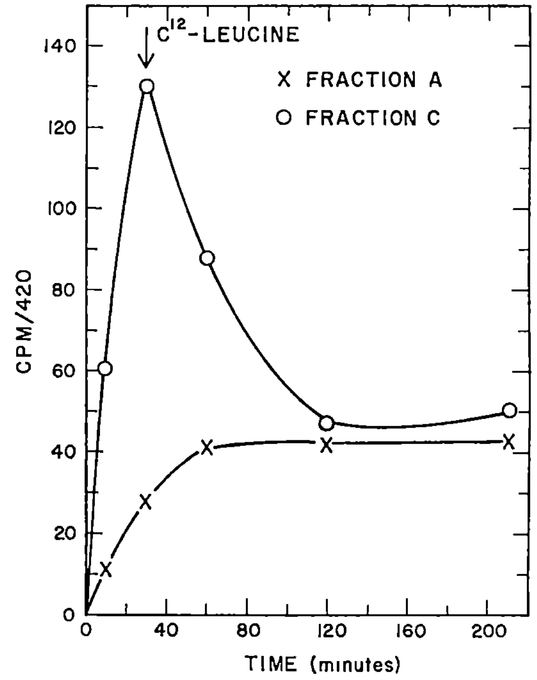

Fig 2 Effect of ^{12}C-leucine chase on incorporation of ^{14}C-leucine into hæmoglobin fractions *A* and *C*

The results of a chase experiment are shown in Fig 2 Reticulocytes were incubated for 30 min in a reaction medium containing labelled leucine, washed with isotonic saline to remove the bulk of the free amino-acid, and re-incubated in the reaction medium containing unlabelled leucine The reticulocytes were sampled at various time-intervals and their hæmoglobin separated into fractions *A* and *C* As shown in Fig 2, the specific activity of fraction *A* increased linearly for the first 30 min, increased further by 35 per cent at the beginning of the chase period and then levelled off The specific activity of fraction *C* increased at nearly a linear rate during the incubation with labelled leucine, attaining a much higher level than fraction *A*, but unlike fraction *A* it lost specific activity during the chase treatment, levelling off at about the specific activity of *A* (the major)

Further analysis of the minor fraction on 'Amberlite *CG*-50' showed that the minor fraction was heterogeneous When fractionation of rabbit hæmoglobin was carried out on 'Amberlite *CG*-50', six components were detected, of which at least three showed a chase effect similar to that shown in

Fig. 2. All the minors behaved on DEAE-cellulose, 'Amberlite *CG*-50' and on starch-block electrophoresis as if they were more acidic than the major. The drop in specific activity during the chase treatment was exhibited not only when the radioactivity was related to hæmoglobin measured at 420 mμ but also at 280 mμ or to dry weight of hæm-free protein.

The possibility that the labile counts in these minors was due to adsorbed leucine was rendered unlikely by the following findings: after treatment with ninhydrin (the leucine was 1-^{14}C), only 0.5 per cent of the radioactivity was recovered in the carbon dioxide; exhaustive dialysis against 0.01 *M* leucine, followed by precipitation with trichloroacetic acid, standing in 1 *N* sodium hydroxide containing 0.01 *M* leucine for 1 hr. at 37° C., re-precipitation with trichloroacetic acid and, finally, extraction with acetone and ether failed to remove counts.

The combined minor fractions, which comprised only 11 per cent of the total hæmoglobin, accounted for 35 per cent of the total counts before the chase treatment. After the chase, there was only 21 per cent of the total counts in the minors. Therefore, 14 per cent of the total counts were labile. This phenomenon clearly is metabolically significant. The results obtained with labelled valine were identical to those with leucine.

The first of these minors to be obtained in sufficient quantity and purity for molecular weight determination was found to have, within experimental error, the same molecular weight as the major.

After adsorbed free leucine had been excluded, there were two obvious explanations for the loss in specific activity of the minors during the chase treatment: (1) dilution of labelled by unlabelled material synthesized during the chase; (2) a simple precursor relationship in which a labelled precursor is replaced by an unlabelled one. Both these explanations appear to be excluded by the following considerations. First, the combined minors comprise 11 per cent of the total hæmoglobin. In order to reduce the specific activity to a half, at least 11 per cent of the total hæmoglobin would have to be synthesized during the chase treatment. In the time interval studied (Fig. 1), there was only a 0.2 per cent increase in total hæmoglobin; this excludes dilution as an explanation. A simple precursor relationship is excluded by the fact that a chase does not reduce the specific activity of any one labile fraction to zero.

One possible explanation for the data is that as hæmoglobin comes off the template it becomes associated with low molecular weight acidic material, which is responsible for the chromatographic and electrophoretic difference of these hæmoglobins from that of the final major or minor forms. This would account for the higher specific activity of the minor fractions. But in order to account for the decrease and final levelling off of their specific activity during the chase, it is necessary to invoke a rapid equilibration of the acidic material with the related pre-existing pool of major and minors; thus a steady-state pool of the more acidic hæmoglobin would exist, and the specific activity of the minor fractions would not drop below that of the major.

Our findings suggest that minors may be classified as either genetic or metabolic. This would help to explain the increasing number of minors which are being found in the fractionation of hæmoglobins.

We thank Dr. Jerome Vinograd for determining the molecular weights. This investigation was supported by grants from the U.S. Public Health Service (*H*-1624), the U.S. Atomic Energy Commission (*AT*(04-3)51) and by a fellowship to one of us (J. B. L.) from the U.S. National Science Foundation.

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DISTRIBUTION OF ZINC-65 IN THE WASP, HABROBRACON, AND ITS EFFECTS ON REPRODUCTION

By Prof. DANIEL S. GROSCH

Genetics Department, North Carolina State College, Raleigh, North Carolina

Radiozinc is second only to radiophosphorus as an ecologically troublesome isotope in the vicinity of reactor sites[1]. Following the atomic weapons tests at the Pacific Proving Grounds, zinc-65 was reported in plankton[2], molluscs[3] and fish[4] of the Pacific, and spectrographic analysis of Marshall Islands natives revealed well-defined zinc-65 peaks[5]. Apparently, the isotope persists through food-chains. When zinc reaches mammals an association with sperm develops[6], for which the prostate gland is responsible[7], but nothing has been published about the effect on fertility.

The investigation reported here was performed with insects uniquely suited for single feedings as well as analysis of fecundity and fertility. The wasp, habrobracon (*Bracon hebetor*), features an expansible crop, four identical polytrophic ovarioles, and haploid parthenogenesis. Samples of about 20 adults from Whiting stock No. 33 were fed measured amounts of zinc sulphate labelled with zinc-65 in sucrose water. For feeding, the sulphate must be used instead of the readily available but unpalatable chloride form. Exposure to an external source was accomplished by depositing labelled zinc chloride on planchets used to cap the lucite chambers described previously[8]. In addition, wasps were reared on host caterpillars (*Anagasta kuehniella*) injected with 105 μc. of labelled zinc chloride made up in 10 ml. of insect Ringer solution. However, the procedure was discontinued when 660 eggs transferred to injected hosts yielded only 20 adults. Apparently, chronic exposure during development has more serious consequences than the main experiment with adults reported herein.

The radioactivity of whole animals, body parts and eggs were assessed using a conventional scintillation well counter connected to an integrating decade scaler An automatic sample changer was used for tissue localization studies Eggs were collected daily and their hatchability scored after 48 hr The records obtained for unfertilized eggs from virgin females give total induced lethality, recessive as well as dominant

Egg production consistently lower than control values was obtained after ingestion of four different doses of zinc-65 Daily averages subsequent to a feeding at the highest level (105 μc /ml) is shown in Fig 1 A curve parallel but lower than controls is more typical of metal poisoning than radiation damage[9] A sixth to tenth day valley characteristic of radiation damage was obtained only by chamber exposures to zinc-65 γ-rays at a level (2 85 mc) far above that of any palatable mixture yet devised This result from the highest of a series of six exposures is also shown on Fig 1

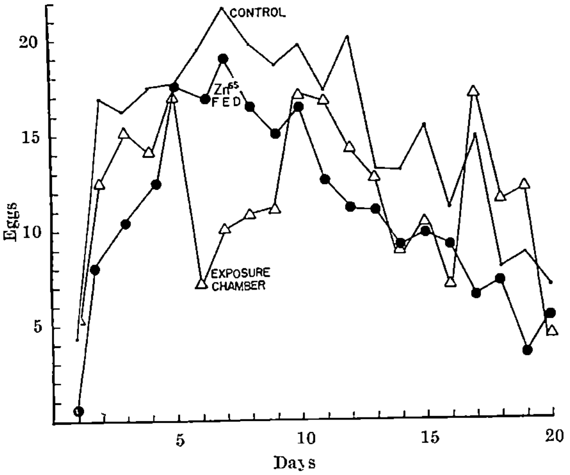

Fig 1 Average number of eggs laid per day by samples of 20 virgin habrobracon Solid circles give results following feedings of zinc sulphate labelled with zinc-65 in sugar mixtures of 105 μc /ml Pre-starvation of these wasps and the sugar-fed controls was at least 96 hr Triangles represent oviposition records of wasps exposed for 96 hr to 2,850 μc of zinc-65 deposited on the metal cap of a lucite exposure chamber

A summary of hatchability for the eggs of Fig 1 is given by Table 1, along with mean life-span The standard errors associated with hatchability range from 5 to 6 except for the third day value of 8 67 for fed zinc-65 Comparison with results from feeding non-radioactive zinc indicates that the decrease in life-span and the inviability of eggs laid late in life may be explained by the metal burden On the other hand, consistently lower hatchability of eggs deposited before senility may be ascribed to radiation damage Most interesting is the exceptionally low hatchability of eggs deposited on the third day, because, as shown in Fig 2, third-day eggs are the most radioactive

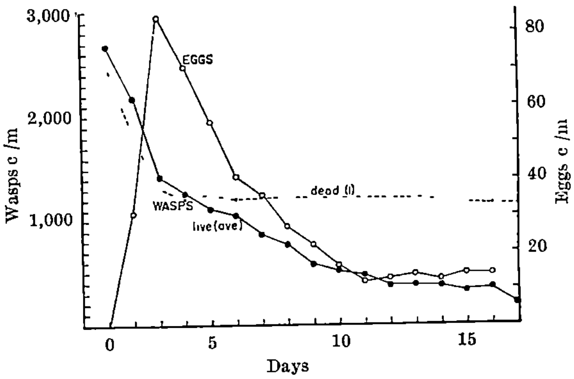

Fig 2 Relative radioactivity of female habrobracon and of eggs laid after ingestion of labelled zinc sulphate in sugar mixture The values are corrected for background but not for physical decay Solid symbols give an average value per wasp based on a sample initially numbering 23. The broken line gives the counts per min of a wasp which died on the fourth day to demonstrate how slight the contribution of physical decay Open circles represent egg radioactivity For convenience, the scale, shown on the right, is calibrated in counts per ten eggs

In addition to depicting the peak in egg radioactivity, Fig 2 also characterizes the decline in radioactivity of ovipositing female wasps The curve shows that the effective half-life for a zinc-65 meal is reached after 2–3 days There is no clear break in the curve, but if one is construed as has been suggested by some ecologists, values later than the third day may indicate the radioactivity of the pool of assimilated zinc-65 Even on this basis the effective half-life would not be more than 7–9 days

In localization studies, samples of 6–8 wasps were killed each day Individuals were counted, transected at the petiole, and then the anteriors and posteriors counted separately The 20 wasps surviving development on radioactive caterpillars showed a relatively homogeneous distribution of zinc-65 with anteriors as radioactive as posteriors A strikingly different pattern of distribution was obtained after adult single feedings Nearly all the radioactivity in the wasp was abdominal, the average rose from 88 to 97 per cent of the total during the first two days During the subsequent week the average abdominal radioactivity dropped to 90 per cent of the total, which held until the fifteenth day Thereafter, higher values became common and by the twentieth day abdominal radioactivity averaged 99 per cent of the total

Anteriorly, muscle and nervous tissue predominate and the digestive tract is represented by only a slender œsophagus In the abdomen, four types of tissue can be separated by dissection digestive tract, fat and urate bodies, gonads and accessory glands, and body wall structures During the first two days, as might be expected, most of the ingested radioisotope was associated with the digestive tract Afterwards the value dropped, but never lower than 50 per cent Counts of the paper disks from the

Table 1 PERCENTAGE OF HATCHABILITY FOR EGGS DEPOSITED DURING DESIGNATED SUCCESSIVE PERIODS
The average life-spans of the ovipositing females are included

Treatment	Days after treatment								Mean life-span (days)
	1	2	3	4	5	6–10	11–15	16–20	
Sugar only	88 0	95 6	94 8	96 8	94 8	91 7	84 8	76 3	18 5
^{65}Zn γ-rays externally*	90 0	88 8	87 7	96 9	94 6	77 7	79 3	70 4	18 9
^{65}Zn fed	88 9	88 0	53 5	88 3	84 8	86 1	73 7	67 7	14 9
Zn fed	93 9	94 5	95 5	96 5	94 9	90 8	80 8	64 5	14 4
Cytological stage when treated	Oocytes progressing through prophase					Transitional cells	Oogonia	Oogonia (senility)	Somatic tissue

* The level used in exposure chamber, 2 85 mc , is greatly in excess of the level of feeding, 105 μc /ml

bottoms of the dishes containing experimental habrobracon demonstrated that significant amounts of zinc-65 were egested. The fæcal route is well known in rats, where it serves even for loss of injected zinc-65 (ref. 10), but habrobracon, an insect, lacks a pancreas.

No attempt was made to determine whether excretion is localized in the Malpighian tubules or in some part of the hindgut. Another tissue of related function, the urate bodies, was found to contain most of the remaining abdominal radioisotope, 20–30 per cent, exceeded by the ovaries only before the third day. The body-wall, along with the heart, œnocytes and tracheal trunks, had very little radioactivity. In males the pattern was similar except that the reproductive system never became appreciably radioactive. This, plus my previous experience that radiophosphorus is relatively ineffective when fed to adult males[11], led me to emphasize female responses here. Even so, a zinc-65 meal seems ineffectual when compared with the impressive decline in second week oviposition after feeding from a 100 μc mixture of phosphorus-32 (ref. 8). Indeed, the influence on egg production as well as such interrelated phenomena as shortened life and poor hatchability of eggs laid during senility seem due to zinc toxicity.

Radiation from ingested zinc-65 may influence the hatchability of eggs laid during youth and middle age, but except for the low value of the third day, results are not as impressive as after a feeding with phosphorus-32. This may be understandable when we consider that less than 20 per cent of the zinc-65 meal is eliminated via the eggs in contrast to 60 per cent of a phosphorus-32 meal taking the route[12]. Furthermore, the radiations differ. Instead of phosphorus-32 β-particles with a range of millimetres we are now dealing with an isotope from which considerable energy is released as more penetrating electromagnetic radiation attenuated only by metres of tissue. Much of the γ-ray energy is dissipated outside an organism measuring only 0.5 mm × 2 mm., or after incorporation and oviposition outside of the smaller eggs. By the use of simplifying assumptions it can be shown that following an isotope meal the energy delivered to the wasp per disintegration is 20–30 times as great from phosphorus-32 as that delivered from zinc-65.

This work was supported by U.S. Atomic Energy Commission contract *At*-(40-1)-2836.

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ŒSTROUS CYCLES IN THE RABBIT *ORYCTOLAGUS CUNICULUS* (L.)

By K. MYERS and W. E. POOLE

Wildlife Survey Section, Commonwealth Scientific and Industrial Research Organization, Canberra

It has long been held that there is no regular œstrous cycle in the rabbit, and that female rabbits remain in a state of œstrus for long periods of time, provided environmental and nutritional conditions are favourable[1–4]. It is also believed that both the domesticated female rabbit and the wild female rabbit[5], given suitable conditions, will accept the buck at any time, although with reduced frequency during pregnancy.

More recently, the occurrence of a cycle of sexual behaviour in confined female wild rabbits was reported[6]. Females became sexually attractive to males at intervals of approximately 7 days (probability of 7-day periodicity < 0.01). The cycle in sexual behaviour has since been correlated with an associated cycle of follicular development, with follicular growth, measured in terms of mean follicular diameter, taking the form of a quadratic regression $y = 0.3794 + 0.1952x - 0.01306x^2$ ($P < 0.05$). Females held separate from males entered into a state of persistent œstrus. The uteri of females developed typical luteal characteristics after a display of strong attraction when mating usually occurred. No females built nests or lactated (Poole, W. E., unpublished results).

In view of this evidence and of the previous findings that the female domestic rabbit has a follicular cycle of about 7 days[7,8] or 7–10 days[9], and of the demonstration of a regular peak in the number of cornified cells in the vaginal smear from unmated does occurring at 4–6 day intervals[10], a reappraisal of the sexual behaviour of female domestic rabbits is clearly necessary.

For the past three months one of us (K. M.) has been observing the behaviour, daily, of 11 female domestic rabbits running with vasectomized males in a ¼-acre enclosure. Two strains of females, lops and cross-bred straight ears, bred and reared at the Institute for Medical and Veterinary Science, Adelaide, are being used. Like their wild counterparts, the domestic females periodically become sexually attractive to the males, but at the shorter intervals of 4–6 days, or multiples thereof ($P < 0.0001$). Vaginal smears taken from the cervical region of the vaginæ of cycling females suggest the occurrence of cyclic changes of epithelial cells. It also seems possible that examination of the ovaries will show a follicular cycle of the same periodicity, thus substantiating the findings of earlier workers[7–10].

It is possible that a shorter cycle exists in domestic rabbits because of the effects of artificial selection during many generations of breeding in captivity

Two of the domestic female rabbits dissected at the height of their sexual attractiveness, and after being subjected to ardent male sexual advances, ovulated without mating Females which mated became pseudopregnant At the end of pseudopregnancy (usually 12 or 18 days) most females dug burrows and covered the entrance with soil Few females built nests or lactated. Two females permitted to mate with fertile males at *post-partum* conceived and carried their fœtuses to full term (litter sizes of 5 and 6) while suckling the first litters

The female wild rabbit and the female domestic rabbit thus appear to be essentially the same animals when placed under similar conditions Earlier observations of persistent œstrus in domesticated female rabbits, usually isolated from males, now fall into better perspective

The occurrence of a regular œstrous cycle in female rabbits appears to be totally dependent on exteroceptive stimuli, of a psychological nature, occasioned by the presence of males and, possibly, other females This is in agreement with the recent suggestion[11] that once the output of pituitary gonadotropin is sufficient to induce follicular maturation there is no sharp division between spontaneous and induced ovulation when exhibited in the same species, the two being regarded as extremes of the same response, determined by the degree of extraneous stimulation As far back as 1935 it was suggested that the regularity or irregularity of œstrous periods in female rabbits depended on the sensitivity of the ovulating mechanism to the stimulus given by the advances of the male[8] The importance of male–female interaction in the œstrous cycle of the mouse has been demonstrated[12,13] The presence of strange males causes the breakdown of corpora lutea in pregnant mice, thus constituting an exteroceptive block to pregnancy[14,15] In like vein it has been shown recently that the smell and sound of rams is sufficient to stimulate œstrus in merino ewes after the spring anœstrus[16]

These findings raise important issues for animal husbandry Domesticated animals like the rabbit have long been the subject of physiological research and an imposing mass of data relating to internal function exists It seems an appropriate time for the naturalist to focus his attention on the ecology of domesticated animals and to study, with the physiologist, the interplay between behavioural stimuli and endocrine response

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RESPONSE OF *NEUROSPORA* MUTANTS TO CARBON DIOXIDE

By Dr H P CHARLES

Department of Microbiology, University of Reading

FAIRLEY reported that α-aminobutyrate or threonine would support growth of a *pyrimidine* mutant of *Neurospora* (*pyr*-3, 1298)[1] This observation was made with liquid cultures

Attempts to repeat this observation using solid media were at first unsuccessful The medium of Ryan, Beadle and Tatum[2] was used, with 0 5 per cent sucrose and 1 per cent sorbose Experiment showed that only rare conidia gave rise to colonies on minimal medium supplemented with α-aminobutyrate Even fewer colonies appeared on threonine-supplemented medium Many of these colonies retained the 1298 mutant character on transfer

Eventually it was observed that when α-aminobutyrate Petri dishes were removed from the incubator and left out on the bench many colonies gradually developed This lead to the discovery that all conidia give rise to colonies on α-aminobutyrate or threonine at 20° C , at 26° colonies are infrequent, and at 30° no colonies develop

The mutant is therefore 'temperature-sensitive' when growing on α-aminobutyrate or threonine It is not temperature-sensitive when supplied with an adequate amount of uridine

Arginine (0 5 mgm /l) prevents growth on α-aminobutyrate (AB) or threonine, but has little effect on growth in the presence of uridine Such an effect of arginine has been observed independently by Boyd and Fairley[3]

The next problem considered was why rare conidia were able to grow at 26° C Eventually our attention was directed[4] to a paper by Borek and Waelsch[5], who showed that temperature sensitivity of *Lactobacillus* was attributable to decreasing solubility of carbon dioxide with increasing temperature This led to the discovery that AB or threonine support growth even at 30° if the Petri dishes are placed in a desiccator having 15 per cent of the air replaced by carbon dioxide The mutant does not grow on unsupplemented minimal medium under these conditions

Preliminary auxanographic tests show that many substances support growth of *pyr*-3 (1298) in an atmosphere of 10–15 per cent carbon dioxide, for example, homoserine[6], α-ketobutyrate, glycine, cysteine, serine, lysine, orotate, β-alanine, propionate[6], succinate, formate, thymine, cytosine, dihydrouracil, methionine Among substances failing to support growth are citrate, malate, aspartate and proline

The mutant grows on unsupplemented minimal medium in the presence of 20–30 per cent carbon dioxide Such carbon dioxide effects are not attributable to a simple disturbance of *p*H

It seems likely that the growth in liquid medium observed by Fairley is attributable to accumulation by the conidia of sufficient carbon dioxide to permit a response to AB or threonine Occasional very dense clusters[7] of conidia on solid medium may partly explain the occurrence of rare colonies on AB or threonine at 26° C It is possible that the many substances supporting growth in 15 per cent carbon dioxide do so by acting as sources of extra carbon dioxide, but it is difficult to explain the inactivity of certain metabolites on this basis It should be noted that none of the active substances increases the growth of *arg*-3 (see below)

If Petri dishes are placed in a sealed desiccator (5 litre) containing 100 ml of 25 per cent potassium hydroxide, the mutant fails to grow on AB or threonine even at 20°, but growth on uridine is not prevented Growth of wild-type *Neurospora* is not prevented by deprivation of carbon dioxide in this way

All the carbon dioxide effects already described are prevented by arginine

It seems probable that growth of a uridine mutant of *Escherichia coli*, examined by Rudner and Ryan[8], is explicable in terms of a response to carbon dioxide

Many variants have been isolated from 1298 Some are probably 'suppressed' mutants, because they grow more slowly on minimal medium than does the wild-type, and growth is prevented by arginine[9–11] This sensitivity to arginine suggested that the suppressed mutants are responding to atmospheric carbon dioxide Experiment confirmed that many of the suppressed strains fail to grow in a desiccator containing potassium hydroxide It is of interest that arginine increases the growth of some suppressed strains if uridine is also present

The most reasonable explanation of the response of mutant 1298 to carbon dioxide is that it requires exogenous carbon dioxide for synthesis of uridine It is known that labelled carbon dioxide enters pyrimidines and other cell constituents of *Escherichia*[12], but one would have expected sufficient carbon dioxide to be available from other metabolic processes Presumably carbon dioxide enters the pyrimidines via carbamyl phosphate[13] This also presents difficulties, because carbamyl phosphate is also a precursor of citrulline and arginine[14] If blocked in carbamyl phosphate synthesis the mutant should therefore require citrulline or arginine, as well as uridine

Examination of five *arginine* mutants showed that one of them (*arg*-3, 30300) responded to carbon dioxide Of the mutants tested this was the only one likely to be disturbed in carbamyl phosphate synthesis, in that it was the only one responding to citrulline but not to ornithine This mutant grows slowly on minimal medium, deprivation of carbon dioxide prevents this slow growth

It is therefore necessary to explain the occurrence of *arginine* mutants which do not require uridine, *uridine* mutants which do not require arginine, and the growth of both types of mutants in the presence of carbon dioxide

Possibly the uridine requirement results from an internal inhibition by arginine This is suggested by the sensitivity of 1298 to arginine inhibition We have been unable to show growth promotion by the arginine antagonists lysine and canavanine, but Mitchell[15] reported that two other *pyrimidine* mutants (*pyr-3a*, *pyr-3b*) grew on lysine when ammonia was excluded from the basal medium Further, some lysine mutants (*ly* 3, *ly* 4) accumulate pyrimidines[10] Davis has suggested that *pyr* 3*a* (37301) is actually arginine inhibited, because the presence of a suppressor mutation is correlated with a 97 per cent reduction in ornithine transcarbamylase activity[16]

Conversely, it may be that some *citrulline* mutants actually suffer from inhibition by uridine precursors This is suggested by the fact that the citrulline requirement is not expressed in the presence of a block in pyrimidine metabolism[17]

Numerous interactions between *pyrimidine, lysine* and *arginine* mutants are known A pathway such as that considered here, leading to both an amino-acid and a pyrimidine, might be the subject of complex controlling mechanisms relating amino-acid and nucleic acid metabolism The mutants discussed here may have disturbances in such controlling mechanisms

It is relevant that various morphogenetic effects of carbon dioxide are known, for example, in *Blastocladiella*[18] and *Hydra*[19] It would be of interest to examine the effects of uridine, arginine, etc , on the development of these organisms

Present experiments follow several lines First, the nature of the lesion in 1298 is being investigated Secondly, we are investigating the interactions between *pyrimidine, arginine* and other mutants Thirdly, we are attempting to isolate, by special methods, mutants of *Neurospora, Aspergillus, Escherichia* and *Salmonella* which require carbon dioxide It is probable that such mutants are often eliminated by the usual isolation methods This approach should reveal whether there are *carbon dioxide* mutants having nutritional patterns different from those studied here

Part of this work was carried out during tenure of an Imperial Chemical Industries research fellowship in the Department of Botany, University College, Swansea *Neurospora* stocks were kindly provided by Prof D G Catcheside

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CORALS AT THE MOUTH OF THE REWA RIVER, VITI LEVU, FIJI

By DONALD F SQUIRES

Division of Marine Invertebrates, Smithsonian Institution, Washington, D C

TWO significant controls on the development and rate of growth of colonial reef corals and of coral reefs are the salinity of the water and the quantity and texture of sediment being deposited in the reef area Throughout much of the Indo-Pacific region these ecological factors are of little concern for atoll islands are small and low and do not possess stream systems or contribute appreciably to the sediment load being transported across the reef High volcanic islands, on the other hand, often do have significant drainage systems which may carry heavy sediment loads, often with deleterious effects on the reef and the reef community One of the largest of these river systems in the Indo-Pacific is the Rewa, which is about 90 miles long and which drains approximately one-third of the island of Viti Levu, Fiji During February 1959, together with Messrs Rob Wright and E S Corbett of Suva, Fiji, I was able to make a brief traverse of the reefs on the eastern side of Nukulau Island in Rewa Roads Although examination was cursory and the area investigated was quite limited, some of the observations are of interest

The well-developed reef of Viti Levu is conspicuously broken in three areas along the southern shore of the island where the Singatoka, Navua and Rewa Rivers discharge Of these three, the greatest disruption of the reef pattern is caused by the Rewa River The lower portion of the river is occupied by a complex of islands, probably deltaic in origin Discharge of fresh water is diffused through a number of channels as a result of this complex, the main areas of outflow being the Navuloa mouth, the Nasoata mouth and the Nukulau mouth This last named, the largest, discharges across a large area free from coral islands called the Rewa Roads, in which there are channels to the delta with depths to 10 fathoms across the strike of the reef To the west, Rewa Roads is bounded by Nukulau Island and to the east by Taituanga Island A traverse was made from the north-east portion of Nukulau Island around the eastern end of the reef surrounding the island to the reefs on its southern shore

On the inner side of Nukulau Island and within Lauthala Bay corals are rare or absent None was seen during these observations Fish of a variety of types were abundant, and a few calcareous algae were seen on the rock surfaces Much of the rock was covered by a layer of fine silt which adhered to the surface In some instances this layer of silt reached 1 in in thickness Characteristic of the rock on the north-eastern end of Nukulau Island reef was an extensive system of tortuous grooves and channels cut into the rock to a depth of about 15–20 ft below mean tide-level In many places buttresses of rock were undercut and there was considerable evidence of the breaking off of pieces of country rock as a result of solution Channels between buttresses contained rounded rock fragments and dead corals loose on the bottom All rock observed was formed of calcium carbonate

During the period of observation, a falling tide, fresh water flowing outward from the Rewa seemed to be largely confined to the upper 3 ft and was underlain by about a foot of visibly mixing water, in turn underlain by saline water No measurements were made of the salinities, but the upper layer was distinctly fresh to taste, while below a depth of 5 ft, more normal salinites were encountered The upper layer of water was turbid and contained considerable quantities of fine sediment and organic particulate matter, some of which was visible to the naked eye Visibility in the upper layer was greatly restricted In the more saline waters below, visibility increased in direct proportion to the decreased turbidity, very little particulate matter being seen Some epifauna was observed at depths of 15 ft or more, the number of animals apparently increasing with depth

Off the east-north-east portion of the Nukulau reef (station 2, Fig 1), the first corals were observed Individual colonies of *Porites* up to 1 ft in diameter tightly encrusted the rock surface, the thickest portion of the colony being only inches deep These scattered colonies were a bone white in colour, some having grey patches on their surface Many of the colonies were dead, although several were collected which

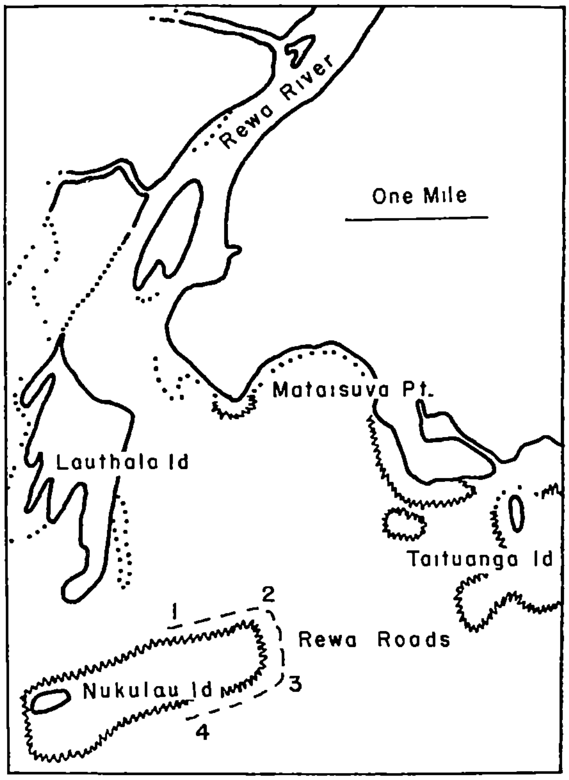

Fig 1 A portion of Viti Levu, Fiji, showing the Rewa River and its relationship to the reef area described Traverse points discussed in the text are numbered 1–4

were in part, at least, alive The colonies were conspicuous through the turbid water as the rock surface is generally a dull brown and is largely devoid of other life Sediment cover on the rock was less than that observed previously Layering of the water was approximately that of the previous description and there was no apparent change in turbidity Most of the coral colonies were found at a depth of about 10 ft Below this depth few corals were seen although alcyonarians (both dead and alive) were noted

Approximately 100 ft farther to the south from the point at which the white corals were seen, similar colonies of *Porites* were found, but which were a bright yellow in colour In general, these colonies were located in a depth-range of 6–30 ft Turbidity decreased, and the water had become better mixed so that downward visibility had greatly increased An interesting feature of these encrusting corals was the severe damage done to the upper surface by intense predation by parrot fish Presumably other fish species known to feed on coral are also responsible for the observed damage, but actual observations of 'grazing' of the coral were limited to the parrot fish Nearly the entire surface of these colonies were deeply grooved in all directions and orientations with the characteristic broad parallel scrape-marks made by these fish

Within a distance of another 100 ft of this point, and in a southern direction, a variety of Faviid corals appeared, but included only small caliced encrusting types Within another 200 ft, that is, within 400 ft of the point at which corals were first observed, a respectable fauna consisting of upwards of 30 species could be found Coral growth then rapidly increased in luxuriousness until the complete reef fauna similar to that found in more normal environments was encountered The coral fauna of the small detached reef patches off the east coast of the Nukulau Reef did not seem to differ in any important respects from that of the main reef to the south of Nukulau, or elsewhere along the southern coast between Nukulau and Suva In this region of rapidly increasing diversity of corals, little of the previously observed effects of the river could be noted Turbidity was not visually noticeable and the waters were well mixed Surge of the breakers on the reef front and of the swell moving up the Rewa channel could be felt Among the corals first to appear were *Archelia* (noted elsewhere on the reefs, generally in shaded areas), meandroid 'brain' corals particularly those with broad calicular vallies, and encrusting forms of *Montipora*

In summary it may be said that

(1) The succession of coral species observed from the central to the outer portion of the western margin of Rewa Roads is that to be predicted from the work of Mayer[1] and Edmondson[2] on the salinity and sediment toleration of various species of coral

(2) Effects of sediment in suspension are mainly in reduction of light penetration restricting the depth of coral growth and may result in the death of symbiotic zooxanthellæ in certain corals

(3) Density layering of waters of differing salinity may impose upward limits of growth on reef corals as well as other stenohaline forms

(4) Reef inhibition adjacent to large rivers is caused in the first instance by deposition of silt, and then by fluctuations in salinity These factors progressively decrease in effectiveness seaward so that a faunal gradient is developed Where swell and breaker action causes complete mixing of waters, flocculation or dispersion of suspended sediment, normal reef conditions may be found

(5) Absence of surface reefs from central Rewa Roads probably results from historical factors and erosion, not from direct inhibition of coral growth

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A PHYSICO-CHEMICAL TREATMENT OF TASTE THRESHOLDS

By Prof THOR R RUBIN
Department of Chemistry, The Ohio State University
AND
FRANCES GRIFFIN and Prof ROLAND FISCHER
Research Division, Department of Psychiatry, The Ohio State University, College of Medicine Columbus 10, Ohio

ADSORPTION as a factor in taste perception has been implied by Cameron[1] It has been discussed explicitly by Ferguson[2] and Beidler[3], who expressed the relationship between concentration and taste intensity through Freundlich- or Langmuir-type equations Beidler recently proposed a Lineweaver–Burk type equation for the possibility of a theoretical prediction of "the intensity of sensation reported by a human subject to certain mixtures of two taste substances of similar taste quality"[4] Using quinine and 6-*n*-propylthiouracil, among other drugs, it was found that high affinity (sorption) of a compound for a protein model receptor could be related to the physiological activity[5], in this case, to the low taste threshold[6,7] of the same compound The sorption process of drug-receptor interaction can be interpreted as occupation by drug molecules of sites of the receptor phase Since the taste threshold of a drug can be regarded as drug-receptor interaction registered as sensory perception[8], it appeared of interest to subject taste thresholds for 6-*n*-propylthiouracil and quinine to a biophysical interpretation

The specific impetus to this line of investigation was prompted by our recent finding that mixtures of 6-*n*-propylthiouracil and quinine produced bitter taste in concentrations well below the threshold for either compound alone Another reason for including 6-*n*-propylthiouracil into our studies was that 'non'-tasting of this and other structurally related antithyroid compounds is considered as a genotypical characteristic (see among others ref 8)

Taste thresholds for quinine sulphate and 6-*n*-propylthiouracil were determined in healthy, normal,

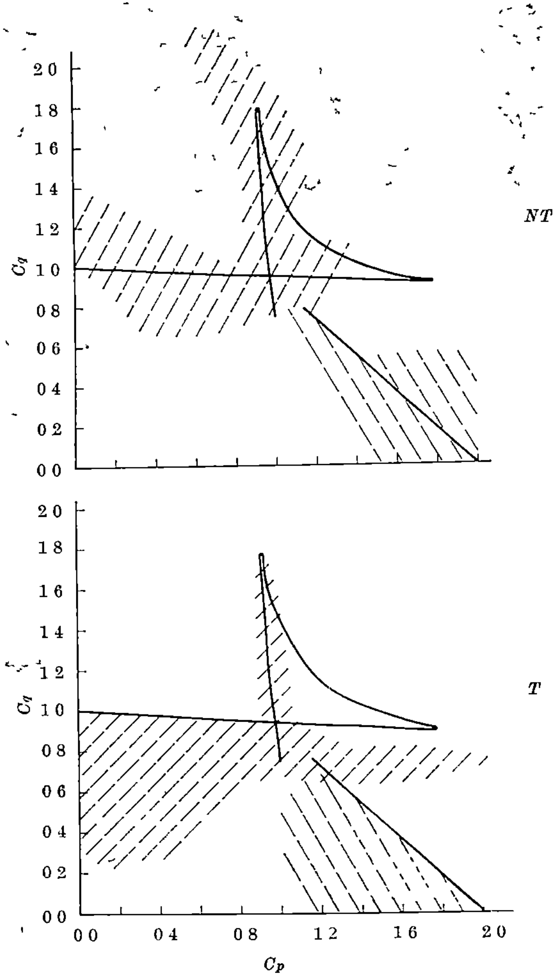

Fig 1 Isothermal taste diagrams for binary mixtures of quinine and 6-*n*-propylthiouracil at room temperature for a typical insensitive ('non'-) taster, called *NT*, and a typical sensitive taster, called *T*, of the compounds $C_q = 1\ 0$ represents the quinine threshold of both *NT* and *T* subjects, whereas $C_p = 1\ 0$ represents 1/2 of the threshold of 6-*n*-propylthiouracil in *NT* subjects and 1/13 of the 6-*n*-propylthiouracil threshold in *T* subjects Representative quinine and 6-*n*-propylthiouracil threshold concentrations for a typical *NT* subject correspond to $1\ 55 \times 10^{-5}$ *M* and $1\ 31 \times 10^{-3}$ *M*, respectively, whereas representative quinine and 6-*n*-propylthiouracil threshold concentrations of a typical *T* subject correspond to $2\ 9 \times 10^{-6}$ *M* and $4\ 37 \times 10^{-5}$ *M*, respectively

Shaded areas indicate concentration combinations of quinine and 6-*n*-propylthiouracil which are not tasted by the subject Note the close correspondence between theoretical curves and experimental data (represented by shaded areas)

trained subjects by the Harris–Kalmus procedure[9,10] This method was used during the training period of a subject which consisted of 1 or 2 threshold determinations per week for at least three weeks The solution numbers represent powers of two, that is, each number denotes a doubling of the concentration, for example, solution number 5 is twice as concentrated as number 4

To increase the resolution of the taste threshold determination, finer concentration intervals based on fractional powers of 2 were prepared, for example, the range between solution Nos 4 and 5 was subdivided into continuous intervals of $2^{1/10}$ With this modification, more refined taste thresholds for series of binary mixtures of quinine and 6-*n*-propylthiouracil were determined The concentration of one compound was held constant while the concentration of the second was decreased in $2^{2/10}$ steps until a taste threshold was reached Then the accuracy of the taste threshold was further increased by the application of the same procedure but using concentration intervals of $2^{1/10}$ steps This procedure was repeated with the order of compounds reversed For each subject approximately 120 combinations of the compounds were tested on two occasions within a five-day period The refined thresholds for quinine and 6-*n*-propylthiouracil (in this order) were determined on each experimental day and used as a base-line The precision of our method is within the limits of a $2^{1/10}$ interval, whereas its accuracy is half as great

In Fig 1 the taste threshold concentrations of a typical insensitive ('non'-) taster for quinine and 6-*n*-propylthiouracil, and a typical sensitive taster for the two bitter compounds are represented as concentrations of quinine plotted against the concentrations of 6-*n*-propylthiouracil The units of concentration in Fig 1 are relative to the values of the particular baseline of the subject on the test day In addition, all concentrations have been adjusted by a constant factor so that the feature of interest appears at values of C_p near one (Fig 1)

The shaded areas represent those concentration combinations of quinine and 6-*n*-propylthiouracil which are not tasted by the subject The unshaded areas (with the exception of the area near the origin) represent concentration combinations of the two drugs which are tasted

Forty combinations were found to be sufficient to define the no-taste areas Between sixty and eighty measurements were necessary to cover the important features of the areas representing tasted combinations The boundary between tasted combinations and non-tasted ones defines a phase diagram for the taste phenomenon Fig 1, in fact, resembles quite closely an isothermal solubility diagram of a pair of salts having an ion in common, both of which are dissolved in a fixed amount of solvent[11]

If drug-receptor interaction is considered as an equilibrium process in taste perception, we can regard the water solution as one of the phases and the taste receptor as the other phase of our system The two components of this system are the two drugs or possibly their positive ions which may combine with sites in the receptor phase and be associated with anions in the solution phase

In order to deal with the taste phenomenon in terms of an isothermal diagram, we have resorted to the regular solution theory of Porter, Heitler and Hildebrand[12–14] We determined the boundary (thresholds) theoretically and compared it with the envelope separating the experimentally determined non-tasted combinations from the tasted ones

In accordance with the notation of Hill[15] we assume that at threshold all available sites of the receptor phase (receptor phase is a hypothetical construct which refers to nodal points in a taste schema attempting to describe the phenomenology of taste[16]) are occupied by N_p molecules of 6-*n*-propylthiouracil and N_q molecules of quinine

For regular solutions then

$$\frac{\mu_q}{kT} = \ln\left(\frac{N_q}{N_q+N_p}\right) - \ln\left[q_q \exp\left(-\frac{CW_{qq}}{2kT}\right)\right] - \frac{CW}{2kT}\left(\frac{N_p}{N_q+N_p}\right)^2$$

and (1)

$$\frac{\mu_p}{kT} = \ln\left(\frac{N_p}{N_p+N_q}\right) - \ln\left[q_p \exp\left(-\frac{CW_{pp}}{2kT}\right)\right] - \frac{CW}{2kT}\left(\frac{N_q}{N_q+N_p}\right)^2$$

where u_q is the chemical potential of quinine in the water phase, q_q is the temperature dependent partition function of quinine in the receptor phase C is the number of nearest neighbours at a site, W_{qq} W_{pp} are the pair interaction energies for quinine and 6-n-propylthiouracil sorbed at adjacent sites W_{pq} is the pair interaction energy at two adjacent sites of one quinine and one 6-n-propylthiouracil molecule, k is Boltzmann's constant and T is the absolute temperature Lastly, $W = W_{qq} + W_{pp} - 2W_{pq}$, p is the chemical potential of 6-n-propylthiouracil in the water phase, q_p is the temperature dependent partition coefficient of 6-n-propylthiouracil

$$\mu_p = kT \ln C_p + \mu_p^0 \qquad (2)$$

$$\mu_q = kT \ln C_q + \mu_q^0$$

where C_p and C_q are the concentrations of 6-n-propylthiouracil and quinine, respectively, in water and μ_p^0, μ_q^0 are the standard chemical potentials of 6-n-propylthiouracil and quinine in the water phase The initial aqueous concentrations given in equations (2) are identical with the concentrations of the taste test solutions since dilution effects by saliva and extraction of the compound by receptor phase are negligible (The volume of a taste test solution is approximately 5 ml , the average volume of saliva secreted during each step of a taste test is approximately 0 04–0 18 ml)

Inserting equations (2) into (1)

$$k_3C_q = \frac{N_q}{N_p + N_q} \exp\left[-\frac{CW}{2kT} \quad \frac{N_p}{N_p + N_q}\Big)^2\right] \qquad (3)$$

$$k_4C_p = \frac{N_p}{N_p + N_q} \exp\left[-\frac{CW}{2kT}\left(\frac{N_q}{N_p + N_q}\right)^2\right]$$

where.

$$k_3 = q_q \exp\left[\frac{\mu_q^0 - \frac{1}{2}CW_{qq}}{kT}\right],\ k_4 = q_q \exp\left[\frac{\mu_p^0 - \frac{1}{2}CW_{pp}}{kT}\right]$$

The values of k_3 and k_4 can be determined from the threshold concentrations of quinine and 6-n-propylthiouracil respectively The value of W was estimated to give a conformation to the theoretical threshold curve which best fits the dividing envelope separating tasted combinations from non-tasted ones

An examination of Fig 1 reveals a qualitative correlation between the leaf-like shape of the theoretical threshold curve and the dividing envelope in the region of high quinine concentrations (for example, those greater than 0 6) The qualitative agreement between the theoretical curves and the experimentally obtained envelopes allows us to make the following conclusions At threshold a certain number of sites in the receptor are occupied by either quinine molecules or an unknown compound formed from quinine and 6-n-propylthiouracil The attraction forces between quinine molecules or the attraction forces between the molecules of the new unknown compound are greater than forces of attraction between molecules of quinine and the new compound This type of interaction in equation 3 gives the leaf-like threshold envelope so long as there are some quinine molecules in the receptor phase

At higher concentrations of 6-n-propylthiouracil (for example, those greater than 1 0) the dividing envelope is a diagonal straight line pointing toward and including the 6-n-propylthiouracil threshold (see lower right portion of both curves in Fig 1) This area, we postulate, corresponds to a receptor phase free of quinine molecules It is not unreasonable, then, to assume that the net attraction forces are zero and the theoretical curve becomes a diagonal straight line corresponding to a perfect solution mixture of 6-n-propylthiouracil and the compound in the receptor phase

The $CW/2kT$ values for a typical insensitive ('non'-) taster and a typical sensitive taster of quinine and 6-n-propylthiouracil are equal to $-$ 3 4 However, the values of the partition coefficients k_3 and k_4 differ for these two taste categories

It should be recalled that there is another important difference between insensitive ('non'-) tasters and sensitive tasters, namely, the significantly greater salivary oxidation rate of the former as compared with the latter[10,17]

The qualitative correlation between the theory of regular solutions applied to the phase rule and experimental evidence allows us to regard taste threshold of a drug or of a mixture of drugs as a specific state of part of the receptor with a given number of receptor sites occupied by drug molecules Taste threshold concentrations of a subject for various compounds are then determined by the number of available sites as well as the magnitudes of the partition coefficients of equations 1

One may speculate about the physical nature of the unoccupied and occupied sites of the receptor phase Volume changes in the latter appear plausible during the sorption process and the concomitant volume change can give rise to mechano-chemical effects, specifically, pressure and swelling[18] of the receptor sites These effects may be part of the chain of events leading to taste sensation

We have introduced this article by regarding taste sensation, with certain restrictions, as drug-receptor interaction Application of the theory of regular solutions to the phase rule allowed us to carry our point farther Fig 1 actually illustrates the dose-response curve for binary mixtures of quinine and 6-n-propylthiouracil Taste or no-taste (shaded) areas correspond to certain combinations of the two drugs and represent phenomena which can be interpreted as synergism and antagonism (as kindly suggested by Dr F W Schueler, New Orleans, La) in the terminology of Gaddum[19]

This work was supported by Grant No M–4694 of the National Institutes of Health

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DIURNAL VARIATION OF THE EARTH'S MAGNETIC FIELD AT SEA

By Dr M N HILL and C S MASON

Department of Geodesy and Geophysics, University of Cambridge

DURING June 20–27, 1961, observations of the total force of the Earth's magnetic field were obtained in four positions with a recording proton precession magnetometer The instrument was contained in a buoy anchored successively at points on the continental slope and at the foot of the continental slope about 120 miles to the south-west of Land's End, in the western approaches of the English Channel The electronics in the buoy consisted of transistorized block units, and the observations were recorded digitally on heat-sensitive paper Dry batteries provided adequate power for up to 15 days recording

A mechanical time-switch cycled the instrument every 5 min and provided hour marks on the record The proton precessional frequency was determined from the time-interval for 2^{10} cycles The accuracy of measurement on land was ± 1γ (10^{-5} œrsted), at sea there may be a larger uncertainty because of motion of the detector coil or because of wave motion

Table 1

Station	Time of operation (B S T)	Position	Water depth (fathoms)
Buoy 1	0900/21/6–1025/22/6	47° 50 5′ N 7° 40 5′ W	450
Buoy 2	1343/22/6–0712/23/6	47° 37 5′ N 7° 44′ W	850
Buoy 3	0956/23/6–0900/25/6	47° 37′ N 7° 43′ W	1,100
Buoy 4	2120/25/6–0855/27/6	47° 22′ N 7° 37 5′ W	2,200
Hartland Point		51° 12′ N 4° 31′ W	
Ivybridge		50° 23′ N 3° 5′ W	

During the period of these measurements at sea, a similar instrument was operated at Ivybridge near Plymouth Total force measurements were available from this instrument at 5-min intervals Advantage has also been taken of the hourly values of the total force obtained by the Royal Greenwich Observatory at Hartland Point, these were computed from the vertical and horizontal hourly values

The positions of the buoy stations and the observatories are given in Table 1

The magnetograms for June 22–23 (buoy 2) and June 26–27 (buoy 4) are shown in Figs 1 and 2 for both the buoy station and Ivybridge, the times shown are G M T + 1 hr Fig 3 shows the average hourly values for all days during June and July 1961, obtained from Hartland Point, and illustrates the relatively steady value of the total force during the night-time hours, typical of land stations in this area

The performance of the buoy magnetometer was checked with a towed proton precession magnetometer The ship steamed on various courses past the buoys and the field values recorded at the point of nearest approach (1–2 cables from the buoys) are shown in Figs 1 and 2

When the conditions are not magnetically disturbed (for example, during the periods of operation of buoys 3 and 4) the daily range is conspicuously greater at sea than at shore (for example, Fig 2) by a factor of about 2 On days magnetically stormy, however, the difference is not so conspicuous (for example, Fig 1)

When the shore observations of the daily variation are subtracted from the buoy observations without

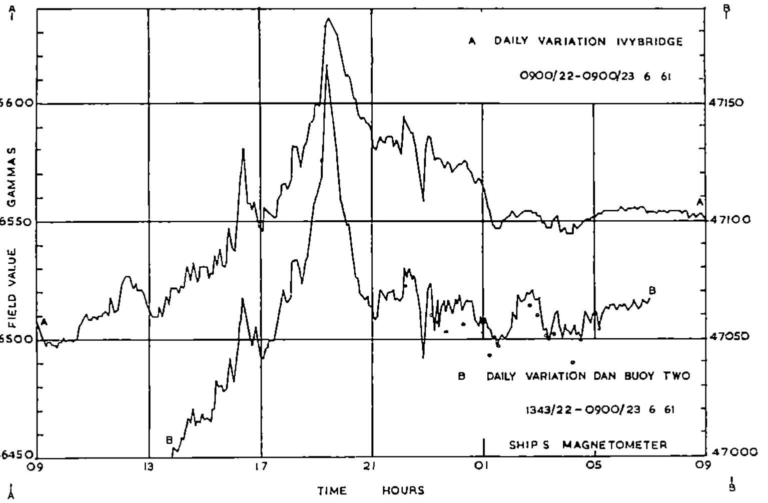

Fig 1

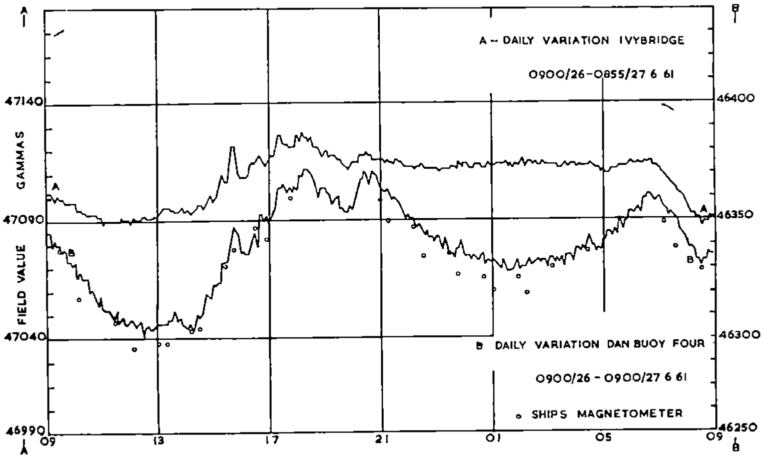

Fig 2

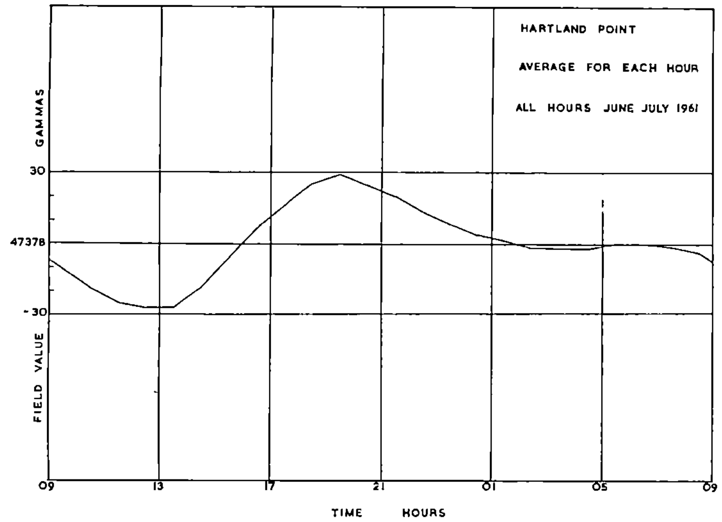

Fig 3

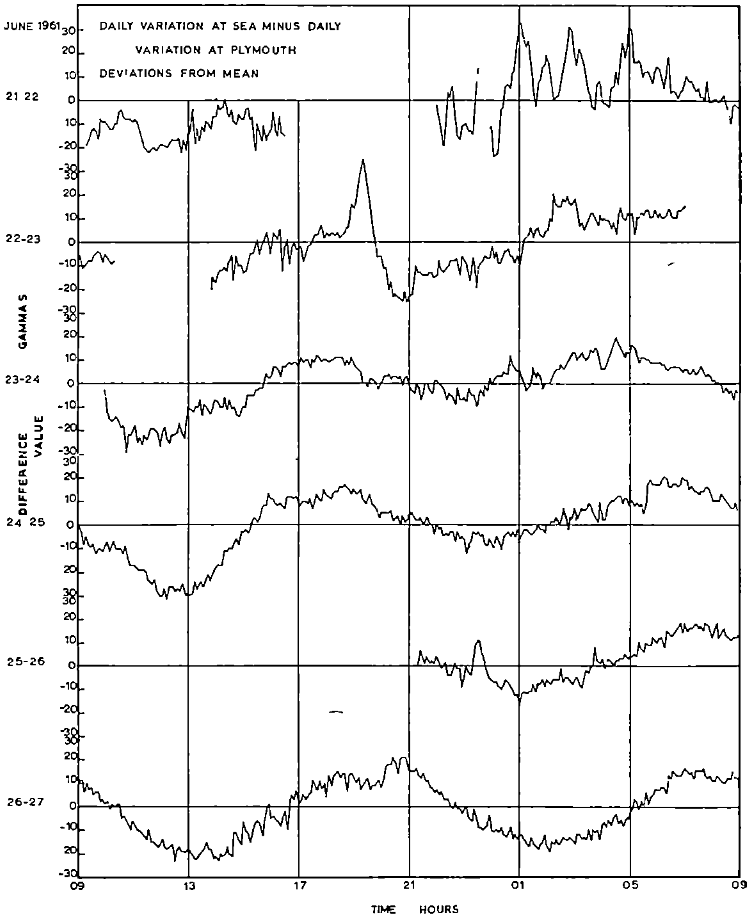

Fig 4

any adjustment for local time difference, the results shown in Fig 4 are obtained These curves (apart from that obtained from buoy 1, where the magnetic storm and the internal failure of the magnetometer between 1630 and 2200 hr obscured the pattern) show a component of change with a period which is probably tidal but which certainly lies between 11 and 14 hr There appears to be a shift to the right of the maxima and minima on the curves of Fig 4 such as would be expected for the semi-diurnal tides Cross-correlation of these curves has not yet been attempted but it might indicate the period with more certainty

The agreement between the ship and buoy magnetometer and the agreement between the magnetic storm details ashore and at sea indicate that the residual effect after the shore variations have been removed is not instrumental It was, however, possible that the variation could be explained by tidal drift of the buoys due to the scope of their mooring wires Precise positioning of the buoys by radio or celestial methods was not possible, but from the known topography and magnetic field around the buoys it has been possible to eliminate buoy drift as an explanation of this semi-diurnal component It is possible that this component, shown in the curves of Fig 4, will only exist near the continental boundary, measurements farther out into the ocean basins are not yet available

These results shown in Fig 4 are unexpected but are probably caused by induced electrical currents in the sea resulting from the tidal motion of the water in the Earth's magnetic field A lunar component of magnetic variation is observed ashore but this is much smaller than that observed in these experiments Theoretical and model studies are now being undertaken in this Department

Because of the electrical conductivity of sea-water some screening effect of magnetic variations might be expected This is not observed either with the normal solar daily variation or with the shorter period (½–2 hr) variations produced by magnetic storms (for example, Fig 1) The effect may exist at periods shorter than about 10 min , this could not have been observed because of the low sampling-rate in the field measurements

We thank Dr H F Finch, Royal Greenwich Observatory, who provided the results from Hartland Observatory, S J E Humphrey, who operated the nuclear precession magnetometer at Ivybridge, the Director of the Marine Biological Association for allowing us to sail in R V *Sarsia*, and the officers and crew for their assistance This work was undertaken under the United States Office of Naval Research Contract *N* 62558–2704, the Shell International Petroleum Co provided a scholarship

LETTERS TO THE EDITOR

ASTROPHYSICS

A New Upper Limit to the Galactic Deuterium-to-Hydrogen Ratio

THE detection of the 21-cm hydrogen line, in 1951, created interest in the possibility of detecting a similar deuterium line at a wave-length of 91 6 cm By comparing the strengths of the two lines, the galactic deuterium-to-hydrogen ratio, N_D/N_H, could be determined This quantity is of astrophysical interest because it will give information about the composition and nucleogenesis of the interstellar medium[1,2]

There have been four previous attempts to detect the deuterium line, and all have given negative results[3-6] The most recent of these, at Jodrell Bank, set an upper limit on N_D/N_H of 1/4,000 in the direction of the Cassiopeia radio source, this value is slightly higher than the terrestrial abundance ratio of 1/6,700

Our work has consisted of attempting to observe deuterium absorption in the Cassiopeia *A* radio source The 85-ft telescope of the National Radio Astronomy Observatory, at Green Bank, West Virginia, was used to track Cassiopeia *A* 12 hr a day for 9 weeks The receiving system has been described elsewhere[7] It incorporates two techniques (1) The autocorrelation function of the signal is determined digitally and Fourier-transformed by a computer to give the power spectrum Use of this method gives high stability and permits multichannel operation (the spectrum is measured at many frequencies during the same time interval) (2) The effective observation time is quadrupled by using four switched receivers to monitor both polarizations all of the time It was thus possible to have 76 5 days (6 6 × 10^6 sec) of effective observation time, even though the antenna was in use only 68 days

The sensitivity of the autocorrelation system has been investigated theoretically[8], and also been checked by computer simulation The root mean square deviation, ΔT, of the spectral measurement is given by

$$\Delta T = \frac{2\ 1}{\sqrt{Bt}}\, T_{\text{tot}}$$

where $B = 3\ 75$ kc /s is the frequency resolution of the measurement $t = 6\ 6 \times 10^6$ sec is the observation time, and $T_{\text{tot}} = 2{,}000°$ is the total noise temperature referred to the receiver input These values give $\Delta T = 0\ 027°$

The spectral dip, T_D, resulting from deuterium absorption, is given by

$$T_D = \tau_D T_p$$

where $T_p = 0\ 46\ \ T_{\text{tot}}$ is the contribution to the receiver input arising from the background source, Cassiopeia *A*, and τ_D is the deuterium optical depth in the angle subtended by the source

The relation relating τ_D to N_D/N_H which we have calculated differs from those given previously by Shklovsky[9] and Adgie[10] The peak optical depth of either gas[11] is given by

$$\tau = \frac{hc^2}{8\pi k}\ \frac{A}{T_s f_0 \Delta f}\ \frac{g_1}{g_1 + g_0}\ N$$

where *A* is the spontaneous emission probability, T_s is the spin temperature, f_0 is the line frequency, Δf is the Doppler-broadened line-width, g_1 and g_0 are the statistical weights of the upper and lower states, and *N* is the total number of atoms per unit cross-section in the two states Calculating the ratio of these quantities for deuterium and hydrogen gives

$$\frac{\tau_D}{\tau_H} = 0\ 33\,\frac{N_D}{N_H}$$

The following assumptions have been made

(1) The value $A_d = 4\ 65 \times 10^{-17}$ sec $^{-1}$ given by Field[12] is correct (this result has been computed independently by A H Barrett of this Laboratory), and the value 6 6 × 10^{-17} sec $^{-1}$ given by Shklovsky is incorrect

(2) The ratio of the Doppler-broadened line-widths $\Delta f_D/\Delta f_H$ would be equal to f_D/f_H if the atoms had the same root mean square velocities However, approximately half the broadening is due to thermal motion in which the deuterium atoms, having twice the mass, would have $1/\sqrt{2}$ of the root mean square hydrogen velocity

$$\text{Thus } \Delta f_D/\Delta f_H = [1/2 + 1/(2\sqrt{2})]\, f_D/f_H$$

(3) The spin temperatures for hydrogen and deuterium are assumed to be equal Justification of this assumption is uncertain, since it depends on estimates of the intensity and detailed profile of the interstellar radiation field at the frequency of deuterium Lyman α-radiation[12]

The value of the peak hydrogen optical depth in the Cassiopeia *A* radio source is also the subject of some controversy, its large value being difficult to measure The first observers, Hagen, Lilley and McClain[13], using a 50-ft paraboloid, report $\tau_H = 2\ 6$ Muller[14], using an 83-ft reflector, gives 4 0, and observers at California Institute of Technology[15], using a single 90-ft telescope, report $\tau_H > 4\ 7$, and $3\ 4 \pm 0\ 4$ when using two 90-ft telescopes as an interferometer Accepting a value of peak hydrogen optical depth of 4, multiplying this by 0 8 to account for reduction because of the 3 75-kc /s band-width, and assuming a detection criterion of twice the theoretical root mean square fluctuation, we obtain a minimum detectable N_D/N_H of 1/18,000

Our results are illustrated in Fig 1 Individual 10-hr runs were examined for interference, corrected for any slope (typical slope correction, 0 03°/kc /s), and averaged into 3-week lots Examination of the 3-week averages indicates that the root mean square deviation is approximately 1 4 times the theoretical value, and thus the minimum detectable N_D/N_H should be raised to ~1/13,000 Our conclusion, then, is that the deuterium-to-hydrogen ratio in the region examined is, with probability 0 977, less than half the terrestrial value

I thank the staff of the National Radio Astronomy Observatory for providing observing facilities and

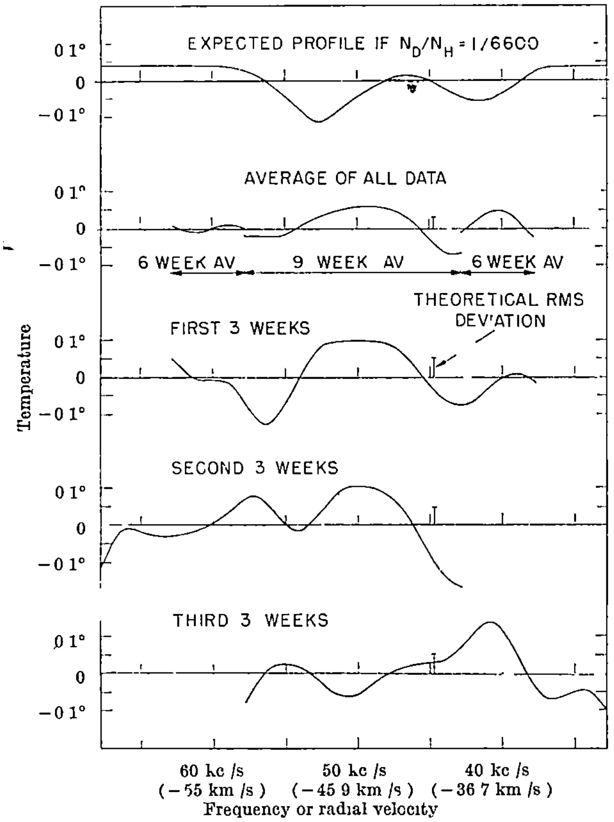

Fig 1 Results of search for 327-mc /s deuterium line absorption in the Cassiopeia *A* radio source

supporting personnel, Prof J B Wiesner for his encouragement and supervision, and Dr H I Ewen for, in many ways, providing the spark that initiated the research I wish to acknowledge the use of the computer facility of the Computation Center, of this Institute in part of this work This investigation was supported in part by the U S Army Signal Corps, the Air Force Office of Scientific Research, and the Office of Naval Research, and in part by the National Science Foundation (grant *G*–13904)

Sander Weinreb

Research Laboratory of Electronics,
Massachusetts Institute of Technology,
Cambridge, Mass

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GEOPHYSICS

Terrestrial Flow of Heat Near Flin Flon, Manitoba

Preliminary measurements of underground temperatures and thermal conductivity have been made in the Coronation Mine near Flin Flon, Manitoba The work is part of a comprehensive geological–geophysical–geochemical investigation of the mine, made possible by the enlightened policies of the Hudson Bay Mining and Smelting Co , who has allowed free use of the mine, even when it interrupted production schedules

Coronation is a copper sulphide orebody in basic volcanics and consists of two lenses, about 300 ft long, which dip steeply southwards The two lenses are separated by about 200 ft of low-grade disseminated ore and extend 900–1,050 ft below the surface The mine is therefore a small one being about 300 ft wide, 900 ft long, and 1,050 ft deep The drift depths are in increments of 150 ft from the surface down to 1,050 ft with an exploratory drift at 1,350 ft

There are numerous drill holes throughout the mine, but only those that were horizontal and open to at least 40 ft from the drift wall were used As the mine was less than 5 years old the distance of 40 ft is well beyond the region of disturbance of temperature due to ventilation[1,2], in some cases penetrations up to 180 ft from the wall were made Water was issuing from some of the holes, but in most of such cases it was possible to push the thermistor temperature sensing element beyond the point of entry of the water so that true rock temperatures could be obtained

Not less than ten readings of temperature were obtained at each drift depth, and the average of these was taken as the temperature of a particular level Between 550 and 600 ft a small but significant change in gradient, which could not be correlated with a change in rock conductivity, was observed, the two gradients together with their respective 95 per cent confidence limits, were 15 3 ± 0 8° C /km to 600 ft and 11 7 ± 1 2° C /km from 600 to 1,350 ft

The thermal conductivities (expressed in c g s units) of rock samples varied from 0 0047 for an andesite to 0 0092 for a tremolite gneiss, showing extensive mineralization The mean for the mineralized rocks is 0 008 and for unmineralized rocks is 0 006, the errors being such that use of the second figure is not warranted Thus from 150 to 600 ft the flow of heat lies between 0 9 and 1 2 µcal /sq cm /sec and from 600 to 1,350 ft it lies between 0 7 and 0 9 µcal /sq cm /sec

Since the temperatures were measured in or near an ore body it is to be expected that the temperature gradient will be low and should be accompanied by a high thermal conductivity For this reason the temperature gradients should be used with the higher value of conductivity given for the mineralized rocks, although without an extensive number of measurements it would be difficult to obtain an accurate mean value

There are a number of factors which could have affected the value of flow of heat[3], in this mine the most important is the probable occurrence of flows of water in the region particularly in the upper parts of the mine The evidence for this, in addition to the flow of water from some of the drill holes, was provided by the tritium dates of two water samples which indicated that the water from the 1,050-ft level was about 25 years older than that from the surface Since this is a point of some importance in determination of flow of terrestrial heat, further water samples will be collected from different levels and their tritium dates found in an effort to determine the pattern and extent of underground water flow before more extensive heat-flow measurements are made.

I thank the National Research Council of Canada for a grant-in-aid, Dr. D. R. E. Whitmore for much information and assistance in the field, and the members of staff of the Hudson Bay Mining and Smelting Co. for their co-operation.

A. E. Beck

Department of Geophysics,
University of Western Ontario,
London, Ontario.

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PHYSICS

X-ray Microanalysis of a Radioactive Particle with the Aid of the Electron Microscope*

In the examination of radioactive fall-out the phenomenon of the radioactive particles has recently attracted growing attention[1,2]. It has become evident that a considerable part of the artificial radioactivity in the atmosphere, caused by atomic explosions, is found in the form of finest 'dust' particles. These may be inhaled or ingested and possibly cause radiation damage.

To make statements on such biological effects an exact knowledge of the physical and chemical characteristics (such as size, structure, composition, solubility) is needed. By means of γ-spectrometric and radiochemical methods the radioactive nuclides in the particles can be detected; the determination of size and shape of a particle is made possible by electron microscopy[3]. Estimates based on activity and diameter of the radioactive particles have shown that they must even at the time of their formation consist partly of inactive material[1,3]. Information concerning the inactive components is important for the question of formation of particles as well as for the problem of their solubility.

Radioactive particles which were collected and examined during a period of three months following the atomic test series of September 1961 were the subject of a recent publication[3]. One of these particles (Fig. 1) was analysed in an electron microscope (Elmiskop I, Siemens) by an X-ray microanalytical method[4]. (The spectra were recorded with the help of Dr. H. Neff and Miss E. Schmiedeskamp with the apparatus of the Siemens Werk für Messtechnik in Karlsruhe.) The aim of this investigation was to obtain information about the composition of the inactive material of the particle. The microanalytical method mentioned uses the X-ray line spectrum of the K-series (characterized mainly by the $K\alpha_1$-quantum energy) which is caused by electron irradiation of the particle in the electron microscope and which is characteristic of each element. The electron beam is focused on the object and the emerging X-ray spectrum is absorbed in a proportional counter. The counter pulses are conducted to an impulse spectroscope from which the spectrum is then photographically recorded with a 'Polaroid' camera. For the time being the method admits the identification of elements of the ordinal numbers 13–40.

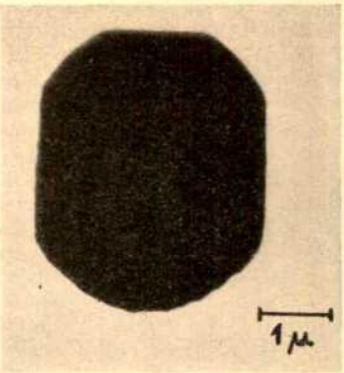

Fig. 1. Outline of a radioactive particle collected in October 1961; activity at the collecting-date 5×10^{-10} c.; largest diameter, 4·4μ. Electron micrograph

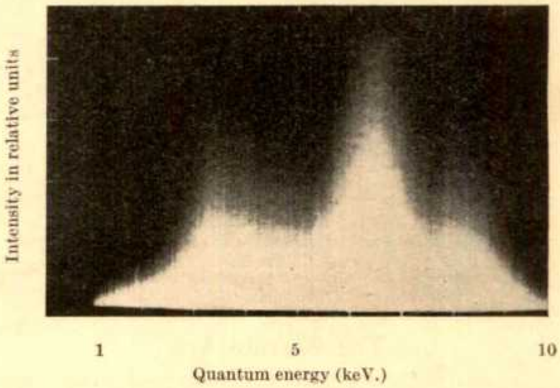

Fig. 2. X-ray microanalysis of the radioactive particle shown in Fig. 1. Beam voltage, 40 kV. A beryllium window diminishes the amount of scattered radiation, but at the same time also the effect of the low-energetic quanta (0–2 keV. range). One easily recognizes the iron peak at 6·4 keV. and the iron escape peak at about 3·5 keV. The peak at about 8·0 keV. (copper) is caused by the bronze grid used and does not belong to the spectrum of the radioactive particle

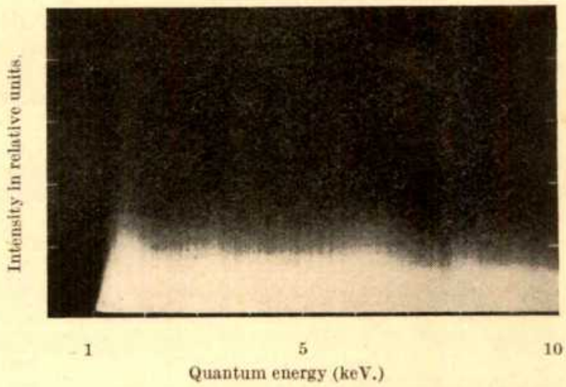

Fig. 3. X-ray microanalysis of the radioactive particle shown in Fig. 1. Beam voltage, 40 kV. Without beryllium window. The amount of scattered radiation is remarkably high but the spectrum covers also the low-energetic range. Due to the high amount of scattered radiation, the time of exposition had to be shorter than in Fig. 2. Therefore the peaks are less distinct. Nevertheless, one now recognizes besides the iron peak and the iron escape peak an additional peak at about 1·6 keV., which is caused either by aluminium (1·48 keV.) or by silicon (1·74 keV.). As in Fig. 2, there is a peak at about 8·0 keV. caused by the copper of the bronze grid used

In the particle analysed we found iron and aluminium or silicon as inactive components. This result was inferred from the spectra shown in Figs. 2 and 3. In the method used there are difficulties in separating elements if their $K\alpha_1$-energies differ only very little. Therefore the existence of iron ($K\alpha_1$-quantum energy 6·40 keV.) can be deduced with certainty from the spectra obtained; there is no clear answer to the question, however, whether the second component is aluminium or silicon. The $K\alpha_1$-energies of these two elements (aluminium, 1·48 keV.; silicon, 1·74 keV.) differ only by 0·26 keV.

A peak at about 8 keV. which is discernible in the spectra given was also found after having focused the electron beam on to the empty methacrylate foil beside the radioactive particle. Without doubt this peak belongs to the copper ($K\alpha_1$-quantum energy, 8·0 keV.) of the bronze grid on which the particle had been introduced into the electron microscope.

In view of these results it appears reasonable to continue with improved methods the examination of radioactive particles with regard to their inactive parts.

MANFRED F. RAJEWSKY
Max Planck Institut für Biophysik,
Frankfurt am Main.

* Dedicated to Prof. B. Rajewsky on the occasion of the twenty-fifth anniversary of the Max Planck Institut für Biophysik.

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The Bertele Arc

UNDER this title, Prof. D. Gabor[1] has made some very interesting observations about an unusual type of low-pressure mercury vapour discharge, which was first observed by Dr. H. von Bertele[2]. The cathode, a water-cooled molybdenum thimble of 1 cm.^{2} area, dips in a trough of mercury, and the 15-amp. arc appears gradually to spread over its entire surface as a "faintly luminous glow".

A significant fact, observed by Prof. Gabor, is that high-speed photographs, with exposure time of 1/15,000 sec., show the glow covering only about 1/10 of the surface, having a "complicated ramified appearance", and increasing in area proportionally to the current. This fact, plus some observations on the "noise" spectrum, forces Prof. Gabor to conclude that the discharge is some kind of a glow discharge, in spite of its impossibly low voltage drop of 10 V., and impossibly high current density of 150 amp./cm.^{2}.

The 10-V. cathode drop supports strongly the suggestion of Dr. A. Robson that this is a normal mercury arc, with a "cathode spot" of current density 10^5 amp./cm.^{2} (ref. 3) running over the surface at high speed. A simple calculation shows that this description fits the observed data remarkably well.

I assume, with Gabor, that the molybdenum is covered with a monatomic film of mercury, and interpret the "forming" period as the time necessary to clean the surface of its oxide layer. It is well known that mercury will not 'wet' molybdenum unless scrupulously clean; in fact, a surface once wetted, for example by cleaving under mercury, becomes quickly 'unwetted' on standing in a fairly good vacuum.

Let us first calculate the velocity of the "cathode spot". This is determined by the time, τ, necessary to evaporate the monatomic film, since the spot must move when it has exhausted its supply of mercury.

The number of atoms in the monatomic film, as given by the closest assembly of possible sites on a 111 molybdenum surface, is $N = 3{\cdot}9 \times 10^{14}$ atoms/cm.^{2}. The rate of evaporation at $T°$ K. and $p_{\text{mm.}}$ mercury pressure is $n = 3{\cdot}51 \times 10^{22}\, p/\sqrt{MT}$ (M = mol. wt.). Hence

$$\tau = \frac{N}{n} = \frac{3{\cdot}9 \times 10^{14}}{3{\cdot}51 \times 10^{22} p/\sqrt{MT}} = 1{\cdot}57 \times 10^{-7}\,\frac{\sqrt{T}}{p_{\text{mm.}}} \quad (1)$$

A second relation between τ and T is given by the time necessary to raise the temperature from 300° K. (temperature of cooling water) to T, namely:

$$\tau = \frac{\pi}{k}\,\frac{(\Delta T)^2K^2}{4Q^2}\ \text{(ref. 4)} \qquad (2)$$

$\Delta T = T - 300$, K = thermal conductivity of molybdenum = 1·38 W./cm. °K., k = diffusivity of molybdenum = 0·73 cm.^{2}/sec., $Q = (V_c + V_i - \varphi_0) \times \frac{I_0}{10}$, where V_c = cathode drop = 10 V., V_i = ionizing potential = 10·4 V., φ_0 = work function = 4·5 V., and $\frac{I_0}{10}$ = positive ion current density[5], I_0 = electron current density = 10^5 amp./cm.^{2}.

Eliminating T (and $p(T)$) between equations (1) and (2) yields:

$$\tau = 1{\cdot}18 \times 10^{-6}\ \text{sec.}\ (T = 422°\ \text{K.}, p = 2{\cdot}75\ \text{mm.})$$

The velocity with which a spot of current i moves over the surface is:

$$\text{vel.} = \frac{\text{diam. of spot}}{\tau} = \frac{2\sqrt{i/\pi \times 10^5}}{1{\cdot}18 \times 10^{-6}} = 3{\cdot}00 \times 10^3\sqrt{i} \quad (3)$$

$= 1{\cdot}17 \times 10^4$ cm./sec. for i = 15 amp.

This velocity is slow enough to be resolved in a 1/15,000 sec. exposure. In fact, it is so resolved, though not as a straight line but as the area of the 'random walk' of the cathode spot.

The motion of the spot is not completely 'random'. When it exhausts the mercury film in one place and has to move, it cannot move backward, since that is where it last came from and the mercury film there is exhausted. But it has an equal probability of moving forward, to the right, or to the left.

The distance moved forward in 1/15,000 sec. will be 1/3 vel. × 1/15,000 = 0·250 cm. This is the total forward distance, a very tortuous line. The distance laterally, at right angles to the forward path, will be a 'random walk' with a mean value ± 0·558 mm. = 1·16 mm. mean width[6].

Hence the area swept over by the spot in 1/15,000 sec., which is the area that is luminous during the time of the photograph, is:

$$\text{area} = 0{\cdot}26 \times 0{\cdot}116 = 0{\cdot}03\ \text{cm.}^2$$

However, since the motion of the spot is tortuous, its ramifications will include a much larger area, and the extent of the "ramified" area should approximate the 0·1 cm.^{2} observed. Also, this area will increase, approximately, in proportion to the current (the square of linear velocity, equation 3), as observed.

Gabor also measured the noise spectrum, and reports: "In the range up to 50 kilocycles the noise power is flat; beyond this there is a rapid fall-off with a complete cut-off at about a megacycle". This is just the spectrum to be expected from plasma ion oscillations, for which Tonks and Langmuir[7] report a maximum frequency "less than 1·5 megacycles", with a rapid fall-off from a frequency 1/25 of this value, namely, 60 kc./s. Cobine and Gallagher[8], who made a systematic study of total noise from arc discharges, found the spectrum for a mercury discharge flat out to a 'transition frequency' of 260 kc./s., with rapid fall-off above that to complete cut-off at 8 mc.

Hence it appears that the spectrum observed by Prof. Gabor is a reasonable one for a normal mercury arc.

Note: If the surface of molybdenum is covered with a thick film of mercury, rather than a monatomic layer, the velocity of the spot will be $6\cdot 1 \times 10^3 \sqrt{i}$[9] rather than $3\cdot 0 \times 10^3 \sqrt{i}$, and the area swept over will be four times as great, which is still closer to the observed value.

A. W. HULL

General Electric Co.,
The Knolls,
Schenectady, New York.

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An Ultra-Rapid Scanning Fabry–Perot Interferometer

IT is well known that the profile of a spectral line may be time-resolved by an arrangement in which light is passed normally through a Fabry–Perot etalon, the spacing of which is varied in time, while a photomultiplier looks at the central fringe of the circular (Haidinger) pattern.

Here we have used a piezo-electric barium titanate element to control the etalon spacing. The method gave a scan speed of one fringe in 3 μsec. to be compared with 100 μsec. obtained by Bradley[1], who suspended the moving mirror in a mechanical vibrating system driven by a solenoid, and with 50 μsec. obtained by Koloshnikov *et al.*[2], who used the linear displacement of a ceramic element.

The apparatus is shown schematically in Fig. 1. Light was passed axially through the hollow cylinder of barium titanate, the two cylindrical surfaces of which had been silvered to form electrodes. To the end of this cylinder was attached a fused quartz optical flat (polished flat to λ/10 and silvered to a reflectivity of 80–85 per cent) which formed one plate of the interferometer. The ceramic tube was clamped at its centre and driven at parallel resonance in its lengthwise mode (a frequency of 27·8 kc./s.) by means of an audio-frequency signal generator. The other plate was an identical quartz flat, mounted in an adjustable stand to allow initial alignment to parallelism.

To test the apparatus experimentally the sodium *D* lines emitted by a standard sodium lamp were scanned; Fig. 2 shows a typical oscilloscope recording. The lower half of the figure shows the voltage applied across the ceramic tube, in this case approximately 30 V. peak-to-peak, while the upper half shows the output of the photomultiplier (*IP*21). In the two regions *AA′*, *BB′*, the rate of change of voltage with time is approximately constant, since the displacement of the ceramic is directly proportional to the applied voltage, and since the wave-length observed by the photomultiplier is directly dependent on the separation of the interferometer plates, in these two regions the linear time-base is also the linear wave-length scale. As three orders of the fringe pattern were scanned per half-period, the second order lies clearly in the region of linear wave-length scale, and so using the doublet separation as a standard wave-length interval of 6 Å. the half-widths of the two *D* lines were easily measured. Both lines had a half-width of about 3 Å. and the free spectral range of the etalon was 14·5 ± 0·5 Å., this interval being scanned in 3·5 μsec.

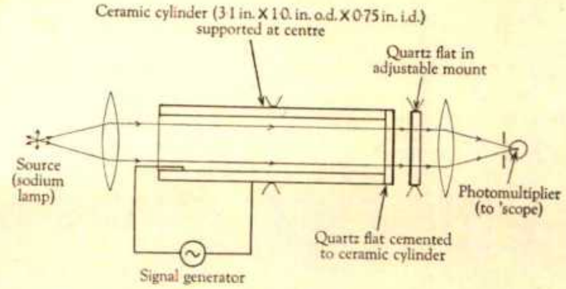
Ceramic cylinder (3·1 in. × 1·0. in. o.d. × 0·75 in. i.d.) supported at centre
Quartz flat in adjustable mount
Source (sodium lamp)
Photomultiplier (to 'scope)
Quartz flat cemented to ceramic cylinder
Signal generator

Fig. 1. Schematic diagram of scanning Fabry–Perot interferometer

With an initial plate flatness of λ/10 the instrumental width due to flatness alone would be approximately 3 Å., and if the effective flatness were increased to λ/15, when only half aperture was used, the instrumental width due to flatness would still be about 2 Å. Therefore, allowing a further instrumental width of 1 Å. for the effects of reflectivity and the rise-time of the amplifier (0·1 μsec.), the instrumental width caused by distortion of the moving plate and lack of parallelism of the movement must be 2 Å. or less, that is, the quality of the movement is as good as the flatness of the Fabry–Perot plates. A second interferometer has already been designed with plates flat to λ/50 so that more stringent tests can be made on this method of scanning.

It is important to realize that although only three orders of the fringe pattern have been scanned in the case of Fig. 2, the power input to the ceramic resonator was less than 0·01 W. With a power input of 1 W., twenty orders were scanned per half-period (that is, in 15 μsec.) so that one order was scanned

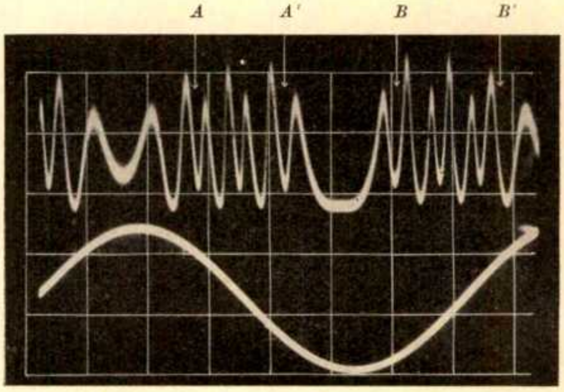

Fig. 2. Oscilloscope recording. Time base 5 μsec. per division

in 0·5 μsec. and a single spectral line could be scanned with this arrangement in about 0·1 μsec. The resolution at such a rapid scanning-rate is limited with the present arrangement by the amplifier rise-time and shot-noise from the photomultiplier.

The maximum possible time resolution is governed by the half-period of the ceramic resonator and the number of fringes scanned in that time. Thus it might be thought that the time resolution could be increased if an interferometer were designed to work at a higher resonant frequency. There is, however, a limit to this process: to prevent the moving Fabry–Perot plate resonating in its flexural mode (such a vibration would of course destroy the Haidinger fringe pattern) the resonant flexural frequency of the moving plate should be at least twice the resonant scanning frequency. With the present system the flexural frequency of the moving plate is only 50 kc./s., which is rather low and probably accounts for the marked increase in resolution when the aperture is decreased by inserting an aperture stop. In the new interferometer the flexural frequency of the moving plate will be 100 kc./s., the scanning frequency remaining at about 30 kc./s.

This instrument, with its time resolution of better than 0·5 μsec. coupled with the large light-gathering capacity, the direct display of the line intensity profile, and other advantages of the scanned Fabry–Perot interferometer, will be especially useful for investigating the physical conditions within pulsed discharges used in controlled fusion research.

J. Cooper
J. R. Greig

High Temperature Group,
Department of Physics,
Imperial College of
Science and Technology,
London, S.W.7.

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Observations on Gaseous Detonation Waves using a Microwave Interferometer

Considerable attention has been devoted to the study of the structure of the reaction zone in gaseous detonation waves with the view of providing information on the duration and quantitative variations occurring in the gas parameters. In order to make these variations amenable to experimental study the waves are usually generated in mixtures at reduced initial pressures so as to prolong the duration of the reaction. If the overall reaction is second order the reaction time is then inversely proportional to the initial pressure.

Unfortunately, at low initial pressures, great difficulty is encountered in some mixtures in establishing an unsupported stable wave. The main criterion of stability is often taken to be the constancy of the wave velocity, which is normally measured over several lengths of the detonation tube preceding the observation section. The desirability of providing a continuous monitor of the velocity over the long lengths of tube, which are necessary at low pressure, is evident. For this purpose the feasibility of using a 3-cm. microwave interferometer[1] has been investigated; this utilizes the interference effect between a microwave beam, launched at the closed end of the detonation tube, and the beam reflected from the dielectric discontinuity at the detonation front.

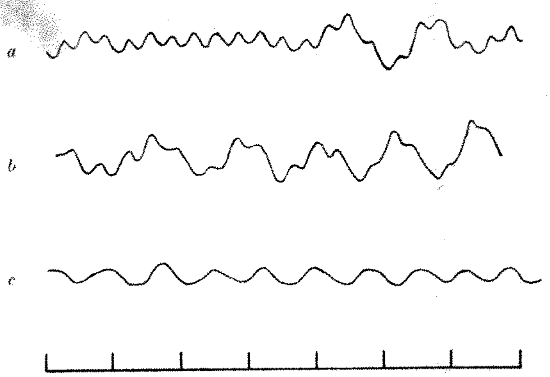

Fig. 1. Single lines of oscilloscope raster sweep display of microwave interferograms obtained from detonation waves in $2H_2 + O_2$ at initial pressures of (*a*) 300, (*b*) 200 and (*c*) 100 mm.

In our preliminary work, the mixture which has been mainly investigated is $2H_2 + O_2$, for which the duration of the reaction zone is ~ 2 μs. in observer's time, at an initial pressure of 100 mm. of mercury. The detonation tube is made of stainless steel, 15 m. long and 5 cm. diameter. Detonation is initiated by a $C_2H_2 + O_2$ mixture, contained in a 1-m. long driving section, which is ignited by an 8-joule spark. The calculated electron density in $2H_2 + O_2$, with the addition of $\frac{3}{8}$ per cent nitrogen as a controlled impurity-level, at 300 mm. of mercury, is $0{\cdot}75 \times 10^{12}$ electrons/c.c. (ref. 2); the 'skin depth', assuming a step profile for ionization for a wavelength of 3 cm., is 10 cm. and the power attenuation of the signal on reflexion is 16 db.

The salient features of the observations made hitherto are the following. At initial pressures down to approximately 500 mm. the reflected power is too small to be detectable with the apparatus available. From about 450–350 mm. pressure, a sinusoidal interferogram is obtained over the whole length of the tube which corresponds closely to the predicted detonation velocity of 2,800 m./s. When a pressure of 300 mm. is reached the regularity of the pattern is intermittently broken by a lower frequency modulation, as shown in Fig. 1*a*; this would correspond to a region moving with a velocity of 850 m./s. This value is well below the mass velocity of the gas at the *C–J* plane of 1,200 m./s.; consequently, its origin is open to conjecture. One may hazard the opinion that it arises from some periodic instability of the front, such as 'spin', which appears to occur frequently at low pressure[3]. The observed reflected power loss at this pressure is 20 db., which agrees reasonably well with the above estimated value. Similar records are obtained at 200 mm. pressure, Fig. 1*b*, except that the lower frequency is always present superposed on a higher frequency corresponding to the detonation front velocity. At 100 mm. the electron density in the reaction zone is insufficient to reflect the microwaves, but reflexions are obtained from a region of ionization travelling with the mass velocity of the gas of 1,200 m./s. Waves in $C_2H_2 + O_2$ exhibit perfectly normal interferograms down to initial pressures of 20 mm.

The non-uniformity of the ionization across the detonation front in $2H_2 + O_2$ provides further evidence[3] of the non-ideality of these waves at fairly high initial pressures. Moreover, the results emphasize the inadequacy of velocity measurements alone as a criterion of wave stability.

D. H. Edwards
E. M. Job
T. R. Lawrence

Department of Physics,
University College of Wales,
Aberystwyth.

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METALLURGY

Substructures in Nickel grown from the Melt

As part of an investigation into the effect of substrate structure on the nucleation and growth of electro-deposits, it was desired to produce in a metal single crystal of high purity various types of imperfection. 'Striation'[1] boundaries and cell boundaries ('corrugation'[2] boundaries) were thought to be suitable as the former should give low-angle boundaries ($\frac{1}{4}$°–5°) with little impurity while the latter should illustrate the effect on nucleation of the impurity contained in cell boundaries.

Floating-zone melting was used to make the single crystals, as contamination is minimized in this way[3]. Preliminary experiments showed that nickel rod of 4 mm. diameter was quite tractable in the electron bombardment apparatus used[4].

A Berg–Barrett X-ray micrographic technique[5] was used for examining the substructures obtained. Specimens of about 2 × 3 × 4 mm. were cut from the rod crystals and these were ground and electropolished to remove the work-hardened layers produced by cutting. A chromium target was used[5], and the radiation was neither filtered nor monochromated. In order to bathe the whole specimen with X-rays, a 10-mm. diameter beam was used and it was found to be necessary to evacuate the long lead collimator tube to reduce absorption of the soft radiation. Kodak *B*10 photographic plates were used to record the images as although these plates have a poorer resolution than Kodak Maximum Resolution plates, they require only short exposures and have proved to be very easy to handle and process.

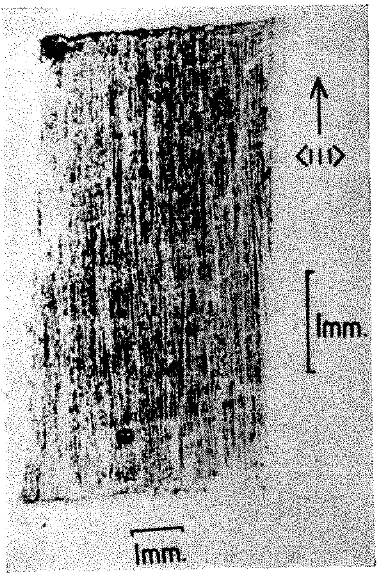

Fig. 1. A Berg–Barrett micrograph of a <111> specimen showing corrugations

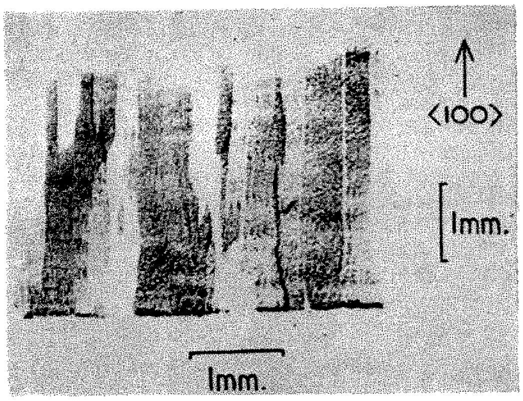

Fig. 2. A Berg–Barrett micrograph of a <100> specimen showing striations and cells

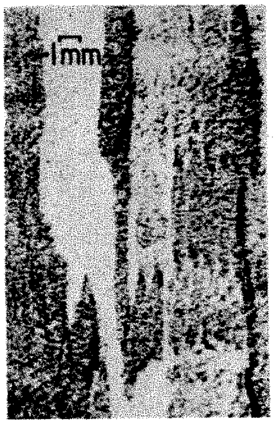

Fig. 3. An enlargement of Fig. 2 showing cells

It is thought that resolution of detail in the image may have been impaired by the distortions induced by sawing and grinding despite the electropolishing treatment, and a chemical saw has been built to eliminate this distortion in future.

In seven experiments, random nucleation of single crystals from polycrystalline rods produced crystal orientations within 10° of <111>. (Orientations refer to the crystallographic direction lying along the rod axis. This is the heat flow axis and hence the direction of growth of the solid front.) This is in accordance with the data of Rosenberg and Tiller[6] which give the preferred growth orientation for pure face-centred cubic metals as <111>. In a <111> crystal grown at 4 mm./min., corrugations were produced, but no striations were seen on the Berg–Barrett image (Fig. 1). The absence of striations in this specimen may be due to the fact that, at fast rates of growth, striations form parallel to a direction <*hkl*>[1], and if this direction is inclined at a large angle to the growth axis, <111> in this case, then 'latent' striations may have grown out of the rod during the incubation period[7]. Aust and Chalmers[8] state that the <*hkl*> for the face-centred cubic aluminium is <100>, which is inclined at 54° 44′ to <111>, and it may also be <100> in the case of nickel.

With the use of a < 100 > seed crystal cut from one of the < 111 > rods, crystals with orientations near to < 100 > were obtained and in these both striations and corrugations were apparent at a growth-rate of 13 mm./min. The Berg–Barrett micrograph (Fig. 2) shows the striations running approximately parallel to the growth direction < 100 > and an enlargement of a region of a striation (Fig. 3) shows a cell structure resulting from impurity segregation.

Although mention is made of the occurrence of striations[9] and corrugations[2] in nickel, no published data have been found on the sub-structure dimensions. The present results are, however, compared in Table 1 with the published data on tin by Chalmers *et al.*

Table 1. SUBSTRUCTURE DIMENSIONS IN NICKEL AND TIN

	Striations			Cells (corrugations)		
	Orientation	Growth-rate (mm./min.)	Width (mm.)	Orientation	Growth-rate (mm./min.)	Width (mm.)
Ni	< 100 >	13	0·25	< 100 > < 111 >	13 4	0·07 0·05
Sn	< 110 >	10	0·25 (ref. 1)		5	0·05 (ref. 9)

The material used for these experiments was 99·965 per cent nickel, the principal impurities being iron and silicon. It has not so far been possible to make measurements of the temperature gradient during growth in the melting apparatus.

Table 1 illustrates some measure of agreement between the results for nickel and the published data for tin and this would suggest that essentially the same processes operate during the solidification of the comparatively refractory metal nickel, as for tin.

We thank Prof. E. C. Rollason for provision of the facilities to carry out the work. The provision of a scholarship by W. Canning and Co., Ltd. (R. B.), and general financial assistance by Joseph Lucas, Ltd., are gratefully acknowledged. We also thank the International Nickel Co. (Mond), Ltd., for provision of the stock material.

R. BROWNSWORD
J. P. G. FARR

Department of Industrial Metallurgy,
University of Birmingham.

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Effect of Strain-Rate on the Tensile-Yield Stresses of Niobium and Tantalum

IN the course of a systematic study of the mechanical properties of the body-centred cubic transition metals which is now being made in this laboratory, measurements have been made of the effect of strain-rate on the tensile-yield stresses of commercially pure niobium and tantalum. Since these measurements have given interesting results, it is proposed to give a preliminary account of them here.

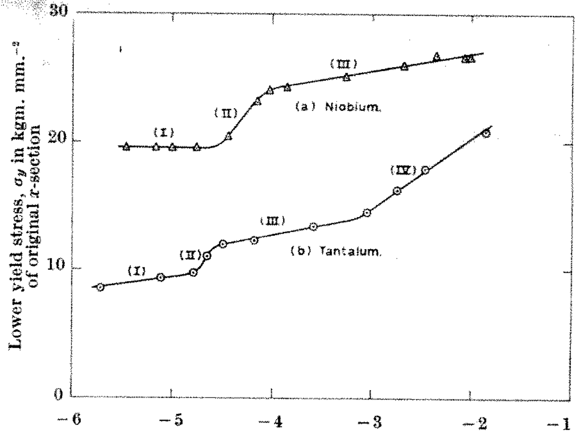

Fig. 1. Effect of strain-rate, $\dot{\varepsilon}$, on the room temperature yield stresses of (*a*) niobium, and (*b*) tantalum

Specimens of average grain diameter about 25μ were prepared from sintered niobium supplied by Murex, Ltd., and tantalum specimens having an average grain diameter of about 400μ from electron beam melted material supplied by the Société Generale de Métallurgie, Hoboken.

Fig. 1 shows the yield stresses of the two materials at room temperature plotted as functions of $\log_{10}\dot{\varepsilon}$, where $\dot{\varepsilon}$ is the strain-rate in sec.$^{-1}$. In the case of niobium three linear ranges are obtained. In range I ($\dot{\varepsilon} < 2 \times 10^{-5}$ sec.$^{-1}$) the yield stress, σ_y, is independent of strain-rate. In range II, ($\dot{\varepsilon} \approx 2 \times 10^{-5}$ to 1×10^{-4} sec.$^{-1}$), and range III, ($\dot{\varepsilon} > 1 \times 10^{-4}$ sec.$^{-1}$), σ_y and $\dot{\varepsilon}$ are related by the equation:

$$\sigma_y = A + A' \log_{10} \dot{\varepsilon} \qquad (1)$$

the values of A and A' being different in the two ranges. In the case of tantalum there are four linear ranges each having different values of A and A'.

An interpretation of the parameter A' can be developed if it is assumed that, in the strain-rate ranges where equation (1) is obeyed, yielding is controlled by stress aided thermally activated dislocation movements (for example, ref. 1). If the assumptions made in this type of theory are accepted, it can readily be shown that A' is related to the first derivative of the activation enthalpy, $\Delta H(\sigma,T)$, with respect to stress at constant temperature, thus:

$$\left(\frac{\partial \Delta H(\sigma,T)}{\partial \sigma}\right)_T = \frac{kT}{A'} \qquad (2)$$

where k is Boltzmann's constant and T the absolute temperature. The left-hand side of equation (2) is known as the 'activation volume', v^*, for the process and is equal to the product of the burgers vector, b, of a slip dislocation and the area swept out by a dislocation segment during activation.

The values of v^* for ranges II and III in niobium come out to be about $7b^3$ and $51b^3$ respectively. In range I, where σ_y is independent of $\dot{\varepsilon}$, v^* is very large, that is, the deformation is controlled by a mechanism for which thermal activation is unimportant. In the case of tantalum, it seems that thermally activated processes control the deformation in all four strain-rate ranges and v^* has a different value for each. The values are $82b^3$, about $6b^3$, $49b^3$, and $16b^3$, for ranges, I, II, III and IV respectively.

The main importance of these results is that they imply that the mechanism controlling the deformation changes with strain-rate in both metals, there being three different mechanisms in the case of niobium and four in the case of tantalum As the result of earlier experiments, it has been suggested that the yielding of iron[1] and possibly other body-centred cubic transition metals[2] is controlled by the Peierls–Nabarro stress of the lattice It is now apparent that this can be so for at most one strain-rate range in each metal

We thank the U K Atomic Energy Authority and the Central Electricity Generating Board for their financial support One of us (G A S) is also indebted to the Department of Scientific and Industrial Research for the award of a research studentship

G A Sargent
P J Sherwood
A A Johnson

Department of Metallurgy,
Imperial College of Science and Technology,
London, S W 7

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RADIOCHEMISTRY

Radium and Fission Product Radioactivity in Thermal Waters

Investigations of the radioactivity of spring waters in Hot Springs National Park, Hot Springs, Arkansas, have been published by Boltwood[1], Schlundt[2], and Kuroda *et al* [3] Particular attention was paid to the radon content, which fluctuated considerably from day to day and from season to season In such waters fission products might be detected, coming from fall-out and from fission of uranium ores

The latter process, for spontaneous fission of uranium, would yield about 8 × 10^{-8} µµc of strontium-89 and -90 activity per litre according to Jonte[4] This is below the present limits of detection If the waters were of meteoric origin, fission-product activity from fall-out could possibly be detected The work described here was performed at the end of 1960 when only long-lived activity from fall-out would still be present, and was in a sense preliminary to later work to be carried out closely following bomb tests Also included is a report on analyses of water from Buie Springs, Marlin, Texas

Samples of water from Buie Springs, and from Display Springs No 1 and No 2 at Hot Springs, Arkansas, were collected in October and November, 1960, and analysed 2-l samples were used for radium determinations 40-l samples were used to search for barium-140, and strontium-89 and -90 fission-product activity The samples were collected 24 months after the atmospheric nuclear test suspension had begun Rain at Fayetteville, Arkansas, in the autumn of 1960 contained no measurable amounts of barium-140 or strontium-89, and about 0 5 µµc /l strontium-90 (Nix, J F , personal communication, 1961)

To measure the radium present in Buie Springs water, inactive barium carrier was added to a water sample, and barium sulphate precipitated This was fused with sodium carbonate, the resulting barium carbonate was washed with large quantities of water and then dissolved in dilute nitric acid Four days later the solution was scavenged with ferric hydroxide, and the precipitate containing radium decay products was counted for β-activity in a gas-flow *CE*-14 Tracerlab counter, having a low background of 1 0 count/min Barium and radium carbonates were precipitated from the filtrate and counted

Radium activities shown in Table 1 were calculated using pitchblende of known uranium content as a standard for radium-226, and Armbrust's[5] relative counting efficiencies for the radium-228 radium-226 chains

Table 1

	Buie Springs	Display Springs No 1	Display Spring No 2
Sampling date	Oct 1960	Nov 4, 1960	Nov 4, 1960
Temperature	64° C	59° C	54° C
*p*H	7 85		
Ca	250	38	38
$SO_4^{=}$ p p m	> 100		
^{89}Sr (µµc /l)		none	none
^{90}Sr (µµc /l)		< 0 12	< 0 2
^{140}Ba (µµc /l)		< 0 07	< 0 005
^{226}Ra (µµc /l)	12 ± 1	3 0 ± 0 5	1 2 ± 0 3
^{228}Ra (µµc /l)	2 1 ± 0 4	none	none

Figs 1 and 2 show the activity of the ferric hydroxide fraction and the barium fraction activity respectively The activity in Fig 1 appears to have a 6 1-hr half-life and may be attributed to actinium-228 Because this activity was less than 2 counts/min and was short-lived, counting accuracy was only within 15–20 per cent

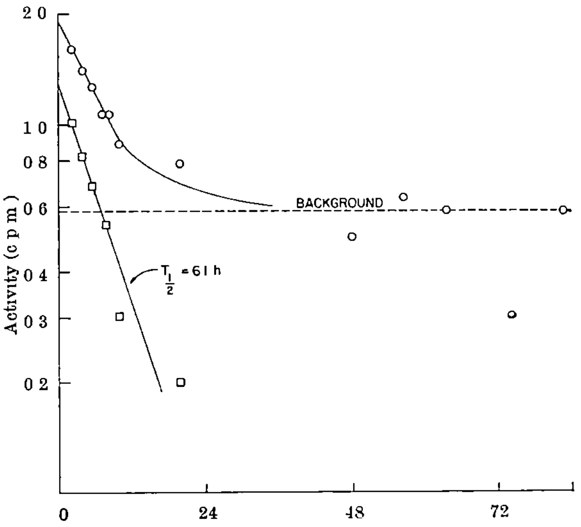

Fig 1 Ferric hydroxide fraction containing radium daughters Buie Springs, Texas ○, Observed counting-rate, □, counting-rate corrected for background

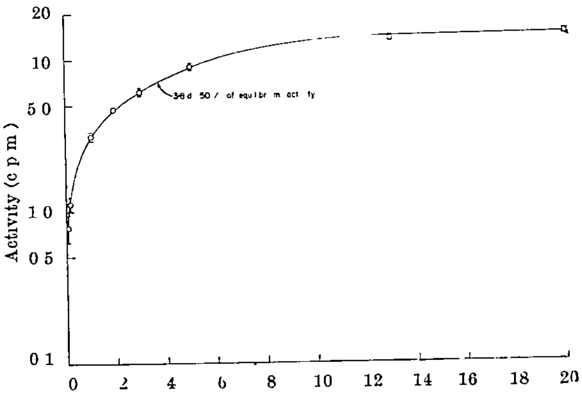

Fig 2 Radium and barium carbonates Buie Springs Texas

Fifty per cent of the equilibrium activity in Fig 2 had grown in 3 8 days, suggesting that radium-226 was present and decaying to ^{222}Rn ($T_{1/2}$ = 3 8 days) In turn the latter decayed to 26 8 m lead-214 and 19 7 m bismuth-214 β-emitters They contributed to the activity in Fig 2, but do not appear in Fig 1. for they had largely decayed away by the time counting was begun

To samples of water from Hot Springs inactive strontium and barium carriers were added, and enough disodium ethylenediamine tetraacetate was added to complex 90 per cent of the calcium present, before precipitating carbonates, to prevent spuriously high strontium yields Further calcium–strontium separation was accomplished satisfactorily by repeated precipitations of strontium in fuming nitric acid After counting the final strontium precipitate (as carbonate) for several weeks to establish that no strontium-89 was observed, strontium-90 was determined by ferric hydroxide scavenging for the yttrium-90 daughter Barium carbonate was counted several weeks, and then scavenged with lanthanum hydroxide to collect any lanthanum-140 and radium daughters

Only short-lived (< 35 min) β-activity was found in lanthanum hydroxide scavengings of water from both hot springs, and the radium parent indicated is radium-226 Radium daughter activities co-precipitated with lanthanum hydroxide would eclipse the low amount of lanthanum-140 that might be present Therefore an upper limit for barium-140 is reported in Table 1, although none was observed

The activity observed for the ferric hydroxide fraction at t_0 was 0 3 c p m above background, and from such poor statistics only an upper limit for strontium-90 in the hot springs could be set It is quite possible that all the activity was in reality due to radium contamination of the strontium precipitate

I thank Prof P K Kuroda for his advice This work was made possible by the support of the U S Atomic Energy Commission

L M Fry

Department of Chemistry,
University of Arkansas,
Fayetteville, Arkansas.

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CHEMISTRY

Formation of Alumina from a 'Hydrogen' Alunite

In a process for making pure alumina which is being investigated by us[1], the intermediate product is a basic aluminium sulphate, averaging 39 5 per cent alumina, 40 5 per cent sulphur trioxide and 20 per cent water This material is isomorphous with the alunite series, giving an X-ray diffraction pattern almost identical with the potassium and sodium compounds, all having the general formula R_2O, $3Al_2O_3$, $4SO_3$, $6H_2O$

However, basic aluminium sulphate differs from its alkali-metal analogues in that it can be converted to alumina by calcination at any temperature above 1,000° C and requires no further purification to remove contaminants such as potassium sulphate and sodium sulphate

At lower temperatures in the range 600°–800° C the basic aluminium sulphate is converted to an amorphous material having the approximate formula $3Al_2O_3$, $4SO_3$ The dehydrated basic aluminium sulphate is much more easily dissolved than the starting material and it is possible to make very basic solutions of aluminium sulphate simply by boiling dehydrated basic aluminium sulphate for 15–30 min with a small amount of dilute sulphuric acid[2]

In the temperature-range 750°–950° C dehydrated basic aluminium sulphate loses sulphur trioxide (in two equal and distinct stages), and recrystallizes to form eta-alumina containing only 2–4 per cent of residual sulphur trioxide The rate of decomposition depends on temperature and bed depth, but with a thin bed (1–2 mm) conversion to eta-alumina is substantially completed within 10 min at 900° C

Eta-alumina is normally prepared by the dehydration of beta-alumina trihydrate at temperatures as low as 300° C [3] At higher temperatures (900°–1,000° C) it transforms to theta-alumina and only reverts to alpha-alumina (the final product) at 1,150° C

It is interesting to note, however, that we have been unable to detect theta-alumina during the further calcination of eta-alumina prepared from basic aluminium sulphate After 1 hr at 1,000° C the X-ray powder diffraction pattern shows only alpha-alumina and residual eta-alumina We believe therefore, that residual sulphur trioxide (0 7 per cent at 1,000° C) tends to encourage in some way the direct conversion of eta-alumina to the alpha form Conversely, it is thought possible that residual sodium (approximately 0 3 per cent sodium monoxide) favours the intermediate transition to theta-alumina when the starting material has been prepared as usual from sodium aluminate solutions

We thank Mrs A B McIlhiney and Mr N F Dyson for help in this work

P T Davey
T R Scott

Division of Mineral Chemistry,
C S I R O , Melbourne,
Australia

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Ancient Iron Nails well preserved from Apparently Corrosive Soils

Some years ago, an examination was made in this Laboratory[1] of samples of well-preserved iron objects of Roman origin discovered at Hungate, York A tentative explanation was given for the absence of corrosion, based on the detection in the surrounding soil of traces of phosphates and tannins and on the presence of a coating of mixed ferrous and ferric phosphates on the objects themselves It was suggested that the tannins were inhibitory of the activity of sulphate-reducing bacteria, which would otherwise have been expected to cause severe corrosion of iron in the anaerobic conditions maintaining It was also considered that the phosphate coating alone would probably not provide sufficient protection against corrosion over such a long period (2,000 years), because it is known that industrially produced phosphate coatings are not, in themselves, usually effective in preventing corrosion completely More recent work[2] has shown, however, that sulphate-reducing bacteria are not rendered inactive unless the concentration of tannin reaches a value of about 5 per cent, far in excess of the traces reported in the soils from York It is necessary, therefore, to look for another cause of the protection of the iron

We have now had an opportunity for some further study of the phenomenon In 1961, a number of very well preserved iron nails of early sixteenth century origin were found in silty gravel on an archæological site at St. Neots, Huntingdonshire Chemical and microbiological examination of the soil showed that it contained sulphide and that sulphate-reducing bacteria were active Such a soil would be expected to be highly aggressive to iron

The nails themselves were of a fairly pure iron and carried a highly adherent grey-black coating about 0 5 mm in thickness A transverse section showed that the coating was very coherent and uniform and that a sharp demarcation existed between the coating and the underlying metal Examination of the coating by X-ray diffraction showed it to consist mainly of ferrous phosphate (vivianite), but with a proportion of other material the nature of which we were unable to determine Significantly, we could not detect any oxides, hydroxides, sulphides, chlorides, sulphates or other phosphates of iron Some oxidation of the outer surface of the coating occurred, however, when the nails were left for some weeks exposed to the laboratory atmosphere

We have obtained electrochemical polarization curves for the nails in anaerobic microbiological medium and compared them with similar curves obtained for mild steel in similar conditions

Cathodic polarization curves were similar in the two cases, giving Tafel slopes of about − 165 mV , but the curves for the nails were about 270 mV more positive throughout their length The nature of the cathodic reaction on the coated nails is not known, but must be possible at the open circuit potential of the nails (− 170 mV E_H) in the absence of oxygen Hydrogen evolution would therefore seem to be excluded

The anodic polarization curves differed strikingly For bare mild steel, a Tafel slope of 37 mV was obtained, in close agreement with the value obtained in like conditions by other workers[3] For the nails, however, in addition to the displacement to more noble potentials, the anodic polarization curves gave a Tafel slope of 200 mV This great increase in slope is typical of anodic behaviour of metals in solutions giving anodic films It can be interpreted as a restriction of the passage of ferrous ions from the metal into solution, through the coating, far in excess of that imposed by an artificially prepared phosphate coating It is clear that this natural phosphate coating has features that require further investigation

A more detailed account of these investigations will be presented elsewhere[4]

G H BOOTH
A K TILLER
F WORMWELL

National Chemical Laboratory,
Teddington, Middlesex

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Borides of Ruthenium, Osmium and Iridium

Buddery and Welch[1] have published approximate compositions for a number of intermediate phases in binary system of boron with the platinum metals The phases were identified by X-ray powder techniques, but crystallographical data were given only for a few of them During the past decade the crystal structures of a number of binary platinum metal borides have been determined, and in Table 1 we have tentatively tried to identify the phases originally reported by Buddery and Welch with the more completely characterized intermediate phases in the ruthenium–boron, osmium–boron and iridium–boron systems

The composition of the various phases as given in Table 1 is only approximate It has been arrived at by examining the powder photographs of arc-melted alloys for each of which only the nominal composition was known The formula for the most boron-rich phase in each system, namely, $RuB_{\sim 2\ 1}$, $OsB_{\sim 2\ 2}$ and $IrB_{\sim 1\ 5}$, corresponds to a metal-rich composition in the homogeneity-range the extent of which is unknown Ru_7B_3 and $Ru_{11}B_8$ appear to have a composition closely corresponding to the ideal structural formula Whether or not this is the case for the more boron-rich phases is uncertain and, besides, it seems largely a matter of opinion which 'ideal structural formula' one prefers to assign to these phases

Table 1 CRYSTALLOGRAPHIC CONSTANTS FOR BORIDES OF RUTHENIUM, OSMIUM AND IRIDIUM

Approximate composition	Structure type	Lattice parameters (Å.)	Ref	Notation according to Buddery and Welch (ref 1)
Ru_7B_3	Th_7Fe_3	$a = 7\ 46_9$, $c = 4\ 71_8$	2	Ru_2B
$Ru_{11}B_8$		$a = 11\ 61$, $b = 11\ 34$, $c = 2\ 83_6$	3	
$RuB_{\sim 1\ 1}$	AlB_2 or WC	$a = 2\ 852$, $c = 2\ 855$	4	RuB
$RuB_{\sim 1\ 5}$	W_2B_5	$a = 2\ 89$, $c = 12\ 81$	4	Ru_2B_3
$RuB_{\sim 2\ 1}$		$a = 4\ 645$, $b = 2\ 865$, $c = 4\ 045$		RuB_2
$OsB_{\sim 1\ 2}$	AlB_2 or WC	$a = 2\ 876$, $c = 2\ 871$	4	OsB
$OsB_{\sim 1\ 6}$	W_2B_5	$a = 2\ 91$, $c = 12\ 91$	4	OsB_2
$OsB_{\sim 2\ 2}$		$a = 4\ 684$, $b = 2\ 872$, $c = 4\ 096$		OsB_2-
$IrB_{\sim 1\ 1}$	α-$ThSi_2$	$a = 2\ 810$, $c = 10\ 26_3$	5	IrB
$IrB_{\sim 1\ 5}$		$a = 10\ 52_6$, $b = 2\ 910$, $c = 6\ 099$, $\beta = 91°$		IrB_2

The phases here referred to as $RuB_{\sim 1\ 1}$ and $OsB_{\sim 1\ 2}$ were tentatively indexed as cubic by Buddery and Welch[1] However, these borides are evidently identical with the AlB_2-type phases which Kempter and Fries[4] recently reported The earlier cubic indexing of these hexagonal phases depends on the c/a ratio being very close to unity Kempter and Fries[4], who prepared ruthenium–boron and osmium–boron alloys by sintering on BN-disks, do not report any deviation from the composition MeB_2, while the observations by Buddery and Welch[1] as well as ourselves indicate a considerable deviation from this composition A more thorough investigation into this problem is being undertaken at this Institute When more precise data are available it will be possible to decide whether these phases should be regarded as crystallizing in the AlB_2- or the WC-type structure

We have confirmed the results of Kempter and Fries[4] that there are phases with the W_2B_5-type structure in the ruthenium–boron and osmium–boron systems and that the boron content of these phases is smaller than 71 4 atomic per cent (Me_2B_5) This formula applies if boron atoms completely occupy the four crystallographical positions in which they can be located without unreasonably short interatomic distances being formed It has not yet been determined experimentally to what extent these possible boron positions are actually occupied in the various phases possessing the same arrangement of metal atoms as W_2B_5 The phases $RuB_{\sim 2 1}$ and $OsB_{\sim 2 2}$ are very probably identical with RuB_2 and OsB_{2+} reported by Buddery and Welch[1] as well as with the phases observed by Kempter and Fries[4] in alloys containing 71 atomic per cent boron These isomorphous phases have an orthorhombic structure the most probable space-group being *Pmmn* The metal atoms are located at 2(*a*) with $z_{Ru} = 0\ 1508$ and $z_{Os} = 0\ 1535$, respectively (The centre of symmetry has been chosen as origin) There is strong experimental evidence that boron atoms occupy a 4(*f*) position with the approximate co-ordinates $x_B = 0\ 059$, $z_B = 0\ 639$ in $RuB_{\sim 2 1}$, and $x_B = 0\ 058$, $z_B = 0\ 632$ in $OsB_{\sim 2 2}$ Considerations of the space available indicate that boron atoms can also be located at 2(*b*) ($z \sim 0\ 8$), but, for the crystals studied, the difference syntheses did not display significant maxima at this position It is not possible to decide to what extent boron atoms may occupy a 2(*b*) position in these phases until very accurate investigations of the homogeneity ranges as well as of the crystal structures have been undertaken

The most boron-rich intermediate phase observed in arc-melted iridium–boron alloys has a composition approximating to $IrB_{\sim 1 5}$ It has a monoclinic structure for which the most probable space-group is *C* 2/*m* The iridium atoms occupy two sets of 4(*i*) positions with the co-ordinates $x_I = 0\ 0995$, $z_I = 0\ 1388$, $x_{II} = 0\ 3576$, $z_{II} = 0\ 2885$ Space considerations indicate that boron atoms can be located in four sets of 4(*i*)-positions All the possible boron positions correspond to positive regions in the electron density difference synthesis, where the contribution of the iridium atoms has been subtracted The approximate co-ordinates of the positions as derived from the difference synthesis are as follows

$x_{III} = 0\ 553$ $z_{III} = 0\ 388$
$x_{IV} = 0\ 180$ $z_{IV} = 0\ 465$
$x_V = 0\ 712$ $z_V = 0\ 309$
$x_{VI} = 0\ 754$ $z_{VI} = 0\ 069$

Complete occupation by boron atoms of the positions V and VI would imply some very short iridium–boron and boron–boron contacts in the structure It would not be incompatible with the experimental results to assume that one or both of these positions are only partly occupied but, as yet, no firm conclusion can be reached on this point

We thank Prof G Hagg for his kind interest This work was financially supported by the Swedish Natural Science Research Council and the Air Force Office of Scientific Research, OAR, through the European Office, Aerospace Research, U S Air Force, under contract *AF* 61(052)–40

B Aronsson
E Stenberg
J Åselius

Institute of Chemistry,
University of Uppsala

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Determination of Polyunsaturated Fatty Acids in *Hevea* Latex

The unsaturated fatty acids expected to be present in *Hevea* latex are very probably oleic, linoleic and linolenic Altman[1], using a method as described by Hazura, succeeded in showing the presence of oleic and linoleic acid in this latex According to this method the unsaturated fatty acids are oxidized by alkaline potassium permanganate solution, converting them to the corresponding oxyacids Altman could identify the dioxystearic acid (m p 131°) and sativic acid (m p 175°)

When the fatty acids mixture consists of saturated fatty acids, oleic and linoleic acid, the method as described by Bertram[2] is applicable These methods are based mainly on the addition of substances, namely, iodine, bromine, etc , to the double bonds, and are indirect, thus making the results not always trustworthy Determination of a mixture of unsaturated fatty acids comprising those having more than three double bonds is more difficult

At present spectrophotometric methods[3] are used to determine the composition of fatty acids mixtures These methods are based on the observation by Moore that linoleic and linolenic acids are isomerized in an alcoholic alkali solution to a conjugated system of double bonds that gives an absorption in the ultraviolet region The Spectroscopy Committee[4] reported extensively on the spectrophotometric method for the determination of polyunsaturated fatty acids by alkali isomerization Equations for the calculation of the polyenoic acid contents were given Micromethods were developed by Hammond and Lundberg[5], Herb and Riemenschneider[6] and Holman and Hayes[7] A method essentially similar to that described by Herb and Riemenschneider has been used by me for the determination of polyunsaturated fatty acids in *Hevea* latex

The reaction vessels used were as proposed by Herb and Riemenschneider and made of 'Pyrex' tubes The vessels have caps with ground joints and are provided with nitrogen gas inlet tubes The lower parts of the vessels had an inner diameter of 16 mm A Beckman model *DU* spectrophotometer equipped with a hydrogen lamp was used for the measurement of the ultra-violet spectra of the samples before and after isomerization The absorbancies were measured in matched quartz cells which had a light path of 2 0 cm The constant-temperature apparatus consisted of an oil bath heated by an electric hot plate and maintained at 180 ± 0 5° C by a Sunvic regulator

The lipids were extracted by adding fresh latex dropwise with mechanical stirring to 2 volumes of 96 per cent ethyl alcohol, previously warmed to a temperature of 40° C , and filtered on a Buchner funnel The coagulum was next re-extracted twice with alcohol at 40° C for 1 hr During the extraction carbon dioxide was bubbled through the solution and 1 c c of 0 1 per cent hydroquinone solution was added per 100 dry rubber content The yellow-coloured alcohol extracts were combined and evaporated to a small volume under reduced pressure and atmosphere of carbon dioxide at a temperature of below 50° C The aqueous residue was extracted 3 times with light petroleum ether (b p 40–60° C), at a volume ratio of 1 8 The combined petroleum ether extracts were washed 5 times with small volumes of distilled water and dried with anhydrous sodium sulphate After filtration, the dried extract was evaporated to dryness at atmospheric pressure and next the remaining solvent was removed at reduced pressure

For the analysis the lipid material was dissolved in light petroleum ether (40°–60° C) and made up to volume A number of ml of the solution were transferred to the reaction vessels and the petroleum ether evaporated under nitrogen 11 gm of ethylene glycol–potassium hydroxide reagent were added, the contents mixed by shaking and for 15 min nitrogen was led in (in later experiments 5 gm of the reagent were used) A vessel containing only the reagent served as blank The vessels were then placed in the oil bath maintained at 180° C After being heated for exactly 15 min , the vessels were chilled in water The reaction mixture was diluted with absolute methanol and made up to volume Absorbancies of these solutions were measured at 233, 268, 315, 346 and 374 mμ against the blank

For the measurement of the absorption before isomerization the lipid material was dissolved in iso-octane or absolute methanol A ten-fold dilution was usually necessary for the determination of the absorbancy at 233 mμ The blank consisted of iso-octane or absolute methanol

The extinction coefficients of the sample before and after isomerization are calculated

$$k_b = \frac{A}{l\ c} \text{ and } k_i = \frac{A}{l\ c}$$

c is expressed as gm lipids per 1 solution The differences, $k = k_i - k_b$, at 233, 268, 315 and 346 mμ are substituted in the following equations

Percentage of dienoic acid $= 1\ 092\ k_{233} - 0\ 573\ k_{268} - 0\ 259\ k_{315} + 0\ 021\ k_{346}$

Percentage of trienoic acid $= 1\ 105\ k_{268} - 0\ 879\ k_{315} + 0\ 306\ k_{346}$

Percentage of tetraenoic acid $= 1\ 650\ k_{315} - 1\ 555\ k_{346}$

Percentage of pentaenoic acid $= 1\ 144\ k_{346}$

Latex tapped from several *Hevea* clones was used for the present investigation In Table 1 the polyunsaturated acids contents of *Hevea* latex are listed The standard deviations of the calculated values are also given

Table 1 POLYUNSATURATED ACIDS CONTENT OF *Hevea* LATEX, TOTAL LIPIDS (PER CENT)

Sample	Pentaene	Tetraene	Triene	Diene	No of determinations
PB 186	0 14 ± 0 09	0 50 ± 0 16	1 75 ± 0 21	22 76 ± 3 23	5
Tjir 16	0 56 ± 0 35	0 88 ± 0 14	1 95 ± 0 90	14 62 ± 0 10	3
Tjir 1	0 14	0 32	2 90	19 08	1
PR 162	0 18	0 37	1 77	8 04	1
Pilm B 84	0 84	0 22	10 04	61 26	1
Av 385	—	—	0 23	11 46	1
Mixed clonal	—	0 83	1 80	17 24	1

It is noteworthy that the polyunsaturated acids content of *PB* 186 are significantly different from those of *Tjir* 16, with the exception of trienoic acid (0 05 > *P* > 0 02) It is further interesting to note that the clone *Pilm B* 84 has an exceptionally high dienoic acid content The findings clearly show that the polyenoic acids contents of *Hevea* latex vary with the clone from which the latex is obtained

I thank Miss Liem Jan Ing for her assistance in the experimental work and Miss Tan Jan Nio for computing the results

NG TET SOEI

Balai Penjelidikan dan Pemakaian Karet,
Bogor, Indonesia

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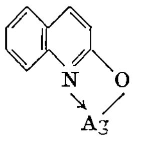
Silver (I) Complexes with 2-Quinolinol and 4-Methyl-2-Quinolinol : a Possible 4-Membered Ring Chelate System

ALTHOUGH the silver (I) complexes of 8-quinolinol (8-hydroxyquinoline) have been the subject of several investigations[1–4], little is known about the complexes formed with 2-quinolinol and its derivatives The 8-quinolinol complexes of silver are rather unusual in that two chelating groups, per metal ion, are present in the complex, or $Ag(C_9H_6NO)\ C_9H_6NOH$ To complicate matters, both a yellow and a green form of the above are known However, we have recently shown that the green form is identical to the yellow except that it contains a small amount of free silver[5] With the above information available, it was thought that the silver (I) 2-quinolinol complexes would be similar to those of 8-quinolinol

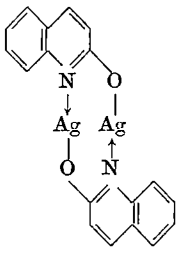
It was found, however, that the composition of the 2-quinolinol complex corresponded to a 1 1 formula, or $Ag(C_9H_6NO)$, rather than a 1 2 composition The complexes were prepared as follows approximately 1 2 gm of silver acetate was dissolved in 250 ml of water, heating the mixture to effect solution The solution was cooled to about 60° C , buffered with 1 gm of sodium acetate, and then 1 gm of 2-quinolinol or 4-methyl-2-quinolinol dissolved in about 30 ml of 95 per cent ethyl alcohol was added Immediately on mixing, a white flocculent precipitate of the silver complex was formed The mixture was then digested for about 30 min on a steam bath After digestion, the precipitate was filtered off, washed several times with water, and then dried in a vacuum desiccator for 24 hr The yield, based on silver ion present, was not quantitative, but averaged about 45 per cent Analysis of the complexes gave $Ag(C_9H_6NO)$, Ag 42 80 per cent theor , 42 10 per cent found, N, 5 56 per cent theor , 5 37 per cent found, $Ag(C_9H_5NOCH_3)$, Ag 40 55 per cent theor , 40 58 per cent found, N 5 27 per cent theor , 5 23 per cent found The silver contents were determined by ignition of the complexes at 800° C , the nitrogen contents by the Dumas method as determined by the Schwarzkopf Laboratory, Woodside, New York

The thermal properties of the complexes were studied by thermogravimetric analysis and differential thermal analysis Both complexes exhibited similar thermogravimetric analysis curves in that they began to lose weight at about 300° C The silver residue weight-levels were obtained at about 700° C The differential thermal analysis thermograms revealed that the decomposition reactions were exothermic as evidenced by a single sharp exothermic peak at 275° and 300° C., for the 4-methyl- and 2-quinolinol chelates, respectively.

It was not possible to determine the molecular weight of either complex due to their insolubility in all the common organic solvents. However, it is of interest to speculate as to their possible structures.



(I)



(II)

Structure (I) would be unique in that a 4-membered ring chelate system is present. This type of ring system is rather rare, with the exception of carbonatoamminecobalt (III) complexes[6]. Recent investigations have shown that the copper 1,3-diphenyltriazen complexes[7] and copper acetate 1-hydrate[8,9] are binuclear structures, and do not involve 4-membered ring chelate systems.

The dimer, as shown in structure (II), is also possible due to the *sp* hybridization of Ag (I) to form linear complexes such as $[H_3N\rightarrow Ag\leftarrow NH_3]^+$. At the present time it is not possible to differentiate between these two possible structures.

WESLEY W. WENDLANDT
JOHN HASCHKE

Department of Chemistry,
Texas Technological College,
Lubbock, Texas.

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Assignment of *cis–trans* Configuration to 1,2-Dialkyl*cyclo*hexanes

PREVIOUS work carried out at the Mass Spectrometry Centre of the University of Liège has shown the specific behaviour under electron impact of *cis* and *trans* isomers in a series of pairs of cyclanic stereoisomers. It has been especially pointed out in 1,2-dialkyl derivatives of *cyclo*propane[1], *cyclo*butane[2], *cyclo*pentane[3] and *cyclo*hexane[4] that in the ionized state the *cis* isomer is less stable than the corresponding *trans* isomer (that is, the relative abundance, expressed as a fraction of total ionic current for 70 V energy electrons, of molecular ions of a *cis* isomer is smaller than that for the corresponding *trans* molecule).

This observation being quite general for the 15 pairs investigated in the works cited gives a rule enabling the *cis–trans* assignment on the basis of the mass spectra in the case of cyclanic stereoisomers for which the assignment has not yet been done.

In 1959 the National Bureau of Standards[5] published mass spectral data for 8 pairs of *cyclo*hexane isomers disubstituted in position 1 by a *cyclo*hexyl or methylene*cyclo*hexyl group and in position 2 by a methyl, ethyl, propyl or butyl group. Those isomers were characterized by their relative boiling points, that is to say one of them was labelled 'high boiling' and the other 'low boiling'. For each isomer of each of those 8 pairs we calculated the abundance of the molecular ions in percentage of total ionization. The results are presented in Table 1.

Table 1. RELATIVE ABUNDANCES OF MOLECULAR IONS IN SEVERAL 1,2-DIALKYL*cyclo*HEXANES (PERCENTAGE OF TOTAL IONIC CURRENT)

Pair No.	Nature of alkyl group		Abundance in low boiling isomer	Abundance in high boiling isomer
1	$—CH_3$	$—$*cyclo*C_6H_{11}	2.33	1.01
2	$—C_2H_5$	$—$*cyclo*C_6H_{11}	1.54	0.66
3	$—nC_3H_7$	$—$*cyclo*C_6H_{11}	1.24	0.51
4	$—iC_3H_7$	$—$*cyclo*C_6H_{11}	0.49	0.08
5	$—nC_4H_9$	$—$*cyclo*C_6H_{11}	1.03	0.42
6	$—iC_4H_9$	$—$*cyclo*C_6H_{11}	0.95	0.41
7	$—CH_3$	$—CH_2—$*cyclo*C_6H_{11}	3.35	2.00
8	$—C_2H_5$	$—CH_2—$*cyclo*C_6H_{11}	1.60	1.02

This shows that molecular ions of so-called 'high boiling' isomers are all less abundant than those of the corresponding so-called 'low boiling' isomers. Referring to the preceding results concerning the 1,2-dialkyl*cyclo*alkanes, as mentioned previously, I suggest, in the present cases, attributing the *cis* structure to the isomers for which the molecular ions are the least abundant and the *trans* structure to the corresponding isomers for which the molecular ions are the most abundant.

This conclusion agrees very well with the *cis–trans* assignment of the isomers already investigated. In the 1,2-disubstituted derivatives of *cyclo*propane, *cyclo*butane, *cyclo*pentane and *cyclo*hexane, the *cis* isomer is always the high boiling one, as has been shown by experimental[6,7] and theoretical[8,9] investigation.

I thank Prof. L. D'Or for his advice.

This work was supported by the Fonds National de la Recherche Scientifique.

P. NATALIS

Faculty of Sciences,
University of Liège.

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BIOCHEMISTRY

Adaptation of the Small Intestine to a High-Fat Diet containing Saturated and Unsaturated Fatty Acids

THE role of exogenous fat is generally evaluated with regard to its caloric value, digestion, deposition, etc. It was, however, revealed that it is of great importance whether or not the fatty component of the diet contains saturated or unsaturated fatty acids.

Marked differences as regards the influence exerted by these substances can be observed, for example, when investigating the weight-curves of realimented

Table 1 CHANGES OF THE SMALL INTESTINE IN RATS AFTER THE ADMINISTRATION OF DIFFERENT HIGH-FAT DIETS

	Stock diet* (controls)	High-fat diet† fatty acids	
		Saturated	Unsaturated
Small intestine (mean values)			
Length (cm)	114 4^{a}	141 3^{a}	130 0^{bb}
Fresh weight (gm)	5 43^{a}	9 49^{a}	6 16^{cc}
Dry weight (gm)	1 26^{b}	1 91^{b}	1 39^{dd}
Protein (gm)	0 86^{a}	1 29^{b}	0 90^{dd}
Enzyme activity (per length unit controls = 100 per cent)			
Peptidases	100^{a}	132^{b}	108^{dd}
Lipase	100^{a}	129^{d}	116^{dd}
Amylase	100^{a}	43^{d}	29^{aa}
Weight of animals (mean)			
At beginning of experiment (gm)	282 4^{d}	287 3^{d}	286 7^{dd}
After 6 days of starvation	222 8^{d}	229 9^{d}	229 2^{dd}
After 17 days of realimentation (at time of decapitation)	306 1^{a}	268 5^{a}	298 4^{dd}

* Composition of stock diet 25 cal. per cent of protein, 22 cal per cent of carbohydrates, 53 cal per cent of fat.

† Composition of high-fat diets 15 cal per cent of protein, 15 cal per cent of carbohydrates, 70 cal per cent of fat, 60 cal per cent of the fat was formed by chemically pure fatty acids, 10 cal per cent of the fat was mostly invisible fat (wheat germ, fatty milk and cod liver oil, etc) Saturated fatty acids = palmitic acid, unsaturated fatty acids = *cis*-isomers of ethylesters linoleic acid (56 per cent), linolenic acid (17 per cent) and oleic acid (27 per cent).

Statistical evaluation

^{a} Controls saturated fatty acids or saturated fatty acids unsaturates fatty acids
^{aa} Controls unsaturated fatty acids $P < 0\ 001$
^{b} ^{bb} ,, ,, ,, ,, $0\ 01 > P > 0\ 001$
^{c} ^{cc} ,, , ,, ,, $0\ 05 > P > 0\ 01$
^{d} ^{dd} ,, ,, ,, , not significant

animals While the high-fat diet containing unsaturated fatty acids renders an optimal course for the possible weight-curve and a similar intake of food as on a stock diet, the administration of saturated fatty acids in a high-fat diet of similar composition led to a significant retardation of the weight-curve The intake of food under these conditions was approximately 25 per cent higher The lipid balance was more positive when the diet contained saturated fatty acids[1]

To elucidate these findings in experimental animals the morphology and some functions of the small intestine, the main active absorption surface of the animal, were investigated

White male albino rats of the Wistar strain were realimented after starvation for 6 days on a standard diet and another group on an isocaloric high-fat diet containing either saturated or unsaturated fatty acids Each group comprised 10–20 animals

Following realimentation, the animals were killed by decapitation, after the organs of the alimentary tract had been removed the length of the small intestine was measured (suspended with a 5-gm weight), and after rinsing its fresh weight was determined Next the dry matter content and protein content of the tissue were estimated The activity of the following enzymes was investigated in homogenates prepared from 10-cm long samples of different sections of the small intestine peptidases (substrate, 1-leucyl-glycyl-glycine[2]), lipase (substrate, tributyrin[3]) and amylase (substrate, starch[4]) The results are presented in Table 1

From the findings, it appears that during short-term realimentation of rats with a high-fat diet morphological and functional changes of organs of the digestive tract occur, particularly of the small intestine, its length increases, which is not due to mere atonia, but hypertrophy or hyperplasia, as indicated by the values for dry matter, the content of protein and the activity of peptidase The lipolytic and amylolytic activity changes, there being an adaptation to the high-fat content of the diet After the high-fat diet containing unsaturated fatty acids the changes described here are less marked

When interpreting these changes the volume and composition of the diets must be considered in particular As to the volume, it was equal in all three types of diets (94, 97, 97 c c /100 gm respectively) In animals on a diet containing saturated fatty acids the increased volume of the ingested food may take part in the morphological changes A significant part is, however, also played by the composition of the diet as these changes occurred also in animals on the diet containing unsaturated fatty acids, where the volume of the ingested food was practically equal to the controls Similar morphological changes were also recorded so far as the fresh weight and dry matter content of the stomach and the large intestine are concerned

These findings indicate that on a high-fat diet under the experimental conditions described morphological and functional adaptations of the organs of the digestive tract occur, and are induced not only by the volume, but also by the composition of the diet The influence of saturated fatty acids is in this respect more marked than that of unsaturated ones

Z SLABOCHOVÁ
Z PLACER

Institute of Human Nutrition,
Budějovická 800,
Prague 4.

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Effect of Fatty Acids, Ketone Bodies, Diabetes and Starvation on Pyruvate Metabolism in Rat Heart and Diaphragm Muscle

IN alloxan diabetes the uptake and oxidation of pyruvate *in vitro* by rat ventricle muscle slices[1] and diaphragm muscle[2] is impaired, and the output of lactate may be increased Moreover, in alloxan diabetic rats[3] the blood concentration of pyruvate may be increased, and in dogs treated with growth hormone pyruvate tolerance is impaired and blood pyruvate elevated[4] In earlier experiments[5], evidence was obtained that impaired phosphorylation of glucose and of fructose-6-phosphate in hearts from alloxan diabetic or starved rats could result from enhanced respiration of fatty acids and ketone bodies and the suggestion was made that this might also be responsible for the high glycogen concentration of the heart in diabetes and starvation and for the myoglycostatic effect of growth hormone In view of these findings, it seemed important to investigate the possibility that enhanced respiration of fatty acids and ketone bodies might also be responsible for abnormalities of pyruvate metabolism in muscles of alloxan diabetic rats and in animals treated with growth hormone

Hearts from male albino Wistar rats weighing 200–260 gm (injected beforehand with 12 mgm 'Nembutal' and 2,500 units heparin intraperitoneally) were perfused for 15 min by recirculation[6] with medium containing (where pyruvate uptake was measured) sodium pyruvate ± fatty acids or ketone bodies or (where pyruvate and lactate production from glucose was measured) glucose and insulin In experiments with palmitate the medium contained, in addition, 2 per cent bovine plasma albumin (Armour Fraction V) freed of insulin and insulin antagonists by passage through Whatman cellulose powder as a 4 per cent solution in phosphate borate buffer *p*H 8 4 (ref 7) followed by dialysis and freeze-drying, and freed of fatty acids by two extractions with heptane glacial acetic acid (20 1 v/v) followed by drying *in vacuo* and dialysis against two changes of 0 9 per cent sodium chloride and six changes of distilled water (ten-fold excess) and freeze-dried Solutions of albumin ± 0 75 m*M* palmitate in perfusion medium were dialysed overnight against a twenty-fold excess of the latter before use Uptake of glucose by the heart was measured by methods given elsewhere[6], pyruvate and lactate were assayed in perfusion medium with lactate dehydrogenase[8] Hemidiaphragms from female albino Wistar rats of 100–150 gm were incubated for 60 min in 2 ml of medium containing pyruvate (± 2-[^{14}C]pyruvate) with or without fatty acids In experiments with palmitate (0 6 m*M*) the medium contained in addition 0 85 per cent bovine plasma albumin $^{14}CO_2$ generated by diaphragm muscle was collected (following addition of sulphuric acid to 1 4 *N*) in *M* hyamine in methanol, and assayed for radioactivity in a Tricarb liquid scintillation counter with addition of 0 4 per cent 2,5-diphenyloxazole and 0 05 per cent 1,4-*bis*-2-(5-phenyloxazolyl)-benzene in toluene[9] Rats were made diabetic by alloxan (60 mgm /kgm) injected into a tail vein and used 48 hr later Starved rats were deprived of food for 40–48 hr , control and diabetic rats were not deprived of food

The uptake of pyruvate by hearts from normal rats perfused with medium containing 4 m*M* pyruvate (µmoles/gm tissue/hr , mean ± *S E* , number of hearts perfused in each group in parenthesis) was significantly reduced by 5 5 m*M* DL-β-hydroxybutyrate from 162 ± 1 to 111 ± 7 6 (14), and by 5 5 m*M* aceto-acetate from 158 ± 17 6 to 78 ± 12 4 (6), and by 2 8 m*M* *n*-octanoate from 181 ± 14 to 84 ± 9 2 (6) Lactate production was not significantly altered by these substrates (the respective changes were 94 ± 5 2 to 108 ± 4 4, 61 3 ± 3 to 68 ± 2 7, and 81 ± 4 2 to 78 ± 6 1) In hearts perfused with medium containing 2 per cent bovine plasma albumin, pyruvate uptake was significantly reduced by 0 75 m*M* palmitate from 79 ± 2 5 to 57 ± 3 4 (11), and lactate output increased from 49 ± 1 8 to 66 ± 6 4 Hearts from alloxan-diabetic or starved rats perfused with medium containing m*M* pyruvate showed a depressed uptake relative to that of controls (from 78 ± 6 4 to 48 ± 4 8 and 43 ± 2 7 respectively, 6 hearts in each group) whereas lactate output was not significantly altered by diabetes (62 ± 12 to 71 ± 5 1) or starvation (62 ± 12 to 68 ± 7 7) In the absence of added pyruvate, 0 75 m*M* palmitate and 5 5 m*M* DL-β-hydroxybutyrate increased the output of lactate from 8 6 ± 2 3 to 45 ± 8 9 (6) and 8 5 ± 1 8 to 20 3 ± 3 4 (6) respectively, whereas pyruvate output was not significantly altered (3 1 ± 0 72 to 3 8 ± 0 69 and 1 6 ± 0 30 to 1 8 ± 0 15 respectively) Since the rate of glycogen breakdown (and therefore of pyruvate formation from glycogen) was not accelerated by fatty acids or ketone bodies, it is concluded that these substrates must impair the utilization of pyruvate in the heart by some pathway other than conversion to lactate (that is, these substrates are presumed to have impaired oxidation of pyruvate)

In hearts perfused with glucose (1 mgm /ml) and insulin (0 1 unit/ml), uptake of glucose (in µmoles/gm /hr) was impaired by alloxan diabetes, starvation and 2 8 m*M* octanoate from 77 ± 1 7 (14) to 36 ± 1 6 (13), 46 ± 1 7 (13) and 46 ± 1 3 (6) respectively This impairment of glucose uptake has been attributed to inhibition of the hexokinase and phosphofructokinase reactions[5,10,11] Glucose uptake was also reduced by similar mechanisms by 0 75 m*M* palmitate carried by 2 per cent bovine plasma albumin, from 66 ± 1 7 (18) to 43 ± 1 6 (18) In spite of the fact that glucose uptake was impaired (and therefore the formation of pyruvate from glucose was presumably reduced, see ref 5) the combined output of pyruvate and lactate was not diminished by diabetes or starvation (changes from 51 ± 7 to 56 ± 11 and 53 ± 9 respectively), and was increased by octanoate (from 51 ± 7 to 74 ± 9) and palmitate (from 41 ± 2 4 to 52 ± 3 7) The output of pyruvate alone was increased by diabetes and starvation (from 4 8 ± 0 27 to 9 0 ± 1 0 and 11 0 ± 1 1), whereas that of lactate was unchanged (from 46 ± 7 to 47 ± 11 and 42 ± 9) Octanoate and palmitate increased the output of lactate (from 46 ± 7 to 72 ± 9 and from 33 ± 2 4 to 46 ± 3 7 respectively) and reduced that of pyruvate (from 4 8 ± 0 27 to 2 1 ± 0 26 and 7 7 ± 0 62 to 5 9 ± 0 47) The lactate to pyruvate ratio was thus decreased by diabetes and starvation (from 9 6 ± 1 8 to 5 2 ± 0 52 and 3 8 ± 0 40), but increased by octanoate and palmitate (from 9 6 ± 1 8 to 29 1 ± 1 4 and from 4 2 ± 0 43 to 8 0 ± 0 90 respectively) We conclude that in rat heart the utilization of pyruvate formed from glucose (by reactions other than conversion to lactate) is impaired by diabetes and starvation and by fatty acids On the other hand, whereas diabetes and starvation decrease the ratio of lactate to pyruvate in the perfusion medium, fatty acids increase this ratio

When hemidiaphragms from normal fed rats were incubated in the presence of 4 m*M* pyruvate ± 2-[^{14}C]pyruvate (4 mµc /ml), 4 m*M* butyrate reduced the uptake of pyruvate from 29 2 ± 1 to 23 5 ± 0 47 (16), diminished the output of $^{14}CO_2$ by 5,600 ± 1,050 c p m /gm /hr (from a control-level of 14,500) and increased lactate output from 17 3 ± 0 56 to 20 3 ± 0 39 The uptake of pyruvate by hemidiaphragms from diabetic rats fed alloxan was lower than that of hemidiaphragms from normal rats (18 5 ± 1, 8 observations) and only slightly further reduced by 4 m*M* butyrate (16 1 ± 0 4, 8 observations) When hemidiaphragms from rats normally fed were incubated in medium containing 0 85 per cent bovine plasma albumin and m*M* pyruvate ± 2-[^{14}C]pyruvate (3 4 mµc /ml), pyruvate uptake was not significantly changed by 0 6 m*M* palmitate (13 1 ± 0 35 to 12 9 ± 0 42, 8 observations each group), but the output of lactate was increased (from 11 4 ± 0 2 to 14 1 ± 0 61) and $^{14}CO_2$ production was diminished by 470 ± 127 c p m /gm /hr (from a control-level of 6,500) We conclude that in rat diaphragm the uptake of pyruvate is diminished by diabetes and by butyrate, and that diabetes, butyrate and palmitate impair pyruvate oxidation

The present studies have confirmed that the uptake and oxidation of pyruvate by rat heart and diaphragm muscle are impaired in alloxan diabetes They have shown further that similar changes occur in the heart following starvation of the rat, that the utilization of pyruvate formed from glucose is also diminished in the heart by diabetes and starvation, and that similar changes can be induced in hearts and diaphragms of normal rats by the *in vitro* addition of fatty acids and ketone bodies It is suggested, therefore, that these particular changes in pyruvate metabolism in heart and diaphragm muscle in diabetes and starvation are due to enhanced respiration of endogenous fatty acids (and perhaps ketone bodies) The present studies have also shown that the metabolism of pyruvate derived from glucose in hearts from diabetic or starved rats differs in one respect from that of normal hearts perfused with fatty acids In diabetes and starvation, the ratio of lactate to pyruvate in the perfusion medium is decreased, whereas in the normal heart perfused with fatty acids this ratio is increased If, as we suggest, enhanced respiration of fatty acids (and perhaps ketone bodies) were responsible for impaired pyruvate utilization in diabetes and starvation, then some other factor must be responsible for the change in lactate to pyruvate ratio in these conditions One possibility in this connexion is that it is brought about by an increase in the ratio of extramitochondrial NAD to $NADH_2$ Glock and McLean[12] have already shown that the ratio of total NAD to $NADH_2$ is increased in diaphragm muscle in alloxan diabetes In view of these findings it seems likely that enhanced respiration of fatty acids and ketone bodies is also responsible for the high blood pyruvate and impaired pyruvate tolerance observed in dogs treated with growth hormone[4]

We wish to thank the British Diabetic Association, the British Insulin Manufacturers and the Medical Research Council for grants which contributed to the cost of this investigation We thank Prof F G Young for his encouragement

P B Garland
E A Newsholme
P J Randle

Department of Biochemistry,
University of Cambridge

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Role of Phagocytosis in the Uptake of Lipid by a Phagocytic Cell

It is well known that free fatty acids are readily taken up by most types of cell, much controversy exists however, concerning uptake of complex lipids It has been recently suggested that triglycerides may enter liver and adipose tissue[1–3] by a phagocytosis-like process The existence of such a mechanism of incorporation of lipid was investigated using suspensions of polymorphonuclear leucocytes capable of phagocytosis *in vitro*

If sequestration of medium represented the chief means of uptake of lipid by such cells a non-selective incorporation might be expected On the other hand, if attachment to the surface of phagocytic cells constitutes a necessary phase in engulfment of particles and macromolecules, incorporation could be selective because of selectivity and competition in attaching to the cell

This communication reports that uptake of lipid by the leucocytes is indeed highly selective, but that sequestration by a phagocytosis-like process does not account for the observed selectivity

Suspensions of polymorphonuclear leucocytes obtained from rabbit peritoneal exudates of rabbits[4] were incubated in the ascitic fluid to which were added one of four kinds of lipids labelled with carbon-14 (1) long-chain fatty acids (as albumin-free fatty acids complexes), (2) triglycerides, (3) mixed phospholipids, biosynthetically prepared by incubating leucocytes with a ^{14}C-1-free fatty acid and subsequent isolation of the radioactive phospholipid fraction of the cell lipids, (4) human chylomicra obtained by thoracic duct cannulation and by feeding a fat meal containing ^{14}C-1-palmitic acid

The radioactivity and lipid content of the total lipid extracts of cells and medium were determined at incubation times of up to 1 hr [5], the appearance of water-soluble radioactivity was sought in the water washings of these total lipid extracts The radioactivity of cell lipid fractions eluted from silicic acid columns was also measured and the $^{14}CO_2$ evolved during incubation was collected in hyamine and counted as described by Isselbacher[6] The effects of inhibition of metabolism on the uptake of lipids by these cells was determined by incubating at 4° C and by exposure to the following agents (*a*) 0 001 *M* sodium iodo-acetate (IAA), (*b*) 0 02 *M* sodium fluoride, (*c*) 0 0025 *M* potassium cyanide, (*d*) 0 0025 *M* 2,4 dinitrophenol (DNP)

In the evaluation of the results a distinction was made between incorporation of medium lipids into the cells by a process dependent on metabolism and a non-metabolic association of radioactive lipid with the leucocytes The following criteria have been viewed as evidence for true metabolic incorporation (1) an increase in the radioactivity of cell lipids during the course of incubation, (2) dependence of uptake of labelled fat on intact metabolism, (3) appearance of increasing amounts of radioactivity as $^{14}CO_2$ and as water-soluble radioactivity which would indicate breakdown of the labelled lipid, (4) appearance of radioactivity into cell lipid fractions other than those in which the label was introduced into the medium

Table 1 shows that incorporation of the carbon-14 label into total lipid extracts of the leucocytes was regularly obtained during incubation with either ^{14}C-1-free fatty acid or with ^{14}C-1-complex lipids added to the ascitic fluid Uptake of ^{14}C-1-free fatty acid was prevented by incubation at 4° C and suppressed by inhibitors of anaerobic glycolysis (IAA and sodium fluoride), these conditions did not affect the uptake of label from ^{14}C-complex lipids Inhibition of oxidative metabolism (with potassium cyanide or DNP) had no suppressive effect on the incorporation of carbon-14 from any of the radioactive substrates Column 2 shows a drop in radioactivity/mgm medium lipids during incubation with ^{14}C-1-free fatty acid, which may be taken to indicate that lipid species other than free fatty acid were taken up more slowly

In addition, following incubation with ^{14}C-free fatty acid, the label also appeared as $^{14}CO_2$ and radioactivity was found in the water washings of the total lipid extracts, however, neither water-soluble radioactivity nor significant amounts of $^{14}CO_2$ could

Table 1 Uptake of Lipids labelled with Carbon-14 by Polymorphonuclear Leucocytes*

Time of incubation (min) at 37° C	Free fatty acids‡ (per cent)† (1)	Free fatty acids‡ (per cent) (2)	Triglycerides (per cent) (3)	Phospholipids (per cent) (4)	Chylomicra (per cent) (5)
0	30 2±12 5 (*n* 20)	100 (*n* 18)	54 8± 6 7 (*n* 6)	111 5±32 5 (*n* 6)	66 7±36 5 (*n* 6)
10			100 0± 0 (*n* 2)	85 3±29 1 (*n* 3)	77 6±29 7 (*n* 5)
80	78 6±10 6 (*n* 10)	56 4±27 0 (*n* 10)	118 4±30 0 (*n* 8)	114 5±30 0 (*n* 6)	75 8±16 1 (*n* 6)
60	100 (*n* 16)	41 0±22 0 (*n* 14)	100 (*n* 11)	100 (*n* 7)	100 (*n* 6)
60+IAA or NaF†	54 2±19 5 (*n* 15)	84 4±11 8 (*n*,12)	114 5±24 2 (*n* 6)	138 0±34 4 (*n* 5)	105 1±35 6 (*n* 7)
60+KCN or DNP‡	110 1±13 7 (*n* 8)	64 5± 4 4 (*n* 6)		108 5± 9 2 (*n* 2)	135 5±43 2 (*n* 2)
60 4° C	36 0±14 9 (*n* 6)	94 8±12 0 (*n* 5)	112 1±20 4 (*n* 8)	126 0±67 0 (*n* 4)	90 0±20 3 (*n* 4)

* The lipid content and composition of the ascitic fluid was not affected by the addition of trace amounts of ^{14}C-1-labelled lipids (free fatty acids triglycerides or phospholipids) In the case of chylomicra, from 200–400 mgm. of fat were added in each experiment to a total volume of 75–150 ml of cell suspension In all experiments, cells and medium were separated by centrifugation The cells were washed with saline to remove adherent or trapped medium and then extracted as previously described (ref 5)

† The results are presented as a percentage of c p m /mgm cell lipid after 60 min incubation at 37° in each experiment and are given as mean ± 1 *S D*

‡ Data shown pertain to ^{14}C-1-linoleic acid, comparable results were obtained with ^{14}C-1-palmitic, stearic and oleic acids

The results shown in column (2) represent the radioactivity found in the medium expressed as a percentage of c p m /mgm medium lipid prior to incubation The figure between parentheses indicates the number of observations

be recovered during incubation with the labelled complex lipids

Table 2 presents the distribution of the carbon-14 label in the lipid fractions of the leucocytes Incubation in the presence of ^{14}C-free fatty acid resulted in the appearance of the label almost exclusively in the phospholipid and triglyceride fractions of the cell lipids In contrast, when complex lipids labelled with carbon-14 were presented to the cells, the label appeared in the fraction in which it was introduced to the suspension These results suggest that the radioactivity found in association with cell lipids after incubation with labelled triglycerides, phospholipids or chylomicra does not represent true entry into the cell but rather adherence of complex lipids to surface sites

Table 2 Carbon-14 in Fractions of Leucocyte Lipids after Incubation with Various Lipids labelled with Carbon-14 for 1 hr

Medium	Cell lipids Total radioactivity per cent incorporated into Phospholipids	Non-phospholipids	FFA
^{14}C-1-free fatty acid	54	43	3
^{14}C-1-triglycerides	3	96	1
^{14}C-1-phospholipids	>90	<10	0
^{14}C-1-chylomicra	<10	>90	0

FFA, free fatty acid

An alternative possibility would be that the labelled complex lipids were sequestered inside the cells after having been phagocytized but without becoming available to the metabolic machinery of the cell This consideration appears unlikely since it has been clearly shown that phagocytosis constitutes an active metabolic event for which anaerobic glycolysis delivers the energy and also that increased uptake of oxygen usually accompanies phagocytosis by polymorphonuclear leucocytes[7] In these experiments low-temperature incubation and glycolytic inhibition had no effect on the incorporation of radiocarbon from complex lipids, while no increase in uptake of oxygen was observed on addition of chylomicra or oil emulsions to the cell suspensions

These investigations therefore indicate that true metabolic incorporation of lipid by polymorphonuclear leucocytes is evident only in the case of free fatty acid, which become esterified during uptake It was found that this esterification was almost complete as soon as association of ^{14}C-1-free fatty acid with cell lipids had occurred, and furthermore, that homogenization of the leucocytes reduced the degree of esterification by 90 per cent or more even in the presence of added co-factors These findings suggest that esterification of free fatty acid occurs at the cell surface during uptake, the rate of transport may be a function of the availability of L-α-glycerophosphate, perhaps generated by the existence of a complete anaerobic glycolytic pathway in the cell membrane as has been suggested for muscle cells[8]

The remarkable selectivity of the process of uptake of lipid by these cells might rest on interaction between carboxyl groups of fatty acids and reactive groups on the cell surface In the case of complex lipids, these carboxyl groups are not available for interaction and no transport takes place

This work was carried out during the tenure of a senior fellowship of the New York Heart Association It was supported by grants from the American Heart Association and the U S Public Health Service

Peter Elsbach

Department of Medicine,
New York University School of Medicine,
New York, 16

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Possible Magnitude of Inhibition of Coenzyme-Substrate Reactions by Competitive Inhibitors in Coenzyme Preparations

An obvious source of error in enzyme kinetic studies is the presence of competitive inhibitors in impure coenzyme preparations, especially perhaps in the unstable reduced phosphopyridine nucleotides In recent years, the availability of coenzymes more than 90 per cent pure has been reassuring except in the unlikely event that an inactive nucleotide, for example, has a much greater affinity for the enzyme than has the coenzyme, it would seem that a few per cent of such a competitive inhibitor in the coenzyme preparation could not produce a significant effect on the rate of reaction—especially if the coenzyme is very firmly bound to the enzyme, as in the case of reduced diphosphopyridine nucleotide (DPNH) and liver alcohol dehydrogenase (*vide infra*)

Consideration of the possible kinetic effects of a competitive inhibitor, present in fixed molar ratio to the coenzyme, suggests that this reassurance may be ill-founded Consider the effect of an inhibitor, I, which competes with the coenzyme, C, by forming an inactive compound, EI, in an enzyme-catalysed reaction which follows a compulsory order mechanism, for example[1]

$$(1)\ E + C \rightleftharpoons E\,C,\quad (2)\ E\,C + S \rightleftharpoons E\,C' + S',\quad (3)\ E\,C' \rightleftharpoons E + C'.$$

In the absence of inhibitor, the relation between the initial rate in the steady state, v_0, the enzyme concentration, e, and the coenzyme and substrate concentrations, c and s, for such a sequence is of the form[2]

$$\frac{e}{v_0} = \varphi_0 + \frac{\varphi_1}{c} + \frac{\varphi_2}{s} + \frac{\varphi_{12}}{cs} \qquad (1)$$

This is true whether or not rate-limiting ternary complexes are postulated in reaction (2) The kinetic coefficients φ_0, etc , are functions of the rate constants in the mechanism, φ_0 is the reciprocal of the maximum rate, V, obtained with infinitely large coenzyme and substrate concentrations, $\varphi_1/\varphi_0 = K_m$, the Michaelis constant for the coenzyme, defined as the concentration which, with infinitely large concentration of substrate, gives half the maximum rate, and $\varphi_{12}/\varphi_2 = K_1$, the dissociation constant of the enzyme–coenzyme complex $E\,C$ (ref 2)

In the presence of concentration i of a competitive inhibitor, the initial rate equation is[2]

$$\frac{e}{v_0} = \varphi_0 + \frac{\varphi_1}{c}\left(1 + \frac{i}{K_i}\right) + \frac{\varphi_2}{s} + \frac{\varphi_{12}}{cs}\left(1 + \frac{i}{K_i}\right)$$

where K_i is the dissociation constant of EI If the coenzyme preparation itself contains an inhibitor, in molar ratio $i/c = r$ then

$$\begin{aligned}\frac{e}{v_0} &= \varphi_0 + \frac{\varphi_1}{c}\left(1 + \frac{rc}{K_i}\right) + \frac{\varphi_2}{s} + \frac{\varphi_{12}}{cs}\left(1 + \frac{rc}{K_i}\right)\\ &= \varphi_0\left(1 + \frac{\varphi_1 r}{\varphi_0 K_i}\right) + \frac{\varphi_1}{c} + \frac{\varphi_2}{s}\left(1 + \frac{\varphi_{12} r}{\varphi_2 K_i}\right) + \frac{\varphi_{12}}{cs}\\ &= \varphi_0\left(1 + \frac{K_m r}{K_i}\right) + \frac{\varphi_1}{c} + \frac{\varphi_2}{s}\left(1 + \frac{K_1 r}{K_i}\right) + \frac{\varphi_{12}}{cs}\end{aligned}$$

This equation is of the same form as equation (1) Experimental determination of the kinetic coefficients in the usual way, by initial rate measurements with various coenzyme and substrate concentrations and double reciprocal plots[2], will give φ_1 and φ_{12} unaffected by the presence of the inhibitor, but the apparent φ_2 will be $\varphi_2\left(1 + \frac{K_1 r}{K_i}\right)$ and the apparent φ_0 will be $\varphi_0\left(1 + \frac{K_m r}{K_i}\right)$ The maximum rate $1/\varphi_0$ in presence of inhibitor will be $V\Big/\left(1 + \frac{K_m r}{K_i}\right)$, and the apparent Michaelis constant for the coenzyme will be $K_m\Big/\left(1 + \frac{K_m r}{K_i}\right)$

Thus the effect produced on the maximum rate by a constant amount of inhibitor in the coenzyme preparation depends on the relative values of the inhibitor constant and the Michaelis constant for the coenzyme The latter bears no simple relationship to, and may be much larger than, the dissociation constant of the enzyme–coenzyme complex If this is so, a small molar proportion of competitive inhibitor in the coenzyme, which binds to the enzyme no more firmly than the coenzyme, may depress the maximum rate considerably

For liver alcohol dehydrogenase at pH 6 0, the dissociation constant[3] for the DPNH compound is 0 21 μM, and the Michaelis constant[4] for DPNH in aldehyde reduction is 14 μM On the basis of these considerations, kinetic measurements with DPNH containing only 3 per cent of a competitive inhibitor with the same affinity for the enzyme as DPNH would give an apparent maximum rate equal to 0 3 V, an inhibition of 70 per cent

Such effects should be detectable by variations of the maximum rate with samples of DPNH of different purity, and this has been reported for liver alcohol dehydrogenase[5] The magnitude of the differences in rate observed with relatively pure coenzyme preparations was surprising It has since been shown[4] that DPNH freshly prepared in the laboratory from 98 per cent DPN (Sigma) gives a maximum rate of aldehyde reduction at pH 6 0 more than 20 per cent greater than those obtained with commercial samples of high purity (94–97 per cent) Moreover, concentrated solutions (300 mgm /ml) of DPNH in water or 0 4 M disodium hydrogen phosphate, kept in the dark for several hours, give maximum rates less than half that obtained with a freshly prepared solution, yet assay shows that no more than 8 per cent of the active coenzyme has been degraded The above considerations make it easier to understand these observations, and indicate that the purity requirements for coenzymes in kinetic studies may be very exacting, especially when the Michaelis constant for the coenzyme is much greater than the dissociation constant of the enzyme–coenzyme complex Another case in which this appears to be so is the lactic dehydrogenase–DPNH–pyruvate reaction[6], it may be remarked also that, in the presence of a competitive inhibitor, the Michaelis constant for DPNH would be under-estimated to the same extent as the maximum rate

It is readily shown that for a single substrate reaction, formulated in the usual way with one or more binary enzyme-substrate complexes, the kinetic effects of a competitive inhibitor present as an impurity in the substrate will be precisely analogous

KEITH DALZIEL*

Department of Biochemistry,
University of Sheffield

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* Sorby Research Fellow of the Royal Society

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Thermal Copolymerization of Protein Hydrolysates containing ^{35}S Amino-Acids and Distribution of Radioactive Sulphur after Application of ^{35}S-labelled Amino-Acid Copolymers in the Rat

THERMAL copolymerization of amino-acids has recently been discussed by Fox *et al* [1–5] The results of these experiments have shown that it is possible to prepare peptides thermally, if aspartic acid or glutamic acid is present in sufficient proportions as a reactant

Thermal copolymerization of amino-acids has yielded anhydropolymers which contain all the eighteen amino-acids common to protein These copolymers have many of the properties of protein[4,6], and after being hydrolysed by hydrochloric acid reveal on paper chromatograms the same amino-acid pattern as protein[2] After chromatography of hydrolysates of proteinoids on ion exchange columns all amino-acids except tryptophan (lost under acid hydrolysis) can be observed on the effluent curve[7,8] Preparations of the same type of proteinoid in different laboratories showed practically the same quantitative amino-acid composition[7,8]

No reports on the administration to mammals of amino-acid copolymers prepared according to Fox *et al* [2,4,5] have been published so far In earlier studies proteinoids (thermal amino-acid copolymers containing all common amino-acids) were seen to be attacked by bacterial proteases[2] and also by proteolytic enzymes of the digestive tract[4-6,8] In further trials the effect of *in vivo* hydrolysis of proteinoids has been tested to obtain information on the breakdown of amino-acid copolymers and on the pathways of the sulphur-containing enzymatic split products of the proteinoids in the mammalian body Experiments have not yet been carried out to test for resulting toxic effects of proteinoids to mammals

Based on these experiences, attempts were made to copolymerize hydrolysates of protein instead of purified amino-acids For this purpose hydrolysates of *Torula utilis* were used This type of yeast was inoculated with sulphate labelled with sulphur-35 to incorporate sulphur-containing amino-acids into the yeast protein[9]

After inoculation *T utilis* was hydrolysed under nitrogen in sealed tubes by 6 N hydrochloric acid for 24 hr to avoid oxidation of sulphur-containing amino-acids The resulting hydrolysates were carefully dried under nitrogen atmosphere and hydrochloric acid was removed by evaporation The residue was dried over phosphorus pentoxide to constant weight

Four parts of aspartic acid, four parts of glutamic acid and one part of dried *T utilis* hydrolysate were dissolved in 5 per cent sulphurous acid

After complete solution of the components, the solvent was evaporated as described for the hydrolysate and the resulting residue was heated under nitrogen for 3 hr at 170° C

The molten mixture was amber in colour After cooling, the vitreous mass was rubbed vigorously with water and after standing overnight the solid was separated by centrifugation Following this the preparation was dissolved in sodium bicarbonate solution and dialysed in cellulose tubing ('Visking' 27/32 in) in an agitated water bath for 4 days Two different types of copolymers could be distinguished One part of the preparation was insoluble (A), while the other part (B) mainly was soluble in water Fraction B is the same as described recently by Fox and Harada[4] Both fractions could be dissolved in sodium phosphate buffer, *p*H 6 8, and precipitations could be achieved as described recently[6] Colour tests which were also positive for protein include biuret, xanthoproteic, Hopkins–Cole, and Salkowski tests as well as the test with azocarmin B These were performed with aliquots of each sample of the polymers All preparations showed a protein-positive reaction and radioactivity

All polymers were pretreated with diluted hydrochloric acid (0 1 N) for 24 hr at room temperature It is assumed that diluted hydrochloric acid removes by-products which may mask peptide bonds when polymerization occurs in the presence of sulphurous acid[8] The diluted hydrochloric acid was removed by dialysis Subsequent to this purification step, samples of the polymers were carefully dried over phosphorus pentoxide and then hydrolysed in sealed tubes under nitrogen The hydrolysates of proteinoids were desiccated to dryness and dissolved in an appropriate volume of sodium citrate buffer, *p*H 2 2 Samples of the hydrolysate containing 2 mgm nitrogen were chromatographed on 'Dowex 50 X 12' columns while the radioactivity was measured by a plastic scintillator spiral flow cell, and the radioactivity was registered on a recorder[10]

The diagram of the sample showed radioactivity at the position of cysteic acid Perhaps some traces of sulphate may appear here also A very small quantity of radioactivity from cystine was measured In spite of the precautions partial oxidation of cystine may have occurred More radioactivity could be observed in the methionine range No radioactive peak appeared at the positions of methionine sulphoxides and methionine sulphone Another radioactive component was eluted from a 150-cm column by 0 2 N sodium hydroxide This active peak was also noticed in hydrolysates of *T utilis* labelled with sulphur-35

These results indicate that it is possible to copolymerize hydrolysates of yeast with dicarboxylic amino-acids and that amino-acids labelled with sulphur-35 can be shown in hydrolysates of the synthesized proteinoid In the feeding trials fraction B of proteinoid was supplied to a male rat (body-weight at the beginning of the experiment was 95 gm) The diet consisted of 86 per cent maize, 10 per cent skimmed milk powder, 3 per cent mineral mixture *USP* XIV and 1 per cent of a complex mixture of vitamins essential for rats

The total mixture of these components could be eaten *ad lib* Twice a day for 7 days, 0 5 ml of a saturated solution of labelled amino-acid copolymer fraction B was administered orally by tube

24 hr after the last proteinoid intake the animal was decapitated After section, no abnormal alteration of the organs could be observed

The tissues in question and the excrements were hydrolysed by 6 N hydrochloric acid in sealed vessels under a nitrogen atmosphere, thus preventing the oxidation of sulphur-containing amino-acids

The hydrolysates were concentrated in a rotary evaporator and dissolved in 0 2 N sodium citrate buffer, *p*H 2 2[11] Aliquots were oxidized according to Denis's method[12], and the radioactivity was measured following the method of v Erichsen and Muller[13] The insoluble residues after hydrolysis were oxidized according to Stotz[14] The results are summarized in Table 1

Table 1 PERCENTAGE OF RADIOACTIVITY RECOVERED

Tissue resp organ	In hydrolysate	In hydrolysate residue	Total
Blood	4 53	0 81	5 34
Skin and hair	0 98	0 19	1 17
Liver	12 04	2 42	14 46
Kidneys	1 08	0 28	1 36
Brain	1 97	0 40	2 37
Digestive tract (tissue)	0 66	0 28	0 94
Rest of body	6 64	2 07	8 71
Excrements			
Digestive tract (content)	1 09	0 84	1 93
Fæces	48 08	10 98	59 06
Urine	3 89	0 67	4 56
Air	—	—	0 10

These results show that proteinoids can be hydrolysed by proteases in the digestive tract *in vivo* and that fragments are absorbed and incorporated in body tissues About 40 per cent of the radioactivity fed was utilized by the rat

The main part of the utilized radioactivity could be found in the liver tissue A relatively high yield of radioactivity was observed in the blood

We thank Dr S W Fox, Institute for Space Biosciences, Florida State University, Tallahassee, Florida, for advice during this work

G KRAMPITZ
F KNAPPEN

Institut fur Anatomie und Physiologie der Haustiere,
Universitat, Bonn, Germany

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Some Plant Glycosidases

A NUMBER of sources of plant glycosidases are known, notably almonds and lucerne seed[1] Having suitable substrates available, we have made a survey of a large number of common garden, farm and wayside plants for different glycosidase activities At an early stage attention was concentrated on seeds, since they were at least as rich a source of glycosidases as other parts of plants The species shown in Table 1 are among the highest in enzyme activity other species tended to display the same general patterns of glycosidases There was little difference in any respect between varieties of single species Enzyme preparations were made by grinding the seeds in a glass mortar with a small volume of water to give a smooth paste after dilution this was centrifuged to remove insoluble debris No appreciable enzyme activity was detected with *p*-nitrophenyl α-L-fucoside[2], phenyl β-D-mannoside[3] or phenyl *N*-acetyl-α-D-glucosaminide[4] as substrate in any species examined The absence of β-D-glucuronidase from most plant species has already been noted[5] It is evident that the seeds of common plants form a convenient source of certain glycosidases, and compare favourably in activity with many other known plant or animal sources

Table 1. GLYCOSIDASE ACTIVITIES OF SEEDS

Species (and variety)	α-D-Mannosidase	β-*N*-Acetyl-D-glucosaminidase	β-D-Galactosidase	β-D-Fucosidase	α-D-Glucosidase	β-D-Glucosidase
French bean (The Prince)	61,000	35,000	22,000	2,800	260	580
Lettuce (Tom Thumb)	40,000	13,000	trace	trace	300	950
Lucerne (Alfa)	25,000	20,000	7,000	2,900	450	1,900
Ryegrass (Irish Italian)	8,000	16,000	1,900	1,600	260	9,000

Results are expressed as μgm aglycone liberated in 1 hr at 37° per gm seed Substrates 6 m*M* *p*-nitrophenyl α-D-mannoside in acetate buffer, *p*H 5 0 (ref 2) 5 m*M* *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide in citrate buffer, *p*H 4 4 (ref 4), 2 5 m*M* *o*-nitrophenyl β-D-galactoside in phosphate-citrate buffer, *p*H 4 5 (ref 2), 1 m*M* *p*-nitrophenyl β-D-fucoside in acetate buffer, *p*H 4 0 (ref 2), 5 m*M* *p*-nitrophenyl α-D-glucoside in phosphate-citrate buffer, *p*H 5 4 (ref 3) 5 m*M* *o*-nitrophenyl β-D-glucoside in phosphate-citrate buffer, *p*H 5 4 (ref 3)

We thank W Smith and Son, Ltd, Aberdeen, for gifts of seeds

G A LEVVY
A McALLAN

Rowett Research Institute,
Bucksburn, Aberdeen

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PHYSIOLOGY

Depolarization and Tension in Crustacean Muscle

WHEN nerve impulses bring about the development of tension in a muscle, depolarization of the muscle fibre membranes can be observed The question arises Is this depolarization directly responsible for initiating contraction ? A considerable amount of evidence supports the view that, in frog striated 'twitch' muscle, depolarization of the muscle fibre membrane is a causal link in the excitation–contraction coupling process[1] However, in many crustacean muscles, contraction can occur when depolarizations of very small magnitude are present[2] It is of interest to know whether or not these depolarizations are responsible for bringing about contraction in a direct manner in crustacean muscles Evidence that this is not the case is provided by the experiments reported here, in which the depolarization of the muscle fibre membrane associated with contraction caused by stimulation of the motor nerve was found to be much less than the depolarization associated with tension development produced by adding solutions containing high potassium concentrations to the muscle

The muscle used in these experiments was the abductor muscle of the first walking leg of the crayfish *Astacus fluviatilis* This muscle is relatively small, consisting of a flat sheet of 30–40 muscle fibres, hence it can be quickly depolarized by adding van Harreveld's solution[3] containing excess potassium All the muscle fibres are innervated multiterminally by a single excitor axon, and electrical responses of different muscle fibres to stimulation via this axon were found to be quite uniform Electrical recordings from single muscle fibres were made by means of glass intracellular electrodes of 5–15 M ohms resistance and low tip potential[4] Usually it was possible to examine several cells from a given preparation during the course of an experiment Mechanical responses of the muscle were recorded isometrically at the tip of the dactyl by means of an *RCA* 5734 transducer

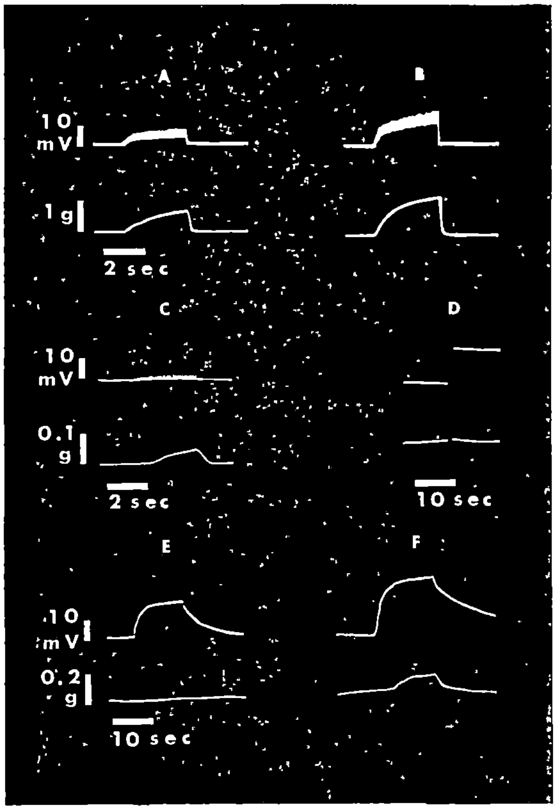

Fig 1 Tension (lower traces) and intracellularly recorded membrane potentials (upper traces) from representative crayfish abductor muscle preparations *A,B*, Responses to a train of stimuli at 70/sec (*A*) and 100/sec (*B*) delivered via the excitor axon, *C*, responses from a different preparation to a train of stimuli at 15/sec , *D*, potassium depolarization in the same muscle fibre, parts of two consecutive traces before and 40 sec after addition of a solution containing 15 m*M* potassium, no tension is developed, *E,F*, responses of another preparation to solutions containing 15 m*M* potassium (*E*) and 22 m*M* potassium (*F*), normal saline is restored after a few seconds in potassium-rich saline

Results obtained from representative preparations during stimulation of the excitor axon at various frequencies are shown in Fig 1 *A,B,C* At low frequencies, small junction potentials of 2–5 mV are produced (Fig 1 *C*), these may grow slightly in size during a train of stimuli As the stimulation frequency is increased, the size of the junction potentials increases and a depolarization 'plateau' is built up (Fig 1 *A,B*) Total depolarization due to junction potentials plus 'plateau' usually amounts to about 15 mV during a three-second period of stimulation at 100 per second However, measurable tension results at quite low frequencies of stimulation (5–15/sec) when the junction potentials are only 2–5 mV , with practically no 'plateau'

When solutions containing excess potassium are added to the muscle, fibres examined in all parts of the muscle show prompt depolarization which can be reversed by returning the muscle to normal 5 m*M* potassium saline Fig 1 *D* shows the electrical response to a solution containing 15 m*M* potassium of the fibre recorded from Fig 1 *C*, the initial resting potential of which was 80 mV A depolarization of 18 mV was produced, but no tension was developed by the muscle In the experiment recorded in Fig 1 *E*, a solution containing 15 m*M* potassium was added to another muscle for a few seconds, after which the normal saline was restored A reversible depolarization of 17 mV was recorded from the fibre examined (which had an initial resting potential of 79 mV) but no contraction occurred The procedure was then repeated using a solution containing 22 m*M* potassium and recording from the same fibre (Fig 1 *F*) A depolarization of 28 mV was recorded, and in this case contraction of the muscle was produced

These experiments, and many others of a similar nature which were performed, indicate that potassium contracture does not occur unless the membrane potential is lowered past a 'threshold' of 55–60 mV. Experiments performed on muscles of *Carcinus maenas* yielded similar results A 'threshold' of this nature does not exist for contractions produced by nervous stimulation, contraction can occur when depolarizations of only a few mV are present It is possible, therefore, that contraction in many crustacean muscles is not brought about by depolarization, but by some other action of the transmitter agent

Apparently there are two separate ways in which the contractile mechanism in crustacean muscles can be activated (*a*) by the nerve impulse and its consequent junctional effects, without necessarily producing much depolarization of the muscle-cell membrane, (*b*) by a sufficiently large depolarization of the muscle fibre membrane In those crustacean muscles which show 'spikes', or in which the electrical responses exceed the depolarization 'threshold', both activating mechanisms may be used, and the contraction-producing transmitter action is probably supplemented by tension developed when depolarization passes the 'threshold'.

H L Atwood

Department of Zoology,
The University, Glasgow

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Effect of Alloxan on the Insulin Content of Micro-dissected Mammalian Pancreatic Islets

The development of techniques for dissection and weighing of µgm pieces of tissues[1] has provided a method for the isolation of frozen dried sections of mammalian islets The availability of a very sensitive bioassay method for insulin[2] has permitted a direct assay of the insulin content of individual islets We have undertaken the examination of the insulin content of micro-dissected islets from normal and diabetic rats at various times after alloxan injection

Pancreas was obtained from normal rats and from animals given alloxan intravenously (50 mgm /kgm body-wt) The rats were killed by decapitation 2, 24, 48 and 72 hr after alloxan injection The pancreas was frozen in liquid nitrogen (– 196° C) immediately on removal The frozen tissue was sectioned in a cryostat at 20µ at – 20° C , the sections were dried under vacuum at – 40° C [1] The dried sections were stored under vacuum at – 20° C Just prior to the time of assay, they were brought to room temperature under vacuum The islets were dissected from acinar tissue under a binocular dissecting microscope and weighed on a quartz fibre balance[1,4] The individual islets, weighing between 0 05 and 0 20 µgm , were suspended in 1 ml of bicarbonate buffer (*p*H 7 4) containing glucose (100 mgm /100 ml), gelatin (2 mgm /ml), and glucose-1-^{14}C (0 2 µc , *S A* = 12 µc /

Table 1 EFFECT OF ALLOXAN ON THE INSULIN CONTENT OF MICRO-DISSECTED RAT ISLETS

Treatment	Time after injection (hr)	No of animals	Total No of islets assayed	Blood sugar at time of death (mgm /100 ml)	Insulin content μunits/μgm dry wt: Average of all determinations	Range of values for individual rats	*S E* of individual islets assayed
Islet tissue							
Saline injected Normal controls	2	3	23	85–103	3,604	2,617–4,137	±436
Alloxan injected	2	4	13	101–207*	4,027	3,267–5,477	±628
Alloxan injected	24	3	10	315–545	3,635	2,917–4,608	±497
Alloxan injected	48	5	20	305–573	155	0–327	—
Alloxan injected	72	3	13	536–846	93	25–285	—
Acinar tissue from normal controls	—	8	—	—	53	0–179	—

* The 2-hr blood sugar values of rats injected with a diabetogenic dose of alloxan may be normal, hyperglycæmic or hypoglycæmic

mgm) The vessels were equilibrated for 5 min with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide, and incubated in a Dubnoff metabolic shaker at 37° C for 3 hr The conversion of glucose labelled with carbon-14 to carbon dioxide, lipid or glycogen was determined, carbon-14 was measured using a liquid scintillation counter

Micro-dissected islets from normal control rats (saline injected) contain an average of 3,600 μunits of insulin per μgm of dry weight tissue If one assumes that 1 μgm of crystalline insulin is equivalent to 25,000 μunits, then the observed insulin content of the normal rat islet is 14 5 per cent of the dry weight In comparison to the islets, the pancreatic acinar tissue contained negligible amounts of 'insulin-like' activity In a preliminary report by Williamson and Lacy[3] similar values have been found in the micro-dissected rabbit islet tissue composed primarily of β-cells

We have also determined the insulin content of the rat pancreas using an acid alcohol extraction procedure and the epididymal fat pad assay method, values of 1 84 units/gm wet wt were obtained This value agrees closely with that reported in the literature (2 units/gm wet wt [5]) obtained by the mouse convulsion assay method Assuming that the islet tissue comprises 1 per cent of the weight of the pancreas and that the water content of this tissue is 70 per cent, the calculated insulin content of the islet tissue is 700 μunits/μgm dry wt, or 2 75 per cent of the dry weight This value is only one-fifth of that obtained for micro-dissected islets

As shown in Table 1, the insulin content of islets obtained from rats killed 2 hr after injection of alloxan contained the same amount of insulin as the islets from control rats At 24 hr, despite elevated blood sugar levels, the insulin content was unchanged However, at 48 and 72 hr after alloxan administration, the insulin content of the islets was markedly decreased to values less than 5 per cent of that of the controls This observation confirms those of Wrenshall *et al* [6], who found a decrease to 5–10 per cent of the control values in the insulin content of whole rat pancreas within 48 hr after injection of alloxan Our finding that the insulin content of the micro-dissected islets is normal at a time when the blood sugar is elevated (24 hr after alloxan administration) suggests that this drug may affect the mechanism of release (or secretion) of insulin The insulin contained in the damaged β-cells may not be liberated into the blood stream because of an alteration in the permeability of the cell membrane, it has been reported that alloxan selectively alters the permeability of the islet tissue in fish[7] The sharp decrease in the insulin content of the islets at 48 hr after alloxan may be attributed to its destruction 'locally'

This investigation shows that the epididymal fat pad method is sensitive enough to determine the insulin content of micro-dissected islet tissue, and to detect changes in the insulin content following alloxan administration It shows that the insulin in the pancreas is largely confined to the islet tissue The findings reported here emphasize the need for further study of the relationship between 'native' insulin as found in the micro-dissected islet tissue, and the insulin which is extracted from the pancreas by acid alcohol

This investigation was supported by research grants *A*–1659 and *A*–1887 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U S Public Health Service

P K DIXIT
I LOWE
A LAZAROW

Department of Anatomy,
University of Minnesota,
Minneapolis 14

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Regulation of Sodium Excretion and Retention by the Intestine in Cows

IT is well known that the kidney is the principal organ regulating sodium retention and excretion in order to maintain a constant sodium-level in the blood and tissues (sodium homeostasis)

With regard to sodium absorption in the gut, it also has been shown that the behaviour of the intestinal wall is not merely passive, so that there would be a tendency towards equal concentrations of sodium in the intestinal and fæcal juices on one hand and in the blood plasma on the other In cows, however, Van Weerden[1,2] showed that, in the lower part of the small intestine and in the large intestine, sodium absorption occurs against a considerable concentration gradient so that relatively little sodium is lost with the fæces This indicates that the cow can subsist on surprisingly small quantities of sodium in the feed Van Weerden[1] stressed the importance of this fact because, ordinarily, pasture and other cattle feeds are very poor in sodium

It seemed of interest to examine whether final concentration of the sodium in the fæcal juice is

Table 1 SODIUM, POTASSIUM AND FREEZING POINT DEPRESSION IN FÆCAL JUICE, URINE AND MILK

Period	Days after beginning of exp	Intake per day (m equiv)		Fæcal juice			Urine (m equiv /100 ml)		Milk (m equiv /100 ml)	
				m equiv /100 ml		Freez point depress (° C)				
		Na	K	Na	K		Na	K	Na	K
				Exp I						
Low sodium	0–20	132	2,534	0 31	3 12		0 07	42 5		
	21–40	127	2,492	0 88	2 74	0 280	1 58	41 9		
	41–58	120	2,576	1 55	2 66	0 278	1 77	32 7		
Moderate sodium	59–77	1,778	2,576	3 89	1 59	0 338	14 86	20 8		
Low sodium	78–93	124	2,577	0 55	2 92	0 356	0 54	38 8		
				Exp II						
Low sodium	0–14	231	4,343	0 53	3 83	0 329	0 18	21 3	1 49	2 80
Moderate sodium	15–24	2,020	4,583	2 24	2 78	0 325	4 26	24 7	1 69	2 76

always the same or whether it is dependent on the amount of sodium in the feed, in other words Does the intestinal wall take part in establishing sodium homeostasis, and if so, to what extent in comparison with the kidney ?

With laboratory animals and human subjects it has already been shown that the specific sodium absorption in the intestine works more efficiently when sodium intake is lower than normal[3–6] So far as we know such observations have not yet been made with cows In this species the problem might be more complicated, because the fæcal juice is always hypotonic as compared with the blood[7]

Two heifers (Exp I) and one milch cow (Exp II) were given sodium-poor rations containing 2 7–5 3 gm sodium per day per animal (low sodium period) In a second period the same rations were fed, but in addition 100 gm of sodium chloride (± 40 gm of sodium) per day per animal was added to the concentrates (moderate sodium period) Finally, the animals received again the low sodium rations (second low sodium period) In Exp I the basal ration consisted of rye straw, hay of mixed grasses, maize meal, linseed meal and calcium carbonate, and in Exp II of rye straw, hay of mixed grasses, potatoes, mixed concentrates and calcium carbonate

Determinations were made of the dry matter in the fæces, and of sodium and potassium in fæces, urine and milk Moreover, press juice (ultra-filtrate) was obtained by pressing the fæces in a membrane press through 'Cellophane' with the aid of nitrogen gas at a pressure of 10 atmospheres[1] In this juice sodium, potassium and osmotic pressure (by freezing point depression) were determined

The average results of the experiments are shown in Table 1 Those of the second low sodium period in Exp II are, however, omitted because in this period the sodium content of the ration was higher than expected

During the low sodium periods, the sodium concentrations in the fæcal juice and in the urine were distinctly lower than during the moderate sodium periods There was also a small but significant decrease of sodium concentration in the milk ($P < 0\ 01$)

In the low sodium periods of both experiments, the potassium concentrations in the fæcal juice were distinctly higher than in the moderate sodium periods In Exp I the same was true for the urine In Exp II, however, there was little difference in this respect

The freezing point depression of the fæcal juice was always within the same range as has been found by Van Weerden[1] thus much smaller than that in the blood In this respect, there was no significant difference between the moderate and the low sodium periods Therefore, the increase of potassium concentration in the low sodium periods can be supposed to be produced by a substitution of sodium by potassium, the former being selectively absorbed from the intestinal contents In dogs such a substitution has already been found by Field *et al* [8] and in human subjects by Spencer *et al* [5]

In Exp II milk, fæces and urine were weighed daily In both periods of this experiment the quantity of fæces amounted to 34–35 kgm a day In the moderate sodium period the average daily excretion of sodium with the fæces amounted to 752 m equiv or 17 3 gm In the low sodium period it was only 196 m equiv or 4 5 gm During the low sodium period the excretion of sodium with the fæces was therefore reduced to about a quarter as compared with the moderate sodium period Thus, 556 m equiv or 12 8 gm sodium was saved from being excreted with the fæces

The figures in Table 1 refer to the whole periods In the latter part of the low sodium period of Exp II the sodium content of the press juice decreased to only 0 20 m equiv /100 ml or even less, that is, about 1/10 of its normal level of about 2 m equiv /100 ml press juice In this case the sodium was absorbed from the intestinal contents against a 75-times higher concentration gradient It is therefore clear that, besides the kidneys, the gut plays an important part in the regulation of sodium metabolism of the cow on sodium-poor diets

J A RENKEMA
TATSUDO SENSHU
BLANCHE D E GAILLARD
E BROUWER

Laboratory of Animal Physiology,
Agricultural University,
Wageningen,
The Netherlands

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Plasma Creatine- and Creatinine-Levels in Sheep

Blanch and Setchell[1] have reported the occurrence of creatinuria in fed, normal sheep They found no increase in creatine excretion occurred during a six-day fast Plasma creatine-levels were found to average 2 62 and 3 01 mgm per 100 ml in four fed and four fasted sheep respectively

During the course of experiments involving starvation of both pregnant and non-pregnant ewes we have also studied plasma creatine- (and creatinine) levels Results substantially different from those of Blanch and Setchell were obtained

The animals used comprised three non-pregnant and twenty pregnant cross-bred ewes Pregnant ewes were about one month from lambing at the beginning of the experiment The animals had been well fed and were in good condition before starvation, which was carried out in semi-exposed pens Drinking water was freely available during the fast Blood was obtained from the jugular vein, heparin being used as anticoagulant Separated plasma was deproteinized with trichloroacetic acid Preformed creatinine and 'total creatinine' (preformed creatinine plus creatine after conversion to creatinine) were determined colorimetrically (Pearce, unpublished method) Creatine was calculated by difference, the results being expressed in terms of creatinine

The results obtained are given in Table 1 It may be seen that in the fed sheep the creatine-levels were similar to the average level of 2 26 mgm /100 ml (expressed as creatinine) found by Blanch and Setchell However, on starvation of pregnant sheep a large increase in plasma creatine was found Non-pregnant sheep responded to starvation with a much smaller initial rise in creatine However, the increase became appreciable by the ninth day of starvation

Some of the pregnant sheep developed, during the course of the fast, certain clinical signs similar to those seen in ovine 'pregnancy toxæmia'[2-5] Of these animals some recovered when re-fed and are included in group *A* in Table 1, together with fasted pregnant sheep in which clinical signs were not observed Other sheep, showing clinical signs, failed to recover although offered food, and died at various times during the refeeding period These deaths, with one exception, occurred prior to parturition Animals of this type are included in group *B* in Table 1 It may be seen that during the fast animals in group *B* showed higher plasma creatine-levels than animals in group *A* On refeeding plasma creatine fell in group *A* to low levels but continued high in group *B* Indeed some animals in the latter group showed the highest levels during the refeeding period The difference between the two groups is still more clearly seen if the average of the last available sample (that is, the last sample before death) for animals in group *B* is considered This was no less than 9 82 mgm creatine (as creatinine) per 100 ml plasma (range 3 6–24 1) Since a gap of several days occurred in some cases between the last sampling and death, even this figure is probably lower than the true terminal average

Results for plasma creatinine are also set out in Table 1 Only slight changes occurred in non-pregnant and group *A* sheep, but a substantial increase occurred during the terminal phase in group *B* The effect is again more clearly seen by considering the last sample obtained before death, which averaged 3 09 mgm creatinine per 100 ml plasma (range 0 76–7 80) It may be remarked that unpublished results for plasma

Table 1 PLASMA CREATINE AND CREATININE IN SHEEP

	Creatine							Creatinine						
	Fasted (days)					Refed (days)		Fasted (days)					Refed (days)	
	0	4	6	9	11	2	10	0	4	6	9	11	2	10
Non-pregnant (3 ewes)	1 99 {1 6–2 4}	2 56 {2 3–3 1}	2 69 {2 4–3 4}	4 50 {3 3–6 8}	3 23 {2 7–3 6}	2 12 {2 0–2 4}	2 36 {2 1–2 6}	0 94 {0 91–0 96}	0 97 {0 82–1 2}	0 98 {0 83–1 1}	0 92 {0 88–0 96}	0 99 {0 96–1 0}	0 96 {0 80–1 2}	1 20 {1 1–1 3}
Pregnant group *A* (9 ewes)	2 01 {1 5–2 6}	3 84 {3 0–8 0}	3 41 {2 8–4 5}	4 18 {3 5–5 6}	4 23 {3 2–6 0}	4 05 {3 1–6 2}	1 63 {0 7–2 3}	1 02 {0 75–1 2}	0 97 {0 82–1 1}	0 96 {0 75–1 1}	1 06 {0 92–1 3}	1 01 {0 80–1 2}	0 99 {0 84–1 2}	0 86 {0 63–1 0}
Pregnant group *B* (11 ewes)	2 20 {1 3–3 0}	3 92 {3 3–5 4}	3 50 {2 4–4 4}	5 40 {4 1–7 5}	6 75 {3 5–24 1}	7 44* {3 6–18 9}	12 70† {5 4–16 9}	0 95 {0 80–1 2}	0 97 {0 77–1 2}	0 99 {0 75–1 3}	1 12 {0 80–1 84}	1 35 {0 88–2 6}	1 83* {0 76–6 1}	5 63† {1 6–7 8}

Results are expressed in terms of creatinine, mgm /100 ml plasma, followed by the range of results in brackets * 8 sheep only † 3 sheep only urea also indicate marked nitrogen retention in animals dying from experimentally induced 'pregnancy toxæmia'

The reason for the discrepancy between our results for creatine and those of Blanch and Setchell is not clear It seems possible that the ewes used by the latter workers may have been non-pregnant—in our study animals of this type showed comparatively little change in plasma creatine after six days starvation (the period used by Blanch and Setchell) in contrast to the larger changes seen in pregnant animals Of possible significance also is the observation that in the pregnant sheep plasma creatine-levels tended to be lower on the sixth day than on the fourth, ninth or eleventh days of the fast A possible difference in specificity of the method used for creatine in our work would also account for the discrepancy but is believed to be rather unlikely

We thank Messrs Imperial Chemical Industries, Ltd , for a grant in support of these experiments

P J Pearce
J S S Inglis

Department of Animal Husbandry
and Veterinary Preventive Medicine,
University of Glasgow Veterinary Hospital,
Bearsden, Glasgow

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Sea Salt and Dental Caries

Experience in the United States and elsewhere has shown that the main source for the fluoride intake in man for the partial prevention of dental caries is the water supply Results from recent investigations in India and Greece strongly suggest that the daily consumption of sea salt by the people of these countries may have been another major source of dietary fluoride Sea salt was reported to contain 40 p p m of fluoride[1] The fluoride content of teeth from India and Greece was found to be considerably high although the level in the water supplies was negligible[1,2] In addition, the prevalence of caries in the two countries has been reported to be low[1,3]

It is generally believed that children up to 12 years of age consuming water containing continuously about 1 0 p p m of fluoride will ingest from water alone approximately 0 4–1 0 mgm of fluoride daily, amounts which will provide considerable protection against caries In order to ingest these levels of fluoride entirely from sea salt, containing 40 p p m of fluoride, a daily intake of about 10–25 gm would be required However, significant reduction in caries has been also observed in children using water alternating in its natural fluoride content from day to day between 0 2 and 1 0 p p m [4] It thus seems likely that intermittent ingestion of sea salt in amounts comparable with the above-mentioned may also result in a fluoride intake sufficient to provide considerable protection against caries

The ingestion of high amounts of salt is not unlikely among people of certain countries because of their dietary habits and food customs It has been reported that the ration of the armed forces of the Republic of Korea included 30 gm of crude sea salt per man per day[5] Furthermore, up to twice this amount was ingested daily from the consumption of native food preparations containing high amounts of sea salt[5] In this connexion, it may be significant that susceptibility to caries among the men of the armed forces was found to be very low[5] In a nutritional survey of the armed forces of Iran it was shown that an allowance of about 30 gm of salt per man per day, obtained from mines, was included in the menu[6] The amount of sea salt produced by salterns in Greece annually indicates that the daily ingestion by the population may be considerable[7] In addition, experiments with laboratory animals indicated that increasing the calcium-level in the diet decreased the amount of fluoride absorbed from the intestinal tract[8] Therefore, in countries where the level of calcium intake is low there may be an increase in the absorption of the ingested fluoride, thus a smaller amount of dietary fluoride would be required daily for protection against caries

In conclusion, it is proposed that serious consideration should be given to the role of sea salt as an important source of dietary fluoride for the prevention of dental caries in areas of the world where the salt consumed locally is prepared by evaporating sea water

D M Hadjimarkos

University of Oregon Dental School,
Portland, Oregon

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Spontaneous Hydronephrosis in Albino Rats

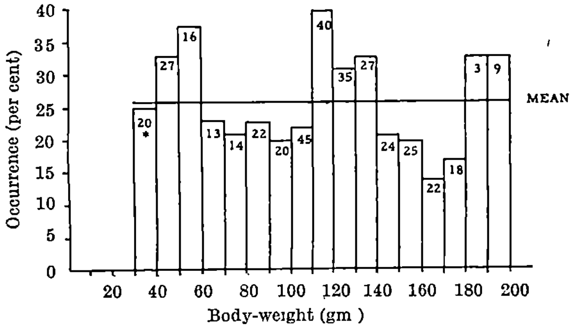
During the course of an investigation on the influence of the pituitary gland on compensatory renal hypertrophy in rats[1], a surprisingly high incidence of hydronephrosis was encountered This made it necessary to discard a great number of animals The failure to find any reference to spontaneous hydronephrosis in rats in the literature has prompted this preliminary report[2–6]

Table 1. Summary of Data on Incidence of Hydronephrosis in Albino Rats

Sex	No of rats	No of rats with hydronephrosis	Percentage of hydronephrosis
Male	415	104	26 3
Female	371	6	1 6

Autopsy findings on 786 rats are presented in Table 1 The rats, which were apparently healthy, were killed because they served as controls in certain experiment or they had to be replaced by younger rats for the maintenance of the colony in the Unit Only kidneys showing gross dilatation of the pelves and calices were considered to be hydronephrotic A careful search for calculi in the urinary passages (pelvis, ureter and bladder) was carried out but none was found Hydronephrosis was almost entirely confined to the right kidney and was much commoner in the male The age of the rats (judged by the body-weight) did not seem to affect the incidence of hydronephrosis

The following findings in two rats is of exceptional interest In one the right kidney was completely absent and the left kidney had increased in size to a considerable extent (left kidney wt mgm /body wt gm 2632/385) The weight of the two kidneys in a rat of the same weight would be 3,000 mgm (ref 7) In the second rat the right kidney was completely replaced by a kidney-shaped transparent cyst filling up the entire lumbar space and containing clear yellow fluid which looked like urine The ureter was greatly dilated and was in its usual anatomical position Both were male rats



* No of rats per group

Fig 1 Hydronephrosis in male albino rats

To our knowledge, this is the first report of spontaneous hydronephrosis in the rat, not associated with calculi or infection The failure to find calculi in the urinary passages, however, does not rule out the possibility of their presence as microscopic crystals in the renal tubules with consequent blocking and atrophy of the renal parenchyma and dilatation of the calices and pelves Although the origin of the rats in this colony is not known exactly, they are believed to be of the Wistar strain They have been maintained as a closed colony for 14 years The fact that no hydronephrosis or renal calculi was found in any of the 400 rats which were autopsied at the Wistar Institute is interesting

One can only speculate about the cause of the spontaneous hydronephrosis which has been observed in these rats The possible presence of intrarenal calculi has already been mentioned Another possibility is that the peristaltic mechanism of the ureter might be unco-ordinated or at fault A genetic stigma which is transmitted in the male rat as a dominant character would appear probable It is hoped that this report will stimulate investigators in the field of renal science to shed new light on the pathogenesis of hydronephrosis

T Astarabadi
E T Bell

Department of Physiology and the Clinical
Endocrinology Research Unit
(Medical Research Council), and
Department of Biochemistry
and the Clinical Endocrinology Research Unit
(Medical Research Council),
University of Edinburgh

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HÆMATOLOGY

Two Types of γ_{1A}-Globulin in Sera from Normals and Patients with Multiple Myeloma

The $\gamma_{1A}(\beta_{2A})$-globulins of normal human serum and the γ_{1A}-myeloma proteins are structurally related to the other immune globulins and Bence Jones proteins[1,2] This communication presents evidence for the existence of two antigenically distinguishable types of γ_{1A}-myeloma proteins Each of these is closely related to one of the major immunologically defined types of Bence Jones proteins[3] and each of them appears to have an antigenically distinct counterpart in the normal γ_{1A}-globulin fraction

An electrophoretically homogeneous preparation of pooled normal γ_{1A}-globulin[4] gave 2 distinct precipitin lines by double diffusion with an antiserum to 7*S*-γ-globulin, while electrophoretically isolated γ_{1A}-myeloma proteins generally gave only one line and could be divided into two antigenically distinguishable groups (Fig 1*A*) Six proteins afterwards classified as belonging to group *A* on the basis of their relationship to group *A* Bence Jones proteins gave a reaction of complete or partial identity with the inner line of normal γ_{1A}-globulin while the other 6, classified as group *B*, fused with the outer line but penetrated through the inner one (Fig 1*A* and *B*) Proteins within each group gave a reaction of complete or partial identity with each other and a reaction of non-identity with those of the other group (Fig 1*A* and *B*) An antiserum specific for γ_{1A}-globulin showed certain common determinant groups on all proteins since all gave a reaction of partial identity (Fig 1*C*) When compared to 9 Bence Jones proteins of groups *A* and *B*, one group gave a reaction of complete or partial identity with Bence Jones proteins of group *A* and was therefore called group *A*, while the others were antigenically related to Bence Jones proteins of type *B* and referred to as group *B*[3] (Fig 1*D* and 1*E*) Antisera prepared against each of the γ_{1A}-myeloma groups reacted only with myeloma and Bence Jones proteins of the same type

The 2 classes of γ_{1A}-globulin did not differ from each other in electrophoretic mobility, sedimentation properties and hexose content All but one of the proteins had a major component with a sedimentation-rate of 7*S*, and 10 had additional peaks with sedimentation-rates of approximately 10, 13 and 15*S* These were unaffected by *p*H 3–4 or 30 per cent urea but were reduced to 7*S* units by 0 1 *M* mercaptoethanol Hexose of normal γ_{1A}-globulin was 5 5 per cent and that of proteins in both groups ranged from 4 to 6 per cent with the exception of one of the group *A* proteins which contained only 1 1 per cent Similarly the 3 5–3 8*S* units produced from the 2 types by papain[5] were similar They migrated as a single component with a mobility similar to the native protein on immunoelectrophoresis Chromatography on CM cellulose yielded only a single peak with the starting phosphate buffer *p*H 7 6, μ0 01 On DEAE cellulose, papain fragments from normal γ_{1A}-globulin and one of the group *B* myeloma proteins could be resolved into two distinct peaks, while those obtained from one other group *B* and two group *A* proteins came off as single peaks with a high ionic strength buffer[6] The papain fragments lost most of the antigenic determinants specific for γ_{1A}-globulin, but they retained sufficient specificity to permit their identifica-

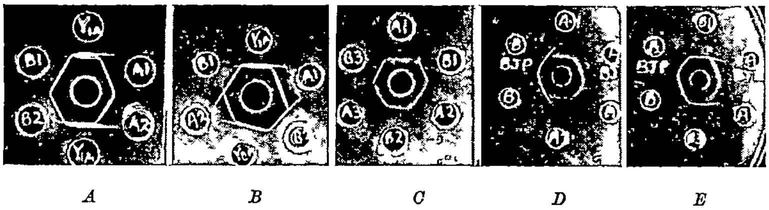

Fig 1 Ouchterlony analyses of *A* and *B*, normal γ_{1A}-globulin (γ_{1A}) and two myeloma proteins of group *A* and group *B* using antiserum to 7*S* γ-globulin in centre well, *C*, three myeloma proteins of each group using antiserum to γ_{1A}-globulin, *D*, two group *A* myeloma proteins (top and bottom) and Bence Jones proteins group *B* (left) and group *A* (right) Antiserum to 7*S* γ-globulin in centre, *E*, two group *B* myeloma proteins (top and bottom) and Bence Jones proteins group *B* (left) and group *A* (right) Antiserum to 7*S* γ-globulin in centre

tion with the same antigenic class as the parent protein

The antigenic similarities noted between the 2 classes of γ_{1A}-globulins and Bence Jones proteins as well as with certain parts of 7*S* and 19*S* γ-globulins suggest that all may contain certain common polypeptide chains of a similar type[7] The genetic control of these is now being investigated[8]

We thank Dr Leonard Korngold for initially classifying two Bence Jones proteins for us This work was supported by the Arthritis and Rheumatism Foundation and U S Public Health Service grants *A*-2594, *A*-1431 and 5141

EDWARD C FRANKLIN

Department of Medicine and
Rheumatic Diseases Study Group,
New York University School of Medicine,
New York, 16

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Hæmoglobin Bart's in Italy

CONTINUING our investigations, already reported[1], we have found 9 cases of hæmoglobin Bart's out of 3,556 samples of cord blood In 7 of these, re-examined after several months, the abnormal hæmoglobin had disappeared In 4 babies, aged from 5 to 10 months, an evident microcythæmic (or thalassæmic) hæmatological picture appeared, in three others, still very young, the hæmatological characters were still uncertain with regard to microcythæmia (or thalassæmia) Examination of the parents, carried out in 8 families, has revealed, in all, the absence of abnormal hæmoglobins, however, in 7 families, one of the two parents showed the hæmatological characters, either pronounced or slight, of microcythæmia, only in one family were the hæmatological characters normal, with the exception of very slight erythrocytic morphological alterations in the mother In our previous communication, we had observed this family only and another one, in which the mother showed rather slight deviations and therefore we thought that we could exclude the existence of any relationship between hæmoglobin Bart's and microcythæmia, however, it must now be that such a relationship exists

The most characteristic observation is that the parents, carriers of microcythæmia, and the sons with hæmoglobin Bart's, re-examined after several months, have never revealed a high portion of hæmoglobin A_2 This fact, which has also been reported by others[2,3], recalls the similar one of families of carriers of hæmoglobin H in which all microcythæmic members and the same carriers of hæmoglobin H always have a normal fraction of hæmoglobin A_2.

The hypothesis may be made that hæmoglobin Bart's and hæmoglobin H appear in the same families in which microcythæmia is present without increase of hæmoglobin A_2, and that is, according to the hypothesis of Ingram and Stretton[3], α-chain microcythæmia

E SILVESTRONI
I BIANCO

Istituto d'Igiene dell' Università di Roma,
Centro di Studi della Microcitemia e
della Anemie Microcitemiche, Roma

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PATHOLOGY

Deoxyribonucleic Acid Measurements on Buccal Cell Nuclei in Megaloblastic Anæmias

MEGALOBLASTIC anæmias, as their name indicates, are characterized by enlargement of the red cell precursors This enlargement affects both nucleus and cytoplasm Some other types of cell are enlarged as well, particularly the immature myeloid cells ('giant band cells') and the squamous epithelial cells of the buccal mucosa Other epithelial cells are sometimes affected, but the changes are inconstant[1]

Interpretations of these findings in terms of the molecular structure of the nuclei are still a matter of speculation Barnard and Lewson[2] have proposed that vitamin B_{12} deficiency results in increased polymerization of DNA, and that this causes nuclear enlargement and hypochromasia Nothing is actually known about the biochemical difference between an affected cell and a normal one Examination of the 'biochemical lesion' in deficiency of vitamin B_{12} and of folic acid has shown that these substances play a part in numerous metabolic processes, not only concerned with nucleic acid metabolism[3,4] The idea that the nuclear changes are due to defective DNA synthesis is not supported by auto-radiographic data[5]

It is not even known whether premitotic megaloblast nuclei contain the same amount of DNA as the corresponding normoblasts Measurements of DNA in interphase nuclei obtained from bone marrow are affected by the proportion of cells which are synthesizing In order to get comparable figures it would be necessary to perform estimations on cells actually in mitosis, and it would not be possible to identify the dividing cells

These difficulties do not apply to the nuclei of buccal epithelial cells Buccal cells are very easy to prepare without the admixture of other cell types, and

Table 1

Case and diagnosis	Treatment and date	Date of sample	Mean nuclear diameter (100 cells) (μ)	Basic DNA value*	No of cells measured	No of tetraploid cells	No of binucleate cells	Basic DNA value of normal controls
E G Pernicious anæmia	B_{12} 18 11 60	18 11 60 9 12 60	9 58 8 84	108 115	38 35	1 0	2 1	106
F H Pernicious anæmia	B_{12} 1 5 61	14 4 61 17 5 61	9 57 9 35	109 112	35 36	1 0	2 0	110
J D Pernicious anæmia	B_{12} 24 4 61	22 4 61 2 5 61	10 13 8 80	103 108	36 30	9 0	1 0	110
A W Pernicious anæmia	B_{12} 28 4 61	28 4 61 20 7 61	9 82 8 86	113 117	30 30	0 0	1 0	113
S E Megaloblastic anæmia of puerperium	Folic acid 12 6 61	17 5 61 19 6 61	10 37 8 73	115 112	30 30	1 0	0 0	115

* Expected normal value = 110

if they are obtained from saliva they are all fully differentiated and incapable of further division As this seemed a favourable material, we have made microspectrophotometric measurements on buccal cell nuclei of four patients with Addisonian anæmia before and after vitamin B_{12} treatment, and of one patient with puerperal megaloblastic anæmia before and after treatment with folic acid

The saliva was centrifuged to deposit the cells The supernatant was decanted and the cellular deposit was spread between two cover-glasses and air dried A few smears were also made on microscope slides, immediately wet-fixed in ethyl alcohol containing 3 per cent of acetic acid, and stained by Papanicolaou's method

The wet-fixed smears were used for micrometer measurements of nuclear diameters These were performed as described by Boddington[6]

The air-dried smears on cover-glasses were used for Feulgen staining In order to provide a standard whereby different smears could be compared with each other and also with control smears of buccal cells obtained from normal individuals, small pieces of fresh human tissue (thyroid or endometrium) obtained at operation were tapped out in physiological saline, and one or two drops of the resulting cell suspension were gently smeared over the cover-glasses, which were immediately fixed by methanol freezing substitution Controls were fixed and stained at the same time as the smears from patients After staining by the Feulgen procedure, measurements were made with the Deeley integrating microdensitometer[7] incorporating the cell-crushing device[8] A random sample of 30–38 buccal epithelial cells and 15–20 'standard' cells were measured on each specimen The mean stain content, in arbitrary units, of those buccal cells which fell within 20 per cent of the main (diploid) mode was calculated, as was the mean of the standard cells Polyploid cells were recorded separately Assigning a value of 100 to the standard cells, the value for the buccal cells was adjusted accordingly, to give the 'basic DNA value' (usually the standard cells were leucocytes or fibroblasts, when epithelial cells were measured, the value of 110 was taken, since in previous measurements on normal human tissues epithelial cells were found to have approximately 10 per cent greater DNA content than leucocytes and fibroblasts)[9]

The results presented in Table 1 show that buccal cells with typical enlarged nuclei due to vitamin B_{12} or folic acid deficiency have a DNA content which is not measurably different from normal, except that tetraploids, and binucleate cells containing two diploid nuclei, are unusually frequent Only 5 of the 330 cells measured had DNA contents which fell outside the diploid (± 20 per cent) or tetraploid (± 20 per cent) regions After treatment, most of the readings show an insignificant rise (perhaps coincidental) and the tetraploids and binucleates disappear

The general nuclear enlargement observed in these cases therefore remains unexplained, but it is now possible to discard the hypothesis that such nuclei are deficient in DNA The apparently light nuclear staining is presumably due to a normal DNA content being distributed over a larger area in the spread-out nucleus

N B ATKIN

Mount Vernon Hospital,
Northwood, Middlesex

M M BODDINGTON
A I SPRIGGS

Churchill Hospital, Oxford

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Induction of Experimental Allergic Encephalomyelitis by Tattooing

TATTOOING has been used in medical practice to introduce certain materials for local effects These have included pigments to modify the appearance of scars, birthmarks or other abnormalities on skin or cornea, titanium oxide to conceal unwanted tattoos, mercuric sulphide to relieve pruritus ani, and steroids to treat local skin lesions The present demonstration that nervous tissue emulsions can be tattooed into the skin of rats in amounts sufficient to cause experimental allergic encephalomyelitis may serve to direct attention to tattooing as an additional method of parenteral inoculation for systemic effect

Experimental allergic encephalomyelitis is induced ordinarily by injection of nervous tissue emulsified in Freund's adjuvant In the present experiments, a 40 per cent aqueous homogenate of spinal cord was emulsified in an equal volume of adjuvant ('Bayol *F*-Arlacel *A*', 85 15) Adjuvants with zero, 4 0 mgm /ml, and 40 mgm /ml killed tubercle bacilli are termed incomplete, complete, and fortified, respectively Female rats of a strain highly susceptible to experimental allergic encephalomyelitis[1] were anæsthetized with ether The emulsion was smeared over the plantar surface of the foot and the electrically operated tattoo needles were passed systematically over this area Some bleeding occurred and the foot was slightly swollen and discoloured for a few days

Among 70 rats tattooed once, experimental allergic encephalomyelitis was detected histologically in 32, two of which had exhibited hind-limb weakness after 13 and 17 days The lesions consisted of mononuclear infiltration of meninges, vessels and perivascular parenchyma with demyelination The incidence varied according to the experimental procedure (Table 1) Tattooing the right hind foot or all 4 feet with an emulsion of rat spinal cord and complete adjuvant produced mild lesions in half the rats Tattooing flank skin, or even ten skin sites on flanks, neck, thighs and base of tail, was almost completely ineffective, thus confirming the superiority of the plantar surfaces of the feet as sites of inoculation[2] Fortification of the adjuvant increased the severity but not the incidence of experimental allergic encephalomyelitis Guinea pig spinal cord antigen was more effective than rat cord It produced moderate or severe lesions in most animals With this antigen, even an emulsion made with incomplete adjuvant was successful, confirming results obtained after injection of corresponding materials[1,3]

Table 1 EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN RATS PRODUCED BY TATTOOING ENCEPHALITOGENIC EMULSIONS

Site of tattoo	Spinal cord antigen	Adjuvant	EAE (histological) Incidence*	EAE (histological) Severity†
1 foot	rat	complete	5/10	0 9
1 flank site	rat	complete	0/10	0 0
10 skin sites	rat	complete	2/10	0 1
4 feet	rat	complete	5/10	0 4
4 feet	rat	fortified	4/10	1 1
4 feet	guinea pig	complete	8/10	1 8
4 feet	guinea pig	incomplete	8/10	0 9

* Numerator, number of rats with lesions, denominator, total number of rats

† Average severity of lesions, graded individually from 0 to 4

At time of death, 3 weeks after a single tattoo, the feet were macroscopically normal, but histological examination revealed a few inflammatory cells and granulomas Popliteal lymph nodes were enlarged 2–4 times normal and contained a few macrophages and epithelioid cell granulomas around oil drops The presence of oil drops (derived from the encephalitogenic emulsion) proved that tattooed inoculum is transported to lymph nodes, a fact already known in Virchow's time However, oil drops and inflammatory reaction in feet and lymph nodes following tattoo were inconspicuous compared with the changes following injection of 0 05 ml encephalitogenic emulsion This suggests that minute volumes of emulsion entered the tissues during tattooing Nevertheless, a high incidence of histological experimental allergic encephalomyelitis resulted It is probable that the fine state of dispersion of the inoculum facilitated efficient utilization of antigenic material In fact, the absence of a 'pool' of inoculum (always present after injections of oily materials) may prove advantageous in studies of experimental allergic encephalomyelitis when it is desired to eliminate the possibility of mobilization of antigen from the 'pool' at unpredictable times during the experiment

It has been stated that the function of oily adjuvant in development of hypersensitivity to aqueous nervous tissue antigen requires a stable water-in-oil emulsion[4] Therefore, our results may be taken as evidence that emulsion stability was not destroyed by the process of tattooing

This investigation was supported by grant No 184–4 from the National Multiple Sclerosis Society

SEYMOUR LEVINE
EUGENE J WENK

Department of Pathology,
St Francis Hospital,
Jersey City, New Jersey

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Use of Fungizone and Polymyxin *B* in Isolation of Naturally Infected Tissues

TISSUES from lung, urogenital, and intestinal tract which cannot be obtained sterile represent a problem to the isolation and establishment of cell lines

In many instances it was not possible to isolate cell lines due to the overwhelming contamination which naturally occurs in these organs In order to overcome this difficulty, the regular procedures of cell isolation were adapted to the special needs

The tissues from lung cervix and other contaminated material were washed three times in Eagle's medium and 20 per cent pooled human serum The following antibiotics were added

Streptomycin sulphate	100 μgm /ml	(Lilly)
Penicillin *G*	100 U /ml	(Squibb)
Neomycin sulphate	160 U /ml	(Upjohn)
Polymyxin *B* sulphate	50 U /ml	(Burroughs-Welcome)
Fungizone-amphotericin *B*	0 02 mgm /ml	(Squibb)

The tissue was then put into new medium with the antibiotics in the same concentration as indicated above and stored overnight at 4° C The tissue was removed from the solution, minced, and trypsinized in a Bellco spinner flask (Bellco Glass Co , Vineland, New Jersey) on a magnetic mixer at room temperature for 30–60 min The trypsinized suspension was centrifuged for 5 min at 1,500 r p m The trypsin was decanted The cells were suspended in Eagle's medium with 20 per cent pooled human serum, streptomycin[1], penicillin[2], neomycin[3], polymyxin *B*[4], and fungizone[5], and then distributed in 10 ml aliquots into 8-oz milk dilution bottles at the desired concentration Polymyxin *B* and fungizone were continually added to the medium for at least one month It was noted that omission of polymyxin *B* and fungizone from the medium at an earlier date was followed by contamination of the primary sterile culture after the first transfer

It was possible to isolate a cell line derived from a normal cervix, a carcinoma *in situ*, an invasive carcinoma of the cervix, and several carcinomas from the intestinal tract The isolation of a cell line from a gastric lesion was not achieved If a carcinoma of the stomach was washed three times and trypsinized immediately, the culture was heavily contaminated However, if stored overnight at 4° C , no growth occurred Also the growth of preputial tissue has been improved by this method (Dr R G Brackett, Parke, Davis and Co)

In case of contamination of an established cell line with moulds or pseudomonas, the following procedures were instituted The medium was removed The cells were washed twice daily with 10 c c BSS containing double strength of the appropriate antibiotic (fungizone or polymyxin *B*) by rotating the bottle gently The solution was then decanted and new medium added containing 0 04 μgm /ml fungizone and polymyxin *B* 100 U /ml , depending on the type of micro-organism present The infection usually cleared up within 2–3 days by this method Neither the polymyxin *B* nor the fungizone in the concentrations used showed any toxic effect on the cells Since we have adopted this method, we have lost very few cell lines

This work was supported by a grant from the U S Army Medical Research and Development Command *DA*-49-007-*MD*-908 and the Michigan Cancer Foundation

H Schneider
J R Brown
E. E Muirhead

Department of Pathology,
Wayne State University College of Medicine,
Woman's Hospital,
Detroit, Michigan

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* Present address Woman's Hospital, 432 E Hancock, Detroit 1, Mich

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IMMUNOLOGY

Simultaneous Intradermal Tuberculin Tests

Rosenthal and Libby[1] have shown that the tuberculin reaction to a 10 T U test is reduced by a simultaneous 100 T U test They suggest that there is competition for the available antibody Stone and Bruce[2] and Bruce[3] assumed, implicitly, that simultaneous tests are independent and, explicitly, that there is a constant antibody level λ throughout an intradermal reaction Both assumptions were inessential and the following extension provides a quantitative treatment of simultaneous test data

The equation[2]

$$D^2 = \bar{\alpha} V \log(4\, d \log_e 10/\pi\bar{\alpha}\tau V) \qquad (1)$$

[where $\bar{\alpha}$ = the clinician's constant, D = induration diameter (mm), V = injection volume, d = tuberculin dose (T U) and τ = minimum areal concentration of tuberculin for induration (T U /mm ^{2})] is unaffected by competition between test sites, if it can be assumed that λ, though decreasing with time, is, at any given time, the same at all sites Since a circulating antibody hypothesis is presumably necessary for competition, this assumption is reasonable

Let $\lambda(T)$ be the antibody-level at time T after injection Suppose any tuberculin that escapes into the bloodstream without local reaction quickly reacts there with antibody Also suppose that, if $d(T)$ is the total unreacted dose in all sites at time T

$$\lambda(T) = \lambda_0 - \theta[d_0 - d(T)] \qquad (2)$$

for T 'such that $\lambda(0) = \lambda_0 > \theta[d(0) - d(T)]$ Due to escape and local reaction

$$d'(T) = -\varepsilon d(T) - \omega\lambda(T)d(T) \qquad (3)$$

With $a = \varepsilon + \omega\lambda_0 - \omega\theta d_0$, (2) and (3) give

$$\lambda(T) = \lambda_0 - \theta d_0 + \frac{a\theta d_0 e^{-aT}}{a + \omega\theta d_0(1 - e^{-aT})} \qquad (4)$$

for T such that (4) is positive Let $t(T)$, $r(T)$ be the areal densities of unreacted tuberculin and of reaction products at a particular point of a site Then

$$t'(T) = -\varepsilon t(T) - \omega\lambda(T)t(T) \qquad (5)$$

$$r'(T) = \lambda(T)t(T) \qquad (6)$$

(4), (5), (6) give $r(\infty)$ for the cases $\lambda_0 \lessgtr \theta d_0$, and, if induration just occurs when $r(\infty) = \rho$

$$\rho\omega\tau^{-1} = 1 - \frac{\varepsilon}{\omega\theta d_0}\log_e\left(\frac{\varepsilon + \omega\lambda_0}{\varepsilon + \omega\lambda_0 - \omega\theta d_0}\right) \qquad (7)$$

if $\lambda_0 > \theta d_0$, and

$$\rho\omega\tau^{-1} = \frac{\lambda_0}{\theta d_0} - \frac{\varepsilon}{\omega\theta d_0}\log_e\left(\frac{\varepsilon + \omega\lambda_0}{\varepsilon}\right) \qquad (8)$$

f $\lambda_0 < \theta d_0$

When $\omega\lambda_0/\varepsilon$ is small, that is, if most of the tuberculin escapes local reaction, (7) and (8) simplify to

$$\varepsilon\rho\tau^{-1} = \lambda_0 - \tfrac{1}{2}\theta d_0 \qquad (9)$$

if $\lambda_0 > \theta d_0$, and

$$\varepsilon\rho\tau^{-1} = \tfrac{1}{2}\lambda_0^2/\,\theta d_0 \qquad (10)$$

if $\lambda_0 < \theta d_0$

With τ determined by (1), equations (9) and (10) are testable even though λ_0 (and possibly $\varepsilon\rho$ and θ) will vary from subject to subject Evidence that $\omega\lambda_0/\varepsilon$ is usually small is provided by data previously reported[2] 39 subjects, vaccinated with a range of *BCG* doses, and tested with 100 T U , gave the results (1) the τ's of subjects who received the same *BCG* dose were close, (2) the whole range of τ's was $10^{-0\cdot5}$–10^{-8}; (3) with higher *BCG* doses, τ's down to 10^{-10} would probably have been attainable With the assumption that the value of λ_0 corresponding to $\tau = 10^{-8}$, say $\lambda_0{}^*$, obeyed $\lambda_0{}^* > \theta d_0$, it can be verified that these results imply $\omega\lambda_0{}^*/\varepsilon < 1/50$ and, *a fortiori*, $\omega\lambda_0/\varepsilon \ll 1/50$ for the lower antibody-levels

An immediate prediction of this model is that, for example, the reduction in size of a 10 T U reaction in association with a 100 T U test should be the same as the reduction of a 10 T U reaction in association with two 50 T U tests For the two cases have the same total dose

M Stone

Department of Statistics,
University College of Wales,
Aberystwyth

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Fate of Attenuated Tubercle Bacilli (*BCG*) in Germ-free and Conventional Mice

In the course of a study of hyper-reactivity to endotoxin of mice infected with *BCG*, the use of germ-free mice was indicated[1] Since little information is available concerning the response of germ-free animals to infectious agents, especially *Mycobacteria*, the fate of *BCG* in these animals was followed carefully and was compared with that in conventional animals of the same strain

Germ-free mice were obtained from the Lobund Institute, Notre Dame University, Notre Dame, Indiana, and were shipped in a specially constructed germ-free isolator They were transferred to an isolator in the laboratory and kept in the germ-free state (except for those infected with *BCG*) until termination of the experiment. Conventional animals were of the same strain and age. Quantitative estimation of the microbial population in the spleen was obtained by determining the number of viable units of *Mycobacteria* which could be recovered from the triturated organs on Loewenstein–Jensen medium. The tissue reaction to the infection was assayed from histological sections of major organs, especially lymph nodes, lungs, liver and spleen. All determinations were done 12 days after intravenous injection of approximately 5 × 10^6 viable units of *BCG*.

The numbers of recovered organisms from individual spleens are recorded in Table 1. Although there may be some indication, though statistically insignificant, of a difference between the means of the two groups, individual animals cannot be placed in either the germ-free or control group on the basis of the mycobacterial load found in the spleens after intravenous infection with *BCG*.

Table 1. RECOVERY OF *BCG* FROM SPLEENS OF CONVENTIONAL AND GERM-FREE MICE

Mouse No.	No. of viable units × 1,000 per 200 mgm. tissue Germ-free	 Conventional
1	0	0·7
2	1·4	1·8
3	4·2	3·0
4	5·3	3·7
5	5·4	3·7
6	5·6	3·9
7	6·4	4·6
8	6·7	5·1
9	6·7	5·1
10	7·2	5·5
11	8·0	5·6
12	8·8	5·6
13	9·7	8·0
14	9·9	8·0
15	12·0	8·3
16	12·0	
17	14·0	
$\bar{x} \pm S.E.$	7·3 ± 3·7 *P* 0·02	4·8 ± 2·2

The cellular response to infection with *BCG* was similar in germ-free and in normal animals. Enlargement of the spleen was accompanied by accumulation of large mononuclear cells with frequent giant cells in spleen and lymph node tissues. Similarly, the number of Kupffer cells in the liver was increased, and many foci of mononuclear cells were present. There was no evidence of necrosis in animals of either group.

The few studies concerned with resistance of germ-free animals to bacterial infection have, in general, shown that these animals are more susceptible than those with a normal intestinal flora. This was the case with infection with *Shigella flexneri* in the guinea pig[2] and with *B. anthracis* in the rat[3]. In the former, the altered resistance is explainable by the absence of bacterial antagonism in the intestinal lumen rather than by decreased reactivity of the tissues. Germ-free animals can be protected against this infection simply by a preceding oral feeding with *Escherichia coli* but not with *Lactobacillus*[4]. A deficiency of the tissue response is assumed to be responsible for the increased pathogenicity of anthrax spores for germ-free rats since the spores are deposited in the tissues. This may be due to the fact that germ-free rats do not contain antibacterial agglutinins or only in very low titre, as against *Staph. epidermidis*[5]. On the contrary, germ-free mice have a relatively high titre of bactericidal antibody against *Escherichia coli*[6]. In addition, the uptake of bacteria by the reticulo-endothelial system of germ-free mice is deficient. This is attributed to the absence of specific opsonins[7].

The results presented indicate that the reticulo-endothelial system of germ-free mice is competent to deal with infection with *Mycobacteria*, possibly because no specific opsonin is necessary for phagocytosis. Furthermore, the capacity to respond with hyperplasia to the presence of tubercle bacilli appears equally well developed in germ-free as in normal mice. The experiments suggest that the germ-free animal represents an excellent tool for the study of natural resistance.

This work was supported by grants *E*–3062 (*A*) and *E*–3789 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

EMANUEL SUTER
EUGENIA M. KIRSANOW*

Department of Microbiology,
College of Medicine,
University of Florida,
Gainesville

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* Medical student trainee supported by grant 2*E*-128 from the National Institute of Allergy and Infectious Diseases of the U.S. Public Health Service.

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BIOLOGY

Water-soluble Polysaccharides of *Porphyra* Species : a Note on the Classification of *P. naiadum*

THE water-soluble mucilages which are present in copious amounts in the red seaweeds (Rhodophyta) are usually sulphated polysaccharides containing galactose, 3,6-anhydrogalactose and, more rarely, 6-*O*-methylgalactose units. These polysaccharides are by no means identical in all red seaweeds, and it has been suggested[1] that the composition of the mucilage might provide a useful additional criterion in classification within the group. We have now isolated and analysed the water-soluble polysaccharides from some fifty different collections of the genus *Porphyra*, and this communication reports the bearing of our results on the classification of the alga hitherto known as *P. naiadum* And.

It is already known[2,3] that the water-soluble polysaccharides of certain *Porphyra* species contain residues of D-galactose, L-galactose, 3,6-anhydro-L-galactose, 6-*O*-methyl-D-galactose and ester sulphate, and that the relative proportions of some of these components vary from sample to sample. It is further known[4,5] that the sulphate ester of the purified polysaccharide (porphyran) from *P. umbilicalis* f. *laciniata* harvested in June is largely present as 1,2- or 1,4-linked L-galactose 6-sulphate. This ester is hereafter called 'precursor sulphate' since it can be transformed into 3,6-anhydrogalactose through the mediation of an enzyme present in the alga[6].

For these investigations a number of *Porphyra* species was collected from sites on the west coast of Scotland and the Pacific coast of North America. The British species[7] included *P. umbilicalis* (L.) Kutz. f. *umbilicalis* and f. *laciniata* (Lightf.) J. G. Ag., *P. linearis* Grev. and *P. leucosticta* Thur. ; the Pacific species[8] were *P. perforata* J. G. Ag., *P. schizophylla* Hollenb. and *P. naiadum* And. Specimens were extracted exhaustively with boiling water, and the extracts dialysed, freeze-dried and analysed for galactose and 6-*O*-methylgalactose by quantitative paper chromatography following complete acidic hydrolysis[9], for 3,6-anhydrogalactose by a colorimetric method[10], for ester sulphate by a spectrophotometric method after destruction of organic matter with nitric acid[11], and for 'precursor ester sulphate' by measurement of the 3,6-anhydrogalactose formed on treatment with sodium hydroxide using conditions under which the polysaccharide is protected from degradation[5]. With the exception of the materials from *P. naiadum*, all the polysaccharide extracts were of similar composition, that is, they contained (i) 6–12 per cent of ester sulphate, most of this being of the 'precursor' type, (ii) 5–20 per cent of 3,6-anhydrogalactose, (iii) usually 9–27 per cent of 6-*O*-methylgalactose, although the amount of this component was occasionally as low as 3–4 per cent, (iv) about two molar proportions of galactose to every one of ester sulphate, and (v) xylose (if at all) in very small quantity. Four samples of *P. naiadum* were examined; one from the Monterey Peninsula, California, one from Friday Harbor, San Juan Islands, Washington, and two from Vancouver Island. These showed mutual resemblance, while differing from the other species, in that they contained (i) 16–20 per cent of ester sulphate, only about one-fifth of which was of the 'precursor' type; (ii) little 3,6-anhydrogalactose (< 3 per cent); (iii) no detectable amount of 6-*O*-methylgalactose (limit of detection about 2 per cent); (iv) less galactose on a molar basis than ester sulphate; (v) 10–20 per cent of the sugar units as xylose (identified by paper chromatography only).

Hollenberg[12] suggested, on other evidence, that there are sufficient grounds for removing *P. naiadum* to a different genus, for which he has proposed the name of *Smithora*. The results described here, namely, that *Porphyra naiadum* differs in certain of its carbohydrate components from other species of *Porphyra*, appear to support this suggestion.

D. A. REES

Department of Chemistry,
University of Edinburgh

E. CONWAY

Department of Botany,
University of Glasgow

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Detoxications of Aromatic Acids with Glutamic Acid and Arginine in Spiders

THE presence of detoxication mechanisms in insects is now well known, and some of these are of significance in the phenomena of resistance to, and selective action of, insecticides[1]. Arthropods, other than insects, are also the object of attack by insecticides for medical or economic reasons, but virtually no information is available on the fate of such foreign organic compounds in these other invertebrates.

Table 1. R_F VALUES AND IONOPHORESIS OF SOME AROMATIC ACIDS AND METABOLITES

	R_F values * in A	B	C	Apparent migration (cm.)†
p-Aminobenzoic acid	0.30	0.85		+ 9.1
p-Aminohippuric acid	0.30	0.68		+ 9.0
p-Aminobenzoylglutamic acid	0.12	0.73		+10.8
p-Aminobenzoylglutamine	0.22	0.45		+ 6.3
p-Aminobenzoylarginine	0.22	0.35		− 1.5
p-Carboxyphenylsulphamic acid	0.29	0.29		+14.0
p-Nitrobenzoic acid	0.73		0.62	+ 9.5
p-Nitrohippuric acid	0.63		0.39	+ 7.1
p-Nitrobenzoylglutamic acid	0.34		0.05	+11.5
n-Nitrobenzoylglutamine	0.60		0.31	+ 4.9
p-Nitrobenzoylarginine	0.70		0.50	− 4.0

* *A*, *n*-Propanol/ammonia s.g. 0.88, 7 : 3; *B*, *n*-butanol/acetic acid/water (4 : 1 : 5); *C*, *n*-butanol/ammonia s.g. 0.88/water (4 : 1 : 5).

† In 0.02 *N* sodium hydroxide for 2 hr. at 10 V./cm.

It was of interest, therefore, to look for detoxication mechanisms in arthropods and compare them with analogous processes in other organisms. *p*-Amino- and *p*-nitro-benzoic acid have been used as test substances since their fate in other animals is well known, and conjugates of these compounds may be easily detected by their reaction with dimethylaminobenzaldehyde, either directly or after treatment with titanous chloride.

When house spiders (*Tegenaria* sp.) were injected with *p*-aminobenzoic acid and their excreta examined on paper chromatograms, three major metabolic products could be detected. One of these was identified as *p*-carboxyphenylsulphamic acid by its delayed action with dimethylaminobenzaldehyde and acetic acid, by its identity with the synthetic reference compound on paper chromatography, and by comparison with the reference material on ionophoresis (Table 1). Acid hydrolysis of eluted zones gave SO_4^{2-} and *p*-aminobenzoic acid.

A second metabolite was thought to be *N*-*p*-aminobenzoylglutamic acid, since it had identical behaviour with synthetic reference material on paper chromatograms and on ionophoresis. Eluates of paper chromatograms showed an absorption maximum at 272 mμ in 0.1 *N* sodium hydroxide, similar to that of *p*-aminobenzoic acid and its conjugates. Acid hydrolysis of such eluates gave *p*-aminobenzoic acid and glutamic acid. Measurement of the fluorescence–*p*H curve[2] gave an apparent pKa_1 of 3.2, identical with that of authentic *p*-aminobenzoylglutamic acid, but differing from those of *p*-aminobenzoylglucoside (1.9) and *p*-aminobenzoic acid (4.5) determined similarly.

A third metabolite is believed to be α-*p*-aminobenzoylarginine, since it had identical behaviour with this compound on electrophoresis and on paper chromatograms. It gave an intense Sakaguchi reaction, and acid hydrolysis of eluates of chromatograms yielded *p*-aminobenzoic acid and arginine. These eluates had a light absorption with a maximum at 272 mμ in 0·1 *N* sodium hydroxide.

Small amounts of *p*-aminobenzoylglutamine were also detected in the excreta by paper chromatography and ionophoresis.

In similar experiments with *Tegenaria*, in which *p*-nitrobenzoic acid was injected, conjugates of glutamic acid, arginine and glutamine with *p*-nitrobenzoic acid were also excreted and identified as above. No reduction of the nitro group was observed.

Other spiders (*Agelena, Lycosa, Epeira* sp.) yielded similar conjugates when dosed with *p*-aminobenzoic acid, but relatively greater amounts of the glutamine conjugates were excreted by *Epeira*, the large garden spider.

A variety of amino-acids may be encountered in the peptide conjugation of foreign aromatic acids[3], but the most widely found reaction is with glycine to form hippuric acids These take their general trivial name from benzoylglycine (hippuric acid), which was first found in the urine of horses A similar process leads to the formation of ornithine derivatives, the so-called ornithuric acids, in some birds[4] Using similar terminology, the excreted glutamic acid conjugates in spiders could be termed arachnuric acids

Experiments (unpublished observations) with other arthropods dosed with *p*-aminobenzoic acid have given no indication of the presence of glutamic acid conjugates Millipedes, centipedes and harvest spiders dosed with *p*-aminobenzoic acid yield a product chromatographically similar to α-*p*-aminobenzoylarginine, and the crustacean woodlouse (*Armadillidium* sp) converts *p*-aminobenzoic acid to the corresponding glycine derivative

J N SMITH

Department of Biochemistry,
St Mary's Hospital Medical School,
London, W 2

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Reversible Inactivation of Acid Phosphatase in Human Prostatic Fluid

FOLLOWING our demonstration that the 'acid phosphatase', in human prostate homogenates, is incompletely and reversibly inactivated by incubation in the presence of calcium ions[1–3], we have confirmed the same reversible inactivation in the fluid obtained by massage of the prostate of individuals with normal prostates The methods used have been described in our previous publications Table 1 gives the results obtained in nine experiments on nine different prostatic fluids. The results are expressed as a percentage of a calculated initial value of 100 As compared with the original values, incubation in the absence of calcium ions resulted in little inactivation (mean 11 4) while, in the presence of calcium ions, inactivation amounted to a mean value of 52 6 per cent When the concentration of calcium ions was increased to 12–18 mgm per cent, inactivation was increased to a mean value of 81 9 per cent

At the end of the incubation in the presence of calcium ions, the partially inactivated samples were deprived of calcium ions by the addition of 'Versene' and further incubated for a period of 60 min This resulted in a partial but definite regeneration of the acid phosphatase activity, which rose from a mean of 18 1 per cent of the original activity to a mean value of 58 5 per cent It is possible that regeneration might proceed further if incubation were prolonged beyond 60 min

Table 1 PROSTATIC FLUID ACTION OF CA^{++} ON ACID PHOSPHATASE

Exp No	Initial value†	Values after incubation*			Reactivation‡ ('Versene')
		Ca^{++} free ('Versene')	Ca^{++} 2–8 mgm per cent	Ca^{++} 12–18 mgm per cent	
1	100 (432)	81	—	10	71
2	100 (5,150)	70	56	14	47
3	100 (1,130)	100	78	58	87
4	100 (4,400)	95	61	36	79
5	100 (6,220)	106	19	1	47
6	100 (1,200)	97	71	29	73
7	100 (3,530)	86	54	9	65
8	100 (3,130)	84	67	3	26
9	100 (3,970)	79	21	3	32
		Mean 88 6	Mean 47 4	Mean 18 1	Mean 58 5

* 90 min at 37° C

† The number between parentheses is the actual value, in Bodansky units per ml

‡ 60 min at 37° C after inactivation

These results confirm the fact of the reversible inactivation by calcium ions of the prostatic acid phosphatase previously established under different experimental conditions and indicate that this inactivation can be produced in normal prostatic fluid as well as in homogenates of the organ

This work was supported in part, by Grant No *DRG–264A(T)* from the Damon Runyon Memorial Fund for Cancer Research, Inc , New York, and by the ' Fondation Christiaens", Brussels (Belgium)

A STEENS-LIEVENS
H J TAGNON
Service of Medicine and Clinical Investigation,
Institut Jules Bordet,
Centre Anticancéreux de l'Université de Bruxelles

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Stimulation of Cell Locomotion and Pseudopod Formation by Heparin

IT has been suggested[1,2] that mucopolysaccharides play a part in cell surface extension and mobility During an investigation of pseudopod formation and chemotaxis in *Amoeba proteus*[3] it has been found that heparin (U S P , Light's Chemical Co) can stimulate the formation of pseudopods when a fine pipette containing 3 × 10^{-5} molar heparin, dialysed against Chalkley's medium, is held near an amœba (Fig 1 *A–E*) Calculation shows that under these conditions the initiating concentration when the amœba is 50μ away from the pipette is approximately 5 × 10^{-7} molar When the pipette contains 10^{-4} molar heparin active pseudopods are quickly inhibited and the amœba reverses away from the pipette Pipettes containing Chalkley's medium, agar, or various concentrations of sucrose do not stimulate or inhibit pseudopod formation There is a mechanical inhibitory effect if fluid is passing rapidly out of the pipette, but this is avoided by using fine pipettes, 10–15μ in diameter, or by making up the solutions in 0 5 per cent agar jelly Glass pipettes held within 1 or 2μ of an amœba will often induce blebs but these do not develop into pseudopods The stimulation of pseudopods by heparin is effective at pipette to amœba distances of the order of 50μ The material in the pipette reaches the amœba by diffusion and not by ejection

It has recently been shown that the anterior portion of an advancing amœba is depolarized, and this fact has been incorporated in a theory of amœboid movement[4]. In an investigation, to be reported elsewhere[5], it has been found that heparin and some other natural mucopolysaccharides also depolarize the amœba membrane, and it is likely that this is the basis of pseudopod stimulation by heparin A derangement of mucopolysaccharide metabolism has been suggested as possibly implicated in malignancy[1] and it may be that malignant cells are those cells the surface membranes of which are by reason of a modification of their composition more easily depolarized by compounds such as heparin normally occurring in body fluids Leucocytes would be cells easily depolarized by heparin-like substances in their natural environment There seem to be two alternative mechanisms

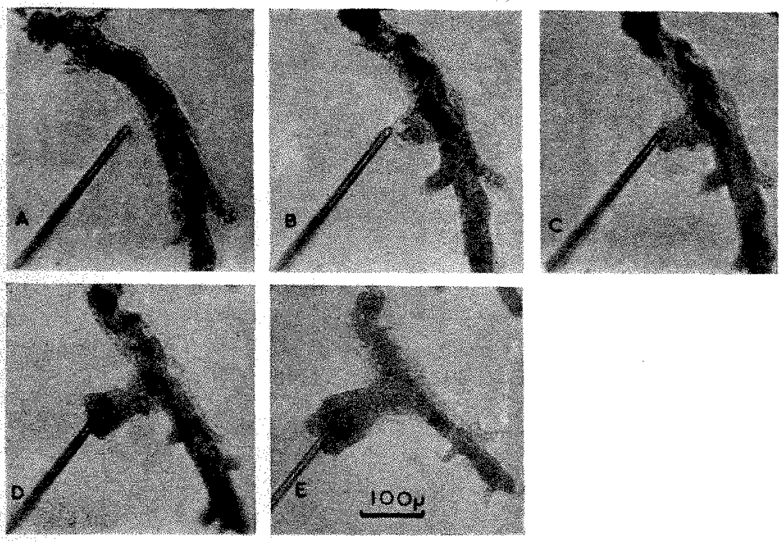

Fig. 1. *A–E*, *Amoeba proteus* in Chalkley's medium. The pipette contains 3×10^{-4} molar heparin, dialysed against Chalkley's medium

(*a*) Normal cells are inhibited by the normal physiological concentrations of heparin-like substances, and malignant cells are less sensitive and so are not inhibited by the normal concentrations. (*b*) The normal level of heparin-like substances is not sufficient to stimulate movement in normal cells, but is sufficient to stimulate the more sensitive malignant cells.

The facts and analysis presented here suggest that compounds such as basic proteins or basic polypeptides which combine with the active groups of mucopolysaccharides may be effective in inhibiting cell movement. This possibility is being investigated in this laboratory. Further work is in progress in the hope of elucidating the action of heparin on pseudopod formation and locomotion.

L. G. E. Bell
K. W. Jeon*

University of London,
King's College,
Strand, W.C.2.

* British Council Scholar.

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Species of Siphonaptera from a British Otter

Siphonaptera have been recorded from the majority of the British wild mammals[1,2]. However, there are several notable exceptions and these are the Cetacea; the Pinnipidea; the Ungulata, with the exception of *Dama dama* L., and the following species: *Myotis bechsteini* Kuhl, *Myocastor coypus* Mol. and *Lutra lutra* L.

On the evening of August 24, 1961, a dead female otter (*Lutra lutra* L.), which was still warm, was found on the road at Faoilean, near Torrin, Isle of Skye. It had been killed presumably by a motor vehicle. The animal was placed in a new polyethylene bag which was then sealed. Because of various circumstances it was not examined until three days later, when a single dead flea was found on the inside of the bag. This flea was a female specimen of *Spilopsyllus cuniculi* Dale. So far as is known, this is the first time that a flea has been collected from a British otter. The principal host of this flea is *Oryctolagus cuniculus* L., although it has been recorded on many other wild mammals including: *Apodemus sylvaticus* L., *Arvicola terrestris* L., *Clethrionomys glareolus* Miller, *Dama dama* L., *Felis silvestris* Miller, *Lepus europaeus* de Winton, *Mustela erminea* Barrett Hamilton, *Mustela putorius* Pocock, *Sciurus carolinensis* Gmelin, *Sorex araneus* Jenyns and *Vulpes vulpes* Bechstein.

The absence of fleas on the Cetacea, which frequent the open seas and are only found on dry land when stranded, is understandable. This is more or less true for the Pinnipidea, because even though they frequent the shores of Britain they never travel far from the sea when on the land. However, it is quite likely that they harbour fleas from time to time. The absence of records from all but one of the Ungulata, and the three species mentioned here, are no doubt because of the lack of investigation, due to various reasons such as rareness or inaccessibility of the species. In time, no doubt, various species of Siphonaptera will be found on all these mammals.

The flea has been placed in the British Museum (Natural History).

Donald I. Chapman

38 Horn Lane,
Woodford Green,
Essex.

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Culture Methods as an Aid to Experiment on Reptile Embryos

Little progress has been made in the application of experimental techniques to reptilian embryology. Panigel[1], however, found that the eggs of the common lizard (*Lacerta vivipara*) could be removed from the mother and cultured in Petri dishes containing cotton wool moistened with saline. He also found that development was more rapid at 27° C. than at room temperature. We have been able to extend these observations and to show that the method has promising applications to future experimental work.

Pregnant females of *Lacerta vivipara* usually contain 5–8 fertile eggs. In 1961 we obtained a total of 122 eggs from lizards taken in the mating season (late April to early June). Of these 93 developed successfully in culture and were kept at various temperatures for periods of 5–40 days. The youngest embryos were at a stage of development corresponding approximately to that of a chick embryo at stage 19 (3–3½ days) in the table given by Hamilton[2] when culture began. Because we wished to investigate several different embryological problems, the embryos were treated in a variety of ways so that an accurate statistical analysis of temperature effects is not possible. The following examples, however, illustrate the effect of high and low temperatures on the rate of development.

First, sibling embryos *a*, *b*, *c*, *d* and *e* were put up in culture at a stage comparable with a stage 19 chick. *a* was cultured for 11 days at 4° C. and 1 day at room temperature (21°–23° C.), and showed no appreciable development: whereas *b*, cultured for 13 days at room temperature, reached the equivalent chick stage 27: *c* and *d* were both cultured for 21 days; *c* was kept at room temperature throughout, but *d* was kept for the first 11 days at 4° C. and then transferred to room temperature: *c* and *d* reached equivalent chick stages of 34 (8 days) and 26 (5 days) respectively: *e*, cultured at room temperature for 40 days, was fixed just before hatching.

Secondly, sibling embryos *f*, *g*, *h*, *i*, *j* and *k* were put up at a stage comparable with chick stage 21: *f*, *g* and *h*, cultured for 9, 23 and 30 days respectively at room temperature, reached equivalent chick stages of 29, 36 and 38: *i* showed no appreciable development after 9 days at 4° C., and *j*, also at 4° C., only reached the equivalent chick stage of 22 after as long as 23 days. On the other hand, *k* reached the equivalent chick stage of 34 after only 8 days of culture at 30° C.

Comparable results were obtained with several other batches of embryos. The 10 embryos kept for some period at 30° C. all showed accelerated development. Conversely, 20 embryos showed an almost complete arrest of development while they were kept at 4° C., although they survived at this temperature for periods of up to 29 days. When some embryos were transferred to room temperature apparently normal development ensued. This finding may have practical significance, since it suggests that large numbers of embryos can be stored at a minimal rate of development until it is convenient to operate on them.

It was found both by Panigel and ourselves that even in embryos kept at room temperature there was considerable mortality around the time of hatching, although up to this point few deaths occurred. None of our embryos kept at 30° C. succeeded in hatching, although some reached near-hatching stages. A few embryos were also kept at 33°–35° C. These failed to survive for longer than about 10 days, but with more stringent precautions against dehydration longer survival might have been obtained.

Operations involving the cutting off of a portion of tail with fine scissors, after making a small cut in the thin shell membrane and underlying embryonic membranes, were carried out on 16 embryos kept at room temperature. These embryos were all at a comparatively late stage of development equivalent to chick stages of 23 (4 days) or later. Only 2 embryos died as an apparent direct result of the operation, the remainder being fixed 5–27 days afterwards. Some of these prolapsed through the rent in their membranes, which appeared to form adhesions with the surface of the body.

In certain cases the results of the operation were difficult to interpret and the tissues near the lesion were disorganized. In other embryos no such complications ensued. None of the embryos showed any obvious signs of tail regeneration although the wound had healed. In some, however, histological examination revealed certain features suggestive of those described in early tail regenerates of geckos by Hughes and New[3]. The apparent lack of tail regeneration in our specimens is of interest and deserves further investigation. It may have been due simply to inadequate survival time, but there are other possible explanations; for example, the power of tail regeneration so characteristic of the adult may not develop until post-natal life.

It was also found possible to culture the eggs of the slow-worm (*Anguis fragilis*), another viviparous species[4]. Temperatures of about 18° C. seemed to be most suitable for their maintenance. Removal of part of the tail was also carried out on 3 late slow-worm embryos; these showed no obvious external signs of regeneration a month after the operation but have not yet been studied histologically.

These observations suggest that Panigel's technique offers a new approach to the problems of experimenting on reptile embryos, and particularly to the study of morphogenesis and regeneration during the later stages of development.

P. F. A. MADERSON
A. D'A. BELLAIRS

St. Mary's Hospital Medical School,
London, W.2.

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Bivalved Gastropod from Jamaica

THE bivalved gastropod *Berthelinia* Crosse (= *Tamanovalva* Kawaguti and Baba) has been reported from Japan[1], Australia[2], and California[3]. A second genus, *Midorigai* Burn, has been described from Australia. The purpose of this communication is to record *Berthelinia* from Jamaica. The first specimen was found on *Caulerpa verticillata* Agardh growing on rocks in an open part of a mangrove channel near Port Royal on January 8, 1962. Small quantities of algæ collected on January 11 and 16 yielded fifty more specimens, so in this small area *Berthelinia* can be described as common. To my knowledge, this is the first time that living bivalved gastropods have been taken from the Atlantic.

The shell is almost identical with that of *B. typica* Gatliff and Gabriel[4]. The present species certainly belongs to this genus, although further work is necessary to decide whether or not it is distinct from those species already described.

I thank Prof. D. M. Steven and the staff of the Department of Zoology, University College of the West Indies, for provision of laboratory facilities, and the Department of Scientific and Industrial Research and the Committee for Commonwealth University Interchange for funds while in Jamaica.

MALCOLM EDMUNDS

Department of Zoology,
University Museum, Oxford.

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Occurrence of *Cystoseira tamariscifolia* (Huds.) Papenf. on the West Coast of Scotland

Cystoseira tamariscifolia (Huds.) Papenf. is generally regarded as characteristic of warmer seas, being particularly abundant on the Atlantic shores of Europe and North Africa and in the Mediterranean[1]. In the British Isles it is frequent on the south-west of England and on the south and west coasts of Ireland[2], extending on occasion to Co. Donegal[3] and Co. Antrim[4]. It has rarely been recorded from Scotland: early records exist from the coasts of Ayrshire and

Kintyre[5] while from the Island of Barra in the Outer Hebrides a single specimen in the drift[6] and one or two dredged specimens[3] have been recorded.

While making a survey of the inter-tidal algae of Barra in July 1961, particular attention was paid to the presence of *Cystoseira*, and near Cliad on the west coast of the Island an abundant growth of *Cystoseira tamariscifolia* was found. The plants were growing in deep tidal pools at a level near mean low water of spring tides and were associated mainly with *Codium fragile* subsp. *atlanticum* and *Halidrys siliquosa*. They ranged from 10 to 25 cm. in height, formed bush-like tufts in the water and showed the marked iridescence normally associated with this species. Several days later, following a severe gale, large quantities of *Cystoseira tamariscifolia* were found in the drift at many localities on the west coast of the Island, suggesting that an abundant growth may occur at sub-littoral levels.

In view of the range in size and the numerous receptacles found on the plants it is likely that this species is vigorously multiplying and that it is present on other Hebridean Islands. This latter suggestion is supported by the finding of fresh drift specimens of this alga on the west coast of Rhum[7] in August 1961. Any further records of *Cystoseira tamariscifolia* from Scotland will be welcomed.

I thank Dr. Helen Blackler of the British Phycological Society's Check-list Committee for confirming my identification of the species.

W. D. P. Stewart

Department of Botany,
University of Nottingham.

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An Annelid Proprioceptor

The structure of the worm *Harmothöe* is in most respects typical of errant polychætes; it has well-developed parapodia armed with bristles and provided with dorsal and ventral cirri, but on the dorsal side of the worm are scales (elytræ) which most polychætes do not have. Methylene blue preparations reveal the following types of sense organs: (*a*) numerous small bipolar cells of ventral cirri and of the elytræ; (*b*) a group of bipolar cell bodies with branched endings in the region where the bristles emerge from the parapodial cuticle; (*c*) numerous and scattered bipolar cells beneath the cuticle particularly on the ventral side of the body; (*d*) bipolar cells of the elytræ; (*e*) concentrated groups of bipolar cells standing at right-angles to the surface in the thickness of the epithelium; (*f*) several bipolar cells each with a long dendrite stretched diagonally across the ventral side of every parapodium. Types (*a*), (*c*) and (*e*) are described by Smith[1].

No other type of peripheral neurone is found and each of the peripheral sensory cells appears to send an axon the whole way to the central nervous system. There is no trace of a peripheral nerve net or running together of axons.

Recording externally from the sensory axons reveals that types (*b*), (*c*) and (*f*) include mechanoreceptors. Types (*a*), (*d*) and (*e*) have not been investigated because of technical difficulties in recording their responses. The group (*b*) are sensitive to any touch of the bristles and respond with an adapting discharge. Of the type (*c*) cells scattered over the body, many, and perhaps all, respond to touch or strain of the cuticle. Most notable of all, large bipolar cells (*f*) respond to strain of the cuticle of the base of the parapodium and hence to any parapodial movement, passive or active. They are therefore proprioceptors. Their responses are readily recorded as large spikes after eliminating the bristle responses by pulling out the bristles. A mechanical deformation of 100μ can be applied by a small stylus on the region occupied by the dendrite of the bipolar cell shown in Fig. 1. A maintained sudden stretch of the dendrite causes a discharge of impulses in the axon of the long bipolar cell, with adaptation over the course of a few seconds. The impulses are thus identified with the (*e*) neurones. The size and regular arrangement of the latter in every segment also suggest their function as proprioceptors.

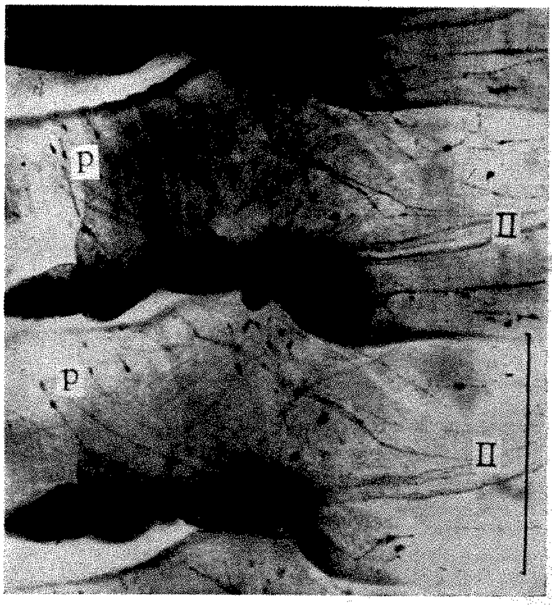

Fig. 1. Bipolar proprioceptor cells (*p*) lying in the cuticle across the ventral side of two adjacent parapodia of *Harmothöe*. Some of the axons of the parapodial nerve (II) are also shown. Anterior is at the top. Scale, 1 mm.

A proprioceptor which adapts so quickly can scarcely be effective in co-ordination of the initial direction of the movement when a resting animal begins to move. One can readily observe how a worm modifies its parapodial posture to suit the irregularities of the substrate, but no evidence has ever been produced to suggest that the basic sequence of locomotory movement is co-ordinated by proprioceptors in polychætes. It has been found that injection of liquid paraffin into the parapodial cavity, or local cuts in the area of the proprioceptors, alter neither the fundamental sequence of movement of the operated parapodium, nor its relationships with the movements of neighbouring segments. The conclusion is therefore that the main sequence of movements is centrally controlled, though it can be appropriately modified in detail by mechanoreceptors of the body wall including proprioceptors of the parapodia.

G. A. Horridge

Gatty Marine Laboratory and
Department of Zoology,
University of St. Andrews.

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ENTOMOLOGY

Artificial Feeding and Rearing of the Aphid, *Myzus persicae* (Sulzer), on a Completely Defined Synthetic Diet

SINCE the classic study of Hamilton[1] in 1935 on the artificial feeding of *Myzus persicae* (Sulzer), many attempts have been made to feed and maintain various aphid species on gels, or on liquids, sometimes under pressure, accessible to the insects via various natural and artificial membranes[2–8]. Although limited uptake of some fluid was demonstrated or could be inferred from slight increases in the survival of the aphids in these studies, no instances of prolonged survival, growth or of development have been reported.

We have also found that *M. persicae* ingests sugary fluids via a membrane. These solutions, with and without neutral red or phosphorus-32 as indicators of uptake, were presented to the aphids in simple cages or in choice-chambers, with the fluids above a membrane of stretched 'Parafilm'—a waxy material made by Marathon, American Can Co., Menasha, Wisconsin. We have been able to demonstrate greater rates of feeding, survival, and nymphal production of aphids fed on 10–20 per cent sucrose solutions, which were also those preferred by *M. persicae*, than on concentrations outside this range. Maintenance of body-weight, the production of salivary sheaths and of honeydew droplets were further found to be related to uptake.

In one experiment with 60 apterous adult *M. persicae* per treatment, the 50 per cent survival-time was 3·5 days on water only, 6·5 days on an 18 per cent sucrose solution and 13 days on a completely synthetic diet having the composition given in Table 1. The average number of nymphs produced per adult was 5 on water, 9 on the sucrose solution, and 19 on the nutrient diet.

Table 1. COMPOSITION OF THE LIQUID DIET FED TO *Myzus persicae*

L-Amino-acids and amides* (mgm.)		Vitamins* (mgm.)	
Alanine	100	Thiamin	2·5
Arginine	270	Riboflavin	2·5
Asparagine	550	Nicotinic acid	10·0
Aspartic acid	140	Pyridoxine	2·5
Cysteine	10	Folic acid	0·5
Glutamic acid	140	Calcium pantothenate	5·0
Glutamine	150	Meso-inositol	50·0
Glycine	40	Choline chloride	50·0
Histidine	40	Biotin	0·1
Isoleucine	30	Ascorbic acid	100·0
Leucine	40		
Lysine	120	Sucrose	18 gm.
Methionine	10	K_3PO_4	500 mgm.
Phenylalanine	10	$MgCl_2.6H_2O$	200 mgm.
Proline	40	Cholesterol	20 mgm.†
Serine	80	Water (to make)	100 ml.
Threonine	140	H_3PO_4 (to give)	*p*H 7
Tryptophan	40		
Tyrosine	40		
Valine	40		

* Relative amounts of amino-compounds and of vitamins based on analyses of pea juice (ref. 9) and on those found satisfactory in locust nutrition (ref. 10), respectively.

† Approximate amount dissolved by boiling in water.

Appreciable numbers of honeydew droplets were excreted by the adult aphids and by their progeny when fed on the 18 per cent sucrose solution and on the diet.

Seventy-one nymphs born to the experimental adults feeding on the diet were in turn confined on a membrane with access to the diet. More than 50 per cent survived for two weeks, during which time all moulted at least twice. Of 40 which survived 16–17 days, 11, 14 and 7 had developed into diminutive fourth instar apterous and alatiform nymphs and apterous adults, respectively.

Improvements clearly need to be made in the chemical and physical properties of the membrane-diet system before rates of feeding, growth, development and reproduction normal for the aphids on their host plants may be reached. However, the limited successes so far achieved indicate the immediate practicability of the artificial feeding method for nutritional and behavioural studies *per se*, as well as for other investigations, such as of form determination and of virus transmission phenomena, which would be facilitated greatly by a prolonged maintenance of aphids divorced from their host plant.

This work was supported in part by U.S. Public Health Service grant *E*-3947 (*R*1).

T. E. MITTLER
R. H. DADD
Department of Entomology and Parasitology,
University of California,
Berkeley, Calif.

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Mosquito Attraction and Repulsion

THERE is uncertainty in the literature as to the exact signals and responses that guide mosquitoes to a warm-blooded host[1]. Reports of specific chemical attractants (for example, lysine[2]) lack confirmation.

Our experiments indicate that *Aedes aegypti* can be steered almost quantitatively to a target, provided: (1) they are activated by breath or carbon dioxide (this is mainly non-directional); and (2) the target is both wet and slightly warm[3]. Some experiments were made in a wind tunnel 2 × 2 × 10 ft. long with air at about 28° C. and 25 per cent relative humidity moving at about 0·5 ft./sec. About 18 in. from the upstream end, three targets were presented, each a cylinder 3 cm. in diameter and 7 cm. long, covered with black woollen cloth and warmed if required (usually to about 35° C.) by an internal heating element. About 25 adult, female *Aedes aegypti* were placed in the tunnel and given at least a day to acclimatize, with water and sugar provided, but no previous blood meal. If human breath or carbon dioxide was suddenly added to the air stream, the insects flew actively and continued to do so for several minutes.

Under these conditions, the number of insects alighting on each target was counted during three 5-min. intervals with the results shown in Table 1.

Table 1. NUMBER OF ALIGHTMENTS

Interval	1	2	3	Total
Warm, wet target	157	103	98	358
Cold, wet target	12	3	7	22
Warm, dry target	2	1	4	7

In other experiments, a stream of warm, humidified air was released into the air stream from a slit. By adding a little inert smoke the location of the 'convection current' could be made visible. Fig. 1 is a time

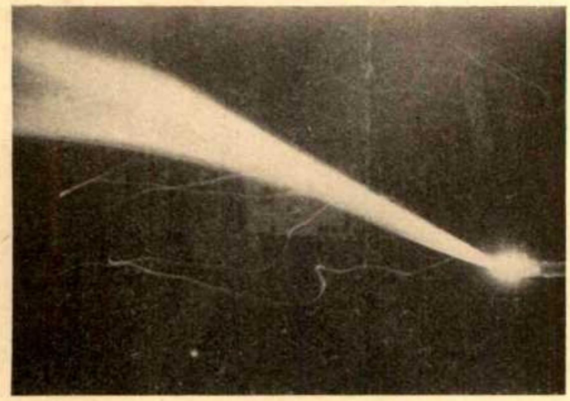

Fig. 1

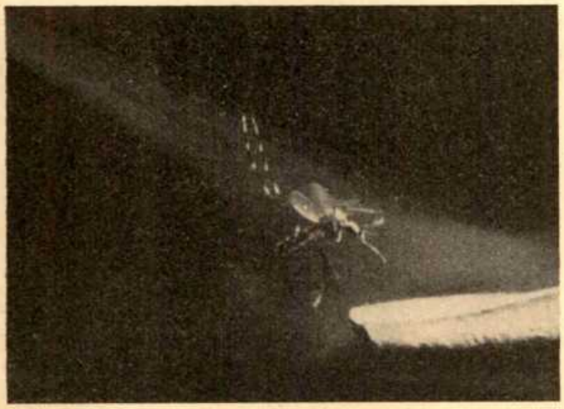

Fig. 2

exposure with dark-field illumination, which shows the tracks of several mosquitoes entering the stream and flying down it to the target. Fig. 2 shows an insect about to alight after having homed in in this way.

These experiments confirm previous reports that warm, wet convection currents play a principal part in guiding *Aedes aegypti* to their hosts[1,3–5].

It follows that the insects must have a sensory organ capable of detecting such currents. In view of the transitory structure of the currents and the flying speed of the insects, the sensing organ must have a very short time constant. The transfer of energy to it is therefore most probably radiative, in which case it will involve the infra-red properties of water vapour, and in particular the far infra-red frequencies which are the only ones that can be activated by collision at ambient temperatures[6].

On the assumption that mosquito repellents interfere with this guidance mechanism, the far infra-red absorption spectra in the 15–36μ region of five commonly used mosquito repellent chemicals were examined in a Perkin–Elmer model 21 spectrophotometer equipped with a cæsium bromide prism. The results are shown in Fig. 3, with the absorption spectra of water vapour and of three non-repellent chemicals included for comparison. (Propylene glycol was run as the pure liquid, the rest as dilute solution in *cyclo*hexane).

There are distinct indications of correlation.

It is not yet known how the repellent interferes with the perception of humid convection currents, but in general anything that would interfere with the radiative transfer of energy would leave only the slower conductive processes to operate and would increase the time-constant of the detector.

This suggestion also accounts for the otherwise curious report[4] that vapours of gasoline and ether are attractive to certain kinds of mosquito. These liquids combine a high volatility with a high molecular weight and thereby bring about a localized reduction in thermal conductivity of the air. A flying mosquito would lose its metabolic heat more slowly in such regions, and this could engender a sensation somewhat similar to that from a warm, moist convection current.

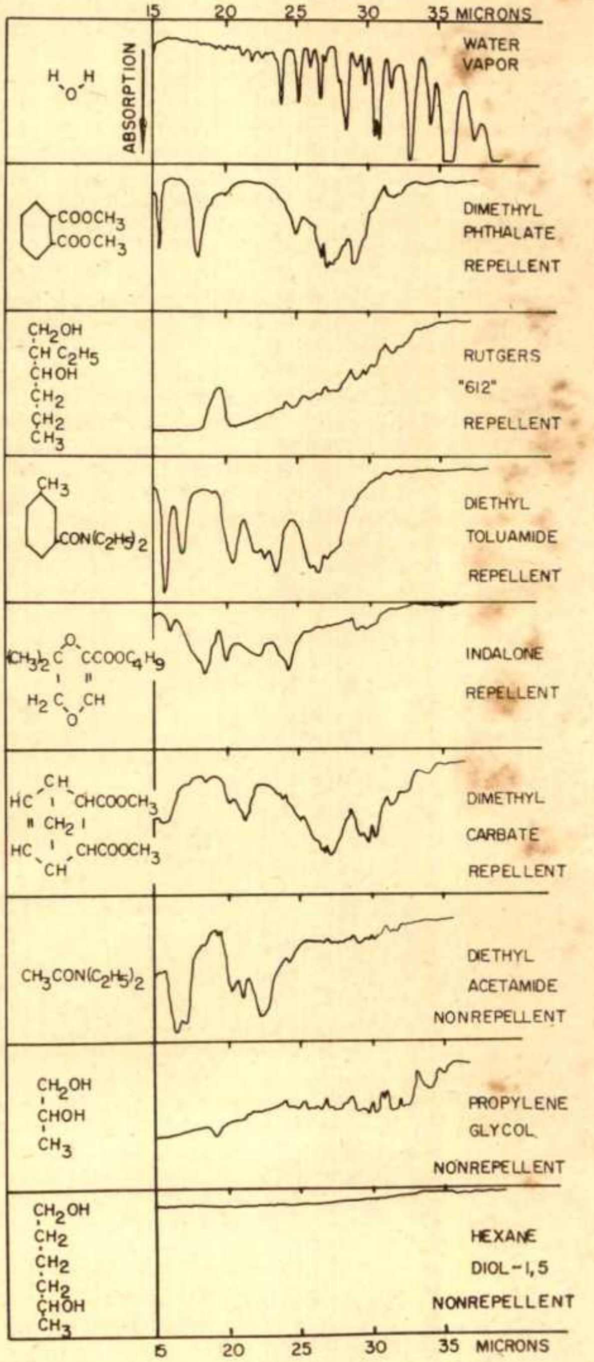

Fig. 3

The guidance system here proposed is naturally incomplete. It takes no account of the effects of target colour and movement, and it does not embrace the fact that when caged mosquitoes are exposed to repellents like dimethyl phthalate or 2-ethylhexane-diol-1,3 they are very difficult to activate by breath or carbon dioxide.

Nevertheless, if the basic requirement for repellency to *Aedes aegypti* is a particular kind of far infra-red absorption spectrum, this is a mechanical rather than a chemical property, and it follows that intrinsic repellency may be looked for in a compound of any chemical class, provided only that the molecule is made up of the right combination of mass-points and elastic forces. If this is so, then the way is open to a rational search for new mosquito repellents, including repellents to be taken by mouth and excrete through the skin.

In case it is thought remarkable that mosquito repellency should be connected with so commonplace a property as a rather general absorption of infra-red radiation beyond about 25μ, it must be remarked that more than a third of the many thousands of chemicals evaluated as repellents at the Orlando Laboratory of the U.S. Department of Agriculture were found to have some repellent properties[7].

We thank Dr. P. N. Daykin for discussions and the Canada Department of Agriculture for a grant which made this study possible.

R. H. WRIGHT
F. E. KELLOGG
British Columbia Research Council,
Vancouver 8, Canada.

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MICROBIOLOGY

Antifungal Activity of *Cylindrocarpon radicicola* Wr.

DURING investigations into the mycorrhizal tuberous roots of the saprophytic orchid *Dipodium punctatum* R. Br., the surface flora of washed root portions were sampled according to the method of Harley and Ward[1]. Isolates from plants of one particular location frequently included a species of *Cylindrocarpon*, afterwards identified as *C. radicicola* Wr.

The accidental contamination of plate culture of this fungus by *Aspergillus niger* revealed that the *Cylindrocarpon* was strongly antagonistic towards this species. Mycelial extension of *A. niger* was inhibited in the proximity of the *Cylindrocarpon* colony. Microscopic examination showed abnormal proliferation of the terminal hyphæ similar to that described by Aytoun[2], as an effect of griseofulvin on *Botrytis* spp.

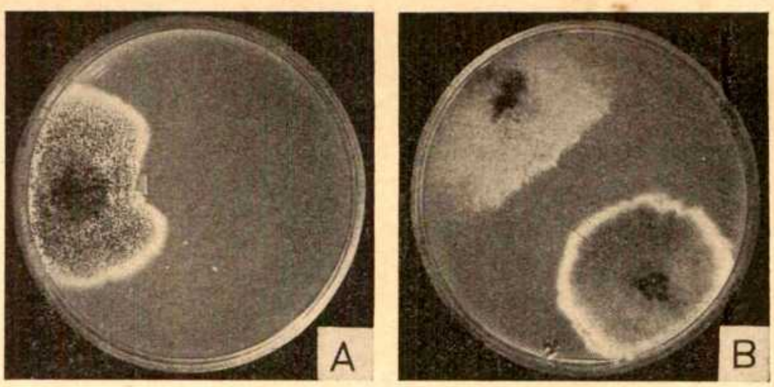

Fig. 1. *A*, Growth of *Aspergillus niger* being inhibited by a sterile agar block removed in front of a colony of *Cylindrocarpon*; *B*, *C. radicicola* inhibiting radial growth of *Botrytis cinerea*

To test the hypothesis that this was caused by an antagonistic substance diffusing ahead of the growing *Cylindrocarpon* colony a small block of agar was cut from a plate culture 1 cm. in front of the advancing mycelium and placed next to a colony of *A. niger* on another plate. The hyphæ of *A. niger* adjacent to the block showed similar microscopic abnormalities and macroscopic evidence of inhibition to those caused by living *Cylindrocarpon* (Fig. 1).

A selection of 66 fungal species, representing the major taxonomic and ecological groupings, were then tested against the *Cylindrocarpon* by simultaneously inoculating opposite sides of agar plates with *Cylindrocarpon* and the test fungus. Suppression of radial growth was used as a simple measure of fungistatic activity. Forty-eight species of 34 genera from all major taxonomic and ecological groups were inhibited. Most of these fungi were extremely sensitive; very few showed intermediate effects. Some of the parasitic and saprophytic species antagonized by *C. radicicola* are listed here: *Alternaria solani* (Ell and G. Martin) Son; *Cercospora viticola* (Ces) Sacc.; *Gibberella fujikuroi* (Saw). Wr.; *Helminthosporium sativum* Pamm. King and Bakke; *Mycosphaerella pinodes* Berk and Blox; *Phoma citricarpa* McA.; *Penicillium notatum* Westling; *Pythium ultimum* Trow; *Schizophyllum commune* Fr.; *Trichoderma viride* Pers. and Fr.

Filtrates from various liquid culture media on which the *Cylindrocarpon* had been successfully grown were also tested for activity. Antibiotic assay disks were dipped into the filtrates and placed on to agar plates seeded with conidia of *A. niger*, a zone of inhibition surrounding the paper disks gave evidence of inhibition of germination and the size of the zone could be used as a measure of fungistatic activity. Other fungi used in this test were *Botrytis allii*, *B. cinerea* and various species of *Penicillium*, all of which showed inhibition of spore germination. Six bacteria and three yeasts were also tested, but the culture filtrate had no effect on the growth of these organisms.

Interesting and potentially useful properties of the fungistatic substance in the culture filtrate were found to be: tolerance to a wide range of *p*H conditions, and extreme heat stability; at *p*H 8·2 the culture filtrate showed no loss of activity after autoclaving at 15 lb./2 in.^{2} for 30 min. These properties, combined with such a broad spectrum of fungistatic activity, warrant further investigation, which is now proceeding and will be reported elsewhere in due course.

A culture of *C. radicicola* obtained from the Commonwealth Mycological Institute, Kew (71577), was shown to have this same antifungal activity, but one obtained from the Centraalbureau voor Schimmelcultures, Baarn, did not. It is interesting in this respect that the local strain and that from Kew coincide in producing a characteristic deep reddish pigment when grown on Czapek–Dox agar while the culture from Baarn lacks this.

This work was carried out while one of us (G. A. C.) held a Commonwealth Scientific and Industrial Research Organization research studentship, and one of us (G. E.) held a Wheat Industry Research Fund studentship.

N. H. White
G. A. Chilvers *
G. Evans

Plant Pathology Laboratories,
School of Agriculture,
University of Sydney.

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* Present address: Botany School, Australian National University, Canberra.

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Lysogenic Conversion in *Staphylococci*

Bacteria carrying a prophage generally differ from the parent strain in one or more properties[1]. The presence of the prophage may produce a new character as in *Corynebacterium diphtheriae*[2] or suppress a property of the parent strain. In *Staphylococci* resistance to erythromycin can be (inversely) related to the presence of a prophage[3]. Blair and Carr[4] have also described relationships between the presence of a prophage and penicillinase or α-toxin production. In our case, lysogenic conversion of *Staphylococci* to loss of β-toxin production is reported. Strain 879 (phage pattern, 3^B, 3^C, 55, 71) is a non-lysogenic strain producing α- and β-toxin (hæmolysin). The phages were derived from staphylococcal strains of phage group II (ref. 5) and propagated on strain 879.

Strain 879 was lysogenized with sixteen phages by spotting the phages on indicator plates, isolating the secondary growth and testing for lysogenicity and immunity by cross-streaking. In eleven cases the lysogenic strains produced only weak hæmolysis on sheep blood. The phages producing this 'conversion' were serologically related (group *F*). No conversion was obtained with the standard phages of group II.

The obligatory relation between lysogenicity and hæmolysis was established in more quantitative experiments. Phage and bacteria were mixed in suitable concentrations, incubated for 5 min. to permit adsorption, centrifuged, and the number of bacteria and plaque-forming units determined in supernate and sediment. Some examples are given in Table 1.

Hæmolysis of survivor colonies was scored on blood-agar plates. Lysogenicity was scored by replicating from the blood-agar plates on indicator plates or by cross-streaking individual colonies. All weakly hæmolytic colonies proved lysogenic. The inverse was not always true. Some fully hæmolytic colonies yielded phage. It was shown by plating out and cross-streaking that these colonies were mixtures of lysogenic (weakly hæmolytic) and non-lysogenic cells. They could have originated from small clumps with a minority of lysogenic cells or, more probably, from late infection of the colonies on the plate with carry-over phage as the phenomenon was always more pronounced in experiments with high multiplicity and could be reduced with antiphage serum.

The fraction of lysogenized survivors increases with multiplicity. However, even with low multiplicity, the rare lysogenic colonies always proved weakly hæmolytic (if a converting phage were used).

The possibility was considered that weakly hæmolytic variants would occur in the parent strain and that the phenomenon was due to *preferential lysogenization* of the minority variant. Spontaneous weakly hæmolytic variants occurred indeed in the parent culture. On purification the parent strain 879 and the variant (879*nH*) proved stable. Preferential lysogenization could be excluded by using purified cultures and by showing that 879 and 879*nH* were lysogenized to the same extent by phage 756 (Table 1, lines 1 and 2). Transduction by phage produced on possible weakly hæmolytic mutants in the propagating strain(s) of the phages was excluded by continual checking of propagating strains. It was, furthermore, shown that phage 756 propagated on 879 and 879*nH* in both cases produced the lysogenic conversion (lines 3, 4 and 5).

The observed conversion is due to loss of β-toxin. Strain 879 produces α- and β-toxin with marked hot–cold hæmolysis on sheep blood agar. Culture filtrates hæmolyse sheep cells to much higher titre than rabbit cells (Table 2). Anti-α-toxin neutralizes the action on rabbit cells but not that on sheep cells, which is, however, neutralized by anti-β-toxin. The lysogenized strain 879(756) produces α-toxin only and shows no hot–cold hæmolysis. The hæmolytic activity of culture filtrates is completely neutralized by anti-α-toxin and not by anti-β-toxin. The spontaneous variant 879*nH* produces mainly β-toxin, with weak action on rabbit cells. The lysogenic strain 879*nH*(756) produces no detectable β-toxin.

Lysogenic conversion to loss of β-toxin by these phages was also observed with three other strains belonging to phage group II. From some of the lysogenic strains, non-lysogenic colonies could be isolated. These reversions which had lost the phage again produced β-toxin.

Table 1

Strain	Phage	Bac./ml. $\times 10^{-7}$	Phage/ml. $\times 10^{-7}$	Per cent ads.	m*	Survivors $\times 10^{-7}$	Colonies		
							Tested	Lysogenic converted	Mixed yielding phage
879	756	7	350	83	41	0·7	81	65	—
879*nH*	756	7	350	83	41	1·2	88	74	—
879	756	11	260	78	20	1·4	37	34	—
879	756/*nH*	9	280	18	6	0·6	51	40	—
879	756/*nH*	7·5	400	43	25	0·7	110	85	—
879	756	5·5	39	78	5·6	1·2	919	216	36
879	756	6·1	10	90	1·5	3·8	174	1	7
879	269	7	85	90	11	2·0	317	31	77
879	269	7·6	10	97	1·3	6·1	2,039	35	—
184	756	1·5	8·5	28	1·6	1·4	195	15	0
184	756	7·4	100	95	13	3·4	556	28	few

*m, multiplicity.

Table 2

Strain	Toxin titre on Rabbit cells	Toxin titre on Sheep cells
879	10	500
879(756)	10	5
879(269)	50	10
184	100	1,000
184(756)	100	10
184(184)	50	5
269	2	5,000
269(269)	2	10
879*nH*	5	200
879*nH*(756)	5	< 2

With another, non-lysogenic, β-toxin-producing strain not belonging to phage group II, no lysogenic conversion was obtained with five phages from the international standard typing set. This strain could, however, be converted with phage 756 after adaptation of this phage.

J. de Waart
K. C. Winkler
Ca Grootsen

Laboratory for Microbiology,
State University of Utrecht,
The Netherlands.

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Growth of Several Strains of *Actinomyces israelii* in Chemically Defined Media

Previous work from this laboratory[1] has described a technique for obtaining uniform and quantitative inocula of the Gram-positive filamentous anaerobe *Actinomyces israelii*. It has also been shown[2-4] that the medium of Howell and Pine[5] could be simplified to give a chemically defined medium containing inorganic salts (including ammonium sulphate), glucose, glutathione, seven L-amino-acids (serine, cysteine, leucine, isoleucine, lysine, tryptophan and glutamic acid) and five growth factors (biotin, inositol, pyridoxal, nicotinic acid and riboflavin) which would support growth of a single strain of *A. israelii* in repeated serial culture. Further work[6] has described some of the chemical and physical changes which occur in a series of defined media during growth of the same Wills strain of *A. israelii*.

The results of experiments on the nutritional requirements of several other strains (Table 1), all of which were macroscopically, microscopically and physiologically typical of *A. israelii*[2,7,8] when grown in the chemically undefined medium *S* of Erikson and Porteous[7], are shown in Table 2. The culture techniques used in the present work and the visual method of assessing growth have been described elsewhere[3]. It is emphasized that previous work has involved exhaustive tests of the ability of the Wills strain of *A. israelii* to survive in various chemically defined media[2-4], or in undefined media[7], for a minimum of five sub-cultivations at 5- to 7-day intervals; in some instances this single strain has been maintained by sub-cultivation for more prolonged periods of several months or years in one or more defined media. The present preliminary results have been obtained from short-term experiments involving only a few serial transfers in any one defined medium.

Table 1. List of Strains of *Actinomyces israelii* examined in the Present Investigation together with the Donors of the Cultures and the Origin of the Cultures where known

Strain	Original donor	Site of infection and date of isolation
1934	Dr. E. Riddell	Abscess of jaw; 1950
Grant	Dr. Jacqueline Walker	Abscess of jaw; 1952
Armitage	Dr. Jacqueline Walker	Simple dermoid cyst, left rectus; 1951
Mullins	Dr. Jacqueline Walker	Abdominal sinus; 1951
Young	Dr. Jacqueline Walker	Abscess of chest (rib)
Balcome	Dr. Jacqueline Walker	Actinomycosis of jaw; 1951
Wills	Dr. Dagny Erikson	Lung lesion; 1951
Yory	Dr. Dagny Erikson	Unknown
Maslin	Dr. Dagny Erikson	Unknown
2147/52	Unknown	Unknown (about 1951)
Hyde	Unknown	Unknown (about 1951)

Of the eleven strains of *A. israelii*, all grew in the undefined media *S* or *A* (ref. 7) (Table 2). Strains 2147/52 and Hyde failed to grow in medium *HP*1, and three other strains (1394, Armitage and Mullins) survived only a few sub-cultures in this medium. All the remaining strains grew in medium *HP*1, but only strain Wills grew in the simplest medium *HP*9 tested[3]. Of the media in Table 2, all except medium *HP*9 contained oleic acid[2] and, unless otherwise indicated, all except medium *HP*9 contained starch[2]. Howell and Pine[5] believed that the sole function of starch in their medium was "to maintain non-toxic concentrations of oleic acid". The present results show that the Wills strain of *A. israelii* would grow in the presence of oleic acid (at least in medium *HP*4) whether starch was present or absent. Other results[2] showed that the same strain would grow in the presence of oleic acid and the absence of starch in media simpler than *HP*4. Strains Yory and Balcome, on the other hand, grew better when starch was present in medium *HP*4.

Table 2. Comparison of the Nutritional Requirements of Eleven Strains of *Actinomyces israelii* of Human Origin (Table 1)

Strain	Medium *S* or *A*	*HP*1	*HP*4	*HP*4 less starch	*HP*5	*HP*5 + adenine + thymine	*HP*9	*HP*9 + adenine + thymine
2147/52	(+)	(0)	(0)	(−)	(−)	(−)	(−)	(−)
Hyde	(+)	(0)	(−)	(−)	(−)	(−)	(−)	(−)
1394	(+)	(±)	(−)	(−)	(−)	(−)	(−)	(−)
Armitage	(+)	(±)	(±)	(−)	(−)	(−)	(−)	(−)
Mullins	(+)	(±)	(−)	(−)	(−)	(−)	(−)	(−)
Wills	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Yory	(+)	(+)	(+)	(+)*	(+)	(+)	(0)	(+)
Maslin	(+)	(+)	(±)	(±)	(0)	(+)	(0)	(±)
Young	(+)	(+)	(−)	(+)	(+)	(+)	(0)	(Ø)
Balcome	(+)	(+)	(+)	(+)*	(−)	(−)	(0)	(0)
Grant	(+)	(+)	(Ø)	(Ø)	(−)	(−)	(0)	(0)

Incubation at 37° in liquid paraffin sealed tubes containing 2 ml. medium (refs. 2–4 and 7); subcultures carried out at seven-day intervals. (+) indicates growth in several serial sub-cultures; (±) indicates failed to survive more than one or two serial sub-cultures; (0) indicates no growth; (−) indicates not tested; (+)* grew better if starch present (ref. 2); Ø consistently gave atypical colonies (ref. 3) if carried for more than a few sub-cultures in this medium but grew typically in more complex media. The results on growth in media *A* and *S* are based on repeated serial sub-cultures over several months; some of the remaining results were obtained from a maximum of five serial sub-cultures in any one medium but the majority of the results were obtained from three serial sub-cultures in any one medium.

It has been noted[4] that growth of the Wills strain of *A. israelii* is inhibited in certain circumstances by 'growth factors' and by purines and pyrimidines; the first indication of this latter effect was obtained during the present study. On the other hand, strain Yory apparently required adenine and thymine to be added to the simplest medium *HP*9 tested (Table 2). Similarly, strain Maslin required adenine and thymine to be added to medium *HP*5, but this strain failed to grow consistently in medium *HP*9 supplemented in the same way; conversely, since medium *HP*4 = medium *HP*5 + adenine + guanine + xanthine + uracil + thymine, it may be deduced that one or more of the bases guanine, xanthine and uracil inhibited growth of strain Maslin in medium *HP*4. Strains Young, Balcome and Grant apparently required increasingly complex media in order to survive, though, as pointed out below, this is not the only possible interpretation of the results.

Since medium *HP*1 was derived[2] from the original Howell and Pine medium[5] by omission of coenzyme *A* and of citrovorum factor, it is possible that strains 2147/52, Hyde, 1394, Armitage and Mullins (Table 2) required the two missing growth factors. This has not been tested nor has a serious attempt been made to grow these strains in a variety of simpler media. It would in any case be rash to conclude from the present results that the eleven strains of *A. israelii* could be divided into two groups, one of which required coenzyme *A* and/or *citrovorum* factor, the other of which did not (Table 2). With some exceptions discussed here it is not possible from present results to state whether failure of any one strain to grow in a particular medium was due to a nutritional deficiency or to the presence of nutritional antagonists[3,4] in that medium. Nor is it possible at present to delineate the basal nutritional requirements of the species *Actinomyces israelii* although it is clear that differences exist in the precise nutritional requirements of individual strains. Detailed experiments of the kind performed for the Wills strain of *A. israelii*[2–4] are required to elucidate these points.

This work was carried out while one of us (A. O. C.) was in receipt of a Department of Scientific and Industrial Research research studentship. The eleven strains of *A. israelii* used were generously donated to one of us (J. W. P.) by Dr. Dagny Erikson.

A. O. Christie *
J. W. Porteous

Department of Biological Chemistry,
Marischal College,
University of Aberdeen.

* Present address: Marine Biology Station, Menai Bridge, N. Wales.

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GENETICS

Differential Response to Mutagens in *Arabidopsis thaliana*

In a series of experiments designed to look for differential response to mutagens in higher plants, more than 800 viable mutations have been induced in the crucifer *Arabidopsis thaliana* (L.) Heynh. This plant was used because of its short life-cycle of 5 weeks, its small size and obligate self-pollination. The mutants were obtained by treating seed with radiation or with mutagenic chemicals. Seed was treated in the X_1 generation and suspected mutants were isolated in the X_2 generation and confirmed as mutants in the X_3 generation. This procedure was necessary because all but one of the mutants are recessive.

The mutagens used were X-rays, X-rays plus 'Synkavit' (a methyl naphthohydroquinone phosphate, known to be a radiosensitizing chemical in tumours[1] and in barley roots[2]), tritiated 'Synkavit', ethylene imine (EI) and ethyl methane sulphonate (EMS). These were the mutagens out of 15 used initially which gave high mutation-rates—a necessary requirement when looking for specificity. EI and EMS are among the alkylating agents known to give differential gene response in *Drosophila*[3].

All mutants were grouped phenotypically into one of 27 classes. This scheme was necessarily empirical in that each class contained several morphologically distinct mutants and because a few mutants could have been placed in more than one class. If only morphologically identical mutants had been placed in any one class, there would have been several hundred classes, and the results could not have been analysed statistically. Because the number of mutants in any one cell of the table was often less than 5, a modified heterogeneity test devised by Smith[4] was used. This showed an overall significant heterogeneity at a level of $P < 0{\cdot}01$, demonstrating that there was a real differential response to the treatments. Subdivision of the table showed significant differences of $P < 0{\cdot}01$ between radiation and alkylating agents and between different concentrations of EMS. There were non-significant differences of $P > 0{\cdot}1$ between X-rays and X-rays plus 'Synkavit', between EMS and EI, between radiation doses and between dates of production of the mutants. These results, particularly the fact that the mutation spectra did not vary throughout the three years of the experiment, show that the classification scheme, although empirical, does have a logical basis.

Apart from classes where there were only few mutants, all treatments produced mutants in all classes, and differences were only of degree. The class for glabrous mutants showed the biggest differences between radiation and alkylating agents: 6 per cent of all radiation-induced mutants being glabrous as opposed to 1·2 per cent for mutants induced by alkylating agents. This gave a difference, significant at $P < 0{\cdot}001$, as evaluated by a χ^2 test. However, since the maximum mutation-rate for EMS was 85 per cent against 12 per cent for radiation, calculated as a percentage of the X_1 plants which gave viable seed, it is clear that for an equal number of X_2 progenies, more glabrous mutants will be produced by EMS than by radiation. Although methods have been calculated, for example by Bodmer[5], for testing the significance of individual classes in contingency tables, it is difficult to ascribe accurate probabilities to a table as used here, where the number of classes is large and also quite arbitrary. Thus it is not possible to state with any certainty that glabrous mutants are preferentially induced by radiation. Sufficient attention has not been paid to this factor in much of the work done elsewhere in which differential responses have been claimed.

Approximately 550 of the mutants have been examined genetically and, of these, only 80 occur at the 25 loci which have mutated more than once in all the experiments, making it impossible to demonstrate whether loci mutate differentially to different mutagens. It can be shown that 25 per cent of all radiation-induced mutants occur at these loci, whereas only 6 per cent of those induced by alkylating agents do so, indicating that alkylating agents may produce mutations at less easily mutable loci, as indicated in *Drosophila*[3].

These results from more than three years of continuous production of mutants in *Arabidopsis* show that although there are differences in the types of mutations produced by radiation as compared with alkylating agents, they are so slight as not to offer any prospects of being able at present to direct the mutation process. Until more information is available from the experiments now in progress about the effect of radiation dose or chemical concentration on the spectrum of mutations in *Arabidopsis*, it is not possible to know whether to ascribe these differences to the mode of action of the mutagens or simply to the overall mutation-rate and preferential elimination of certain types of mutants. There is a high probability ($P < 0{\cdot}01$) that the spectrum of EMS-induced mutants varies with the concentration of chemical used.

Evidence put forward by Ehrenberg *et al.*[6] that the spectrum of chlorophyll mutations in barley is dependent on the mutagen fails to take into account adequately this dose factor. The low percentage of chlorophyll mutants which are 'albina' type after treatment with alkylating agents compared with radiation may be due to the fact, as may be deduced from their results, that this percentage always decreases with increased overall mutation-rate, perhaps due to preferential elimination of 'albina' mutants at high mutation-rates.

The results reported here show that in forward-mutation experiments, based on a broad spectrum, it is difficult to demonstrate differential response to mutagens, and that many factors must be taken into account. Previous claims for mutation specificity in higher plants must be regarded with caution as the results have been interpreted in ways which might not be agreed to by all statisticians.

This work was carried out under a Nuffield Foundation Grant.

A. D. McKelvie

Plant Breeding Institute,
Trumpington,
Cambridge.

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AGRICULTURE

Use of Mechanical Barriers instead of Guard Rows in Field Experiments

A MAJOR disadvantage in manurial and other field experiments on trees and other perennial crops is the requirement for 'guard rows' between plots to restrict the roots of the experimental trees to the treated areas of soil. As a consequence, the plots are dispersed over a considerably larger area than would be required for the treatments alone.

A new technique, probably of wide application, is to use 'Vinyl' copolymer foil as a barrier to root extension across plot boundaries and to lateral movement of water from irrigated plots. This has been made possible by the development of a machine to lay the foil.

A special tine, fitted to a sub-soiler, encloses a pivoted laying leaf which feeds, horizontally, plastic foil 30 in. wide down into the soil so that it emerges as a continuous vertical strip within the channel formed by the tine (Fig. 1).

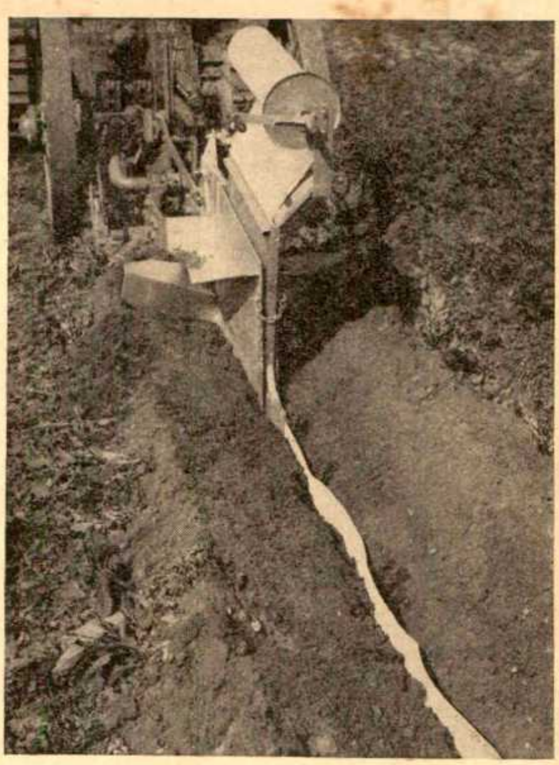

Fig. 1. Plastic foil being laid vertically in soil, to a depth of 34 in., to form a barrier to root extension across experimental plot boundaries

The technique has been developed in connexion with a large field experiment to determine the effects of different nitrogen and water régimes, and their interactions, on the growth and cropping of apple trees over many years. Vertical strips of foil 0·015 in. thick have been laid successfully down to a depth of about 34 in., delimiting the various plots on all four sides. The elimination of the need for guard trees has reduced the area required for the experiment from 11 to 4·7 acres.

The field method of laying the root barriers is to mark the boundaries of the plots with furrows and then to make all the runs in one direction before attempting the transverse ones. As the first runs are made, the laid foil is cut where the intersections will occur to enable the implement to pass through at right angles on the subsequent runs. Usually the gaps left at these intersections are not greater than 3 or 4 in. wide. Continuous laying is achieved by sticking the end of each new roll of foil to its predecessor with a double-sided transfer tape, using a little heat if necessary.

Normally a speed of about 0·75 m.p.h. has been maintained while laying at a mean draught of 12,100 lb. in a fine sandy loam soil passing into a clay loam over Kent ragstone, but occasional maxima of more than 20,000 lb. have occurred when the tine struck rock. In the course of four clear working days some 2·75 miles of foil were laid for a material cost of £900, which compares favourably with the £1,700 which would have been the cost of the 500 cu. yd. of concrete required to make equivalent barriers in the soil.

J. E. Goode

East Malling Research Station,
East Malling,
Maidstone, Kent.

W. T. B. Marchant

National Institute of Agricultural Engineering,
Wrest Park,
Silsoe, Beds.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Lecturer in Organic Chemistry—The Registrar, The University, Manchester 13 (August 1)

Research Assistant in the Department of Psychology—The Registrar, The University, Sheffield 10 (August 1)

Lecturer (with a good honours degree in physics or electrical engineering, together with experience in the field of communications (rather than in electronics)) in Telecommunications—The Clerk to the Governors, Woolwich Polytechnic, London, S E 18 (August 3)

Tutorial Student (graduate or about to graduate) in Mechanical Engineering—The Registrar, King's College (University of London), Strand, London, W C 2 (August 3)

Lecturer in Physics—The Principal, Nottingham and District Technical College, Burton Street, Nottingham (August 4)

Lecturer or Assistant Lecturer in Mathematical Statistics—The Registrar, The University, Manchester 13 (August 4)

Assistant Lecturer (interested in physical organic or inorganic chemistry) in the Department of Chemistry—The Secretary, The University, Edinburgh (August 8)

Imperial Chemical Industries Research Fellow, to carry out original research in Chemistry, Physics, Engineering (including Chemical Engineering) Metallurgy, Pharmacology or related subjects—The Registrar, The University, Sheffield 10 (August 8)

Research Assistant (graduate in agricultural botany, botany, biochemistry or agricultural chemistry, with interests in plant physiology or anatomy) in the Department of Agricultural Botany, to undertake research on silica uptake and deposition by grasses—The Registrar, University College of North Wales, Bangor, North Wales (August 8)

Assistant in Zoology—The Secretary of University Court, The University, Glasgow (August 10)

Technician in the Department of Zoology, Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, August 10)

Curator (with a university degree in geology) of Museum and Departmental Collections in the Department of Geology, to be responsible for cataloguing and curating the Departmental collections of rocks, minerals and fossils—The Secretary, The University, Edinburgh (August 11)

Lecturer in Mathematics—The Secretary, University of St Andrews, Queen's College, Dundee (August 11)

Lecturer in Physiology—The Secretary, The London Hospital Medical College (University of London), Turner Street, London, E 1 (August 11)

Lecturer (preferably with special interests in fluid dynamics or numerical analysis) in Applied Mathematics in St Salvator's College—The Secretary, The University, College Gate, St Andrews, Fife (August 11)

Lecturer (with special interests in igneous or metamorphic petrology) in the Department of Geology—The Secretary, The University, Edinburgh (August 11)

Lecturer in Computer Science—The Secretary, Birkbeck College (University of London), Malet Street, London, W C 1 (August 13)

Lecturer in the Department of Mathematical Physics, for duties which will include the teaching of mathematical physics and applied mathematics, to undergraduate level and assistance in the postgraduate and research activities of the Department, which are at present primarily concerned with the quantum theory of fields and nuclear theory—The Secretary, The University, Edinburgh (August 15)

Lecturer (with good qualifications and teaching experience in physics and/or chemistry, and preferably a higher degree in education) in Education—The Registrar, The University Keele, Staffs (August 18)

Temporary Assistant Lecturer in Psychology (for two years)—The Registrar, The University, Keele, Staffs (August 18)

Lecturer (with an interest in physical metallurgy) in Chemical Engineering at the University College of The West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (August 20)

Lecturer or Assistant Lecturer (with a degree in veterinary medicine or physiology) in the Department of Physiology—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N W 1 (August 25)

Assistant Lecturer (with a good honours degree in physics and some postgraduate experience) in the Department of Natural Philosophy, to take part in the plasma physics research programme of the Department—The Secretary, Royal College of Science and Technology, Glasgow, C 1 (August 31)

Lecturer in Chemistry at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (August 31)

Professor of Chemistry at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, University of Canterbury (August 31)

Research Fellow in Chemistry at the University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (September 1)

University Assistant Lecturer (preferably with a knowledge of either East or Central Africa, or the Pacific area) in Social Anthropology—The Secretary of the Appointments Committee, Faculty of Archæology and Anthropology, The University Downing Street, Cambridge (September 1)

Chair of Soils, and Chair of Agriculture (Crop Production) in the University of the West Indies (Imperial College of Tropical Agriculture, Trinidad)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 5)

Lecturer (preferably with experience in vertebrate zoology, insect ecology, or marine biology) in Zoology at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, September 7)

Reader in Mathematics at Royal Holloway College—The Academic Registrar, University of London, Senate House, London, W C 1 (September 10)

Lecturer in the Department of Chemical Engineering—The Secretary, University College, Gower Street, London, W C 1 (September 17)

Reader (with special interests in mineralogy and petrology) in Geology at King's College—The Academic Registrar, University of London, Senate House, London, W C 1 (September 20)

Reader in Mathematics at Royal Holloway College—The Academic Registrar, University of London, Senate House, London, W C 1 (September 20)

Chair of Forestry and Natural Resources—The Secretary, The University, Edinburgh (September 30)

Lecturer in Forest Inventory at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Reader-in-Charge of Forestry, University of Melbourne (Australia and London, September 30)

Entomologist, Grade A/B (with a good honours degree in entomology (zoology) and a particular interest in biological control work)—The Director, Commonwealth Institute of Biological Control, Gordon Street, Curepe, Trinidad, W I (October 15)

Lecturer (graduate in chemistry and preferably with experience in the food industry), for duties which will include the teaching of chemistry and food quality control—The Principal, National College of Food Technology, St George's Avenue, Weybridge, Surrey

Lecturer (honours graduate) in the Department of Mechanical Engineering—The Registrar, The University, Liverpool, quoting Ref CV/142

Neurochemistry Research Fellow (honours graduate in biochemistry or chemistry), for biochemical investigations on muscle disease in the Neurochemical Research Laboratory of the Regional Neurological Centre, Newcastle upon Tyne—The Secretary, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne 4

Research Assistant (with a degree or H N C in electrical engineering or applied physics, or an equivalent qualification and experience in electronics) in the Radio Astronomy Research Group—The Secretary, Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge

Research Fellow (with at least three years postgraduate research experience) in the Department of Electron Physics, to lead a small group either in work related to the Space Research Programme or to associated laboratory ionic physics problems—The Deputy Registrar, The University, Edgbaston, Birmingham 15

Scientific Officer or Senior Scientific Officer (honours graduate, preferably with a Ph D in biochemistry, with interests in the isolation and chemistry of proteins, and in studies of enzymes) in the Physiology Department—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, near Reading, quoting Ref 62/8

Senior Biology Master, to take charge of the Biology Department—The Headmaster, King's College, Taunton

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

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SCIENTIFIC RESEARCH FOR INDUSTRY IN BRITAIN

A MAJOR theme of the report of the Council for Scientific and Industrial Research for the year 1961* is service for industry, and particularly the ways in which industry can be helped and encouraged to apply the results of scientific research Of two major questions on which indication of the Council's policy is awaited the report gives no guidance the report on aid for research and training in universities and colleges promised last year is still in the press, and the problems of providing large-scale equipment for universities and other institutions and of centralization of research facilities are no more than mentioned However, the report is almost enthusiastic about the possibilities opened up by Prof M Stacey's work at the University of Birmingham on the synthesis and properties of organic fluorine compounds—studies which could profoundly influence polymer, dyestuffs and biological chemistry Apart from reporting the progress of a scheme for demonstrating how research results can be applied in industry to improve efficiency and increase productivity, there is no indication of what is contemplated with regard to the research associations, beyond greater flexibility and the acceptance of a recommendation from the Industrial Grants Committee that further grants 'earmarked' for special activities should be offered These would be aimed at increasing the efforts of research associations in fields judged to be of particular importance, industrial health and safety being instanced

Under the special assistance scheme just mentioned, expenditure during the year was some £88,000, making a total of £264,000 on projects from twenty associations since the scheme was started Most of the scheme involved the extension of information services, and in general there has been a good response from industry to the introduction of the additional services Clearly this aspect of the work of the research associations which is related to their educational functions is of growing importance However, the Robbins Committee may well have some valuable comments here in due course, of which full account should be taken of any modifications in the structure or functions of the research association system which may result from the inquiry the Council has already instituted

The Industrial Grants Committee also supervises, through a sub-committee, the work of the Industrial Operations Unit, which seeks to show in practical terms how the systematic study of operational methods can lead to greater efficiency without any basic change in technology An example of the Unit's work during the past year is seen in the special course in simplification of work presented for the British Steel Castings Research Association. Other research associations are showing increasing interest in the application of mathematical methods to the making of decisions These activities, which originally were financed from Conditional Aid Funds, are part of the Department's policy of securing rapid dissemination and application of the results of research

They are also linked with the information activities of Headquarters which the Council has carefully reviewed over the past three years Little more information, however, is forthcoming in the present report on this aspect of the Department's work, although the Council set up in 1959 a Committee on the Dissemination of Scientific and Technical Information, with as a first main task the consideration of the regional organization of technical information services While the Department's branch offices in Scotland and Wales have been strengthened and grant-aid for a further three years is being given to six regional technical information centres in Birmingham, Bristol, Liverpool, Manchester, Newcastle and Portsmouth, there is no discussion of the implications of the proposals in the Roberts Report on the public library service, and no indication that the new centres have been planned with any regard to the potentialities of the public library service or to existing university or technical college libraries

No co-ordination of public information services can be expected to avoid waste of effort or finance if due regard is not paid to such potentialities, the reluctance of the Council to provide sufficient information for an informed judgment to be made on this aspect of the Department's work is somewhat puzzling On the other hand, the Department is giving increasing attention to its publications and is seeking to make its publications more effective and attractive Besides improving lay-out and typography, publications specially designed for industry have been launched One series, for example, puts the results of selected studies in the human sciences into a concise form suitable for use by industrial management Another features various technical developments, often stemming from the work of the research stations and research associations, and sometimes providing concise surveys of particular fields of technology Besides this, the Information Committee is now considering specifically the improvement of documentation techniques and what further action the Department can usefully take in this field

The Council's report directs particular attention, however, to two relatively new types of activity which also bear on this general problem of assisting industry to utilize the results of research First, it emphasizes the value of the series of detailed studies of particular industries which the Economics Committee has been making in recent years The formation during the year of the new British Ship Research Association, which will take over the facilities of the former Parsons and Marine Engineering Turbine

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* Department of Scientific and Industrial Research Report of the Research Council for the year 1961 Pp 60 (Cmnd 1734) (London H M Stationery Office, 1962) 4s net

Research and Development Association and undertake a more extensive research programme is a direct result of the Economics Committee's recommendations in its report on *Research and Development Requirements of the Shipbuilding and Marine Engineering Industries* A new organization, continuing the name "Pametrade", will take over design work for marine turbine manufacturers and the Council is considering proposals for strengthening the national effort in research and development in marine engineering The Economics Committee, in conjunction with the Board of Trade, is making a similar study of requirements for research and development in textile machinery It has completed investigations concerning research and development in foundry engineering and equipment, a study of research and development relating to other metal-forming processes is also contemplated

The second matter is the placing of development contracts in industry Support has already been given for the development of a large gear-grinding machine incorporating entirely novel mechanical principles and embodying some of the latest techniques developed at the National Engineering Laboratory. A second project in the machine-tool field is under consideration, and a project designed to increase the overall effort on research and development in computers and to improve co-ordination of work in this field in industry and Government laboratories is being discussed with the firms concerned This project will cover, in the first place, research and development on new ranges of components and techniques for the next generation of digital computers, later, it will include the study of problems arising in the design of computers using such components

The industrial recipients of development contracts will contribute substantially to the cost, and normally a return will be sought on the Government contribution, usually by means of a levy on subsequent commercial sales Other projects which may warrant support are being examined and close liaison with the National Research Development Corporation is being maintained The Minister for Science has repeatedly expressed the view that more use should be made of development contracts, and the system has attracted noticeable support when questions of research and development have been debated in Parliament in recent sessions The position of the National Research Development Corporation was specifically mentioned recently by Lord Hailsham in referring to the Treasury Committee now examining the Government organization for civil research It is possible that the relations of the Corporation with the Department as well as with the Board of Trade are receiving fresh and independent scrutiny

The Council expresses the hope, in conclusion, that by economic studies of research requirements, by the award of development contracts, by the continued support of research associations, and by the work done in its own research establishments, the Department makes a significant contribution to increased industrial efficiency and, therefore, higher productivity On the evidence of this report, subject to all the reservations already made, that hope seems to be justified On certain subjects the interest at the moment appears to have shifted outside the Council's own responsibility the Minister for Science would seem to be looking to the Trend Committee for guidance on some matters of organization and responsibility, though it is curious that the Council for Scientific and Industrial Research in this report makes no reference to the findings of the Zuckerman Committee on the management of research Again, it may be that the Minister is looking to the Advisory Council on Scientific Policy for guidance when his inquiry into the functions of the research associations is complete Certainly that body is better placed for consideration of the general co-ordination of information activities and the extent to which the Council of Scientific and Industrial Research, or other Research Councils, should follow the lead of the National Science Foundation and enter into the study of documentation problems Meanwhile, however, the Council could well expedite its own account of its activities in support of university research and training, for even if such activities have only an indirect effect on industrial efficiency, that effect is vital, and the Department's contribution to the supply of Britain's trained manpower and its most effective use is of increasing rather than diminishing importance

NEWTON'S CORRESPONDENCE, 1688–1694

The Correspondence of Isaac Newton
Vol 3 1688–1694 Edited by Prof H. W. Turnbull, F R S Pp xviii + 445 + 6 plates (Cambridge At the University Press Published for the Royal Society, 1961) 147*s*. net

THE review of this third volume of *The Correspondence of Isaac Newton* must open on a melancholy note Its preface is still written by Prof H W Turnbull, but a note tells us that he died before the volume was published, and the preface itself ends with a paragraph recalling the sudden death in 1960 of Mr W H Robinson, who had seconded him in the preparation of the first three volumes In starting the great undertaking with such devoted care and brilliant scholarship, they have earned the gratitude of many generations of historians of science We may be sure that the continuation of the work, in the hands of Dr J F Scott, will fully uphold the high standard of excellence set by the initial volumes

In its outlay the present volume follows the same pattern as those preceding it A new feature is a list of errata and addenda to the latter, which in particular contains important corrections to the notes on chemical matters Even so, that these errata do not fill more than three pages is a striking testimony to the quality of the editorial commentary, a quality which is as high as ever in the volume under review, in spite of the greater variety of the topics occurring in it

In fact, there is a noticeable change in the general character of the correspondence of this period, which corresponds to an actual change in Newton's preoccupations after the publication of the *Principia* One may say that this publication marks the end of his scientific creative period, certainly he remained active in science until his last days, but the work he did —mainly centred around the preparation of two further editions of the *Principia* and the publication of several editions of the *Opticks*—was no more than an elaboration of his early ideas Moreover, his active participation in politics, and afterwards his anxiety to obtain some administrative office, show his determination to abandon his studious college life However, it would be wrong to think that the later correspondence would not have the same interest for the study of Newton's scientific work as that contained in the two first volumes For Newton is so reticent in his writings, especially about his more general conceptions, that a full understanding of his thought cannot be gained from his books and published papers alone no scrap of evidence in this respect may be neglected, however indirect it may seem, and since to the last Newton's meditations did not cease to concentrate on the same basic ideas, late documents are just as precious as early ones in helping to elucidate these ideas Thus, this third volume, which covers the six years following the appearance of the *Principia*, is just as packed with material of scientific importance as those preceding it Besides, it contains, in greater amount than the latter, documents illustrating Newton's personality and his wide interests in human affairs

Much of this documentation is published here for the first time, some had been published before, but only in comparatively inaccessible places and often in imperfect form and even the pieces well known since Brewster's time acquire an enhanced value by appearing in such a complete, chronologically arranged collection of authentic texts Of special importance for the history of Newton's mathematical and astronomical investigations are the memoranda drawn up by David Gregory after conversations he had with Newton We must be grateful for the editor's decision to include them, and yet regret that he left out a most interesting manuscript (mentioned in note 10, p 339) given by Newton to Gregory, of which only extracts are available in print, these fragments, containing early drafts of some parts of the famous *Scholium generale* which concludes the later editions of the *Principia*, are remarkably revealing for the evolution of Newton's philosophical outlook, and an authentic reproduction of the whole document is highly desirable About this vexed question of including pieces which are not strictly letters, the editor apparently had second thoughts, he has inserted in the present volume three early documents of the greatest historical importance, the regular place of which would have been in the first volume Such inclusions, even belated, can only be encouraged, however, later insertions ought not to be made at arbitrary places (as in the present instance of Nos 347, 348, 349) but preferably at the end of the volumes, otherwise, their location is likely to cause much inconvenience

The impression made by the appearance of the *Principia* is well illustrated in this volume, in which we can study the reactions not only of specialists like Huygens, Leibniz or Gregory but also of cultivated laymen such as Locke and Bentley Of particular value in this respect is the correspondence of Fatio de Duillier with Huygens, Leibniz and Newton (the latter hitherto unknown), Fatio was an enterprising young man (he was born at Basle in 1664) studying at first hand the new discoveries in mathematics and astronomy—a thrilling experience, which may be visualized by those of us who made their first steps in physics in the twenties He must have been of a particularly engaging disposition, for he is treated by the three great men who knew him with more than usual kindness and esteem The letters exchanged between him and Newton reveal to us an unexpected, and rather pathetic, fatherly attitude of the latter towards the candid youth who trusted him and showed intelligent interest in his ideas

Among the many points of interest in the correspondence relating to the *Principia* I shall especially mention the surprising error committed by Newton in the 37th proposition of the second book, in which he asserts that the velocity of flow of a liquid from a hole at the bottom of a vessel is the same as that acquired by a body falling freely from rest through a height equal to *half* that of the liquid in the vessel Although Huygens immediately saw (see No 344) the contradiction between this result and the law of conservation of energy (which he had recognized as the main principle of mechanics), we learn (note 3 to No 371) that Fatio de Duillier, who likewise discovered the error, could only dissuade Newton from his opinion by performing experiments to the purpose No more convincing proof could be found of how foreign ideas of conservation were to Newton's conception of force, in contrast to the Continental tradition of Stevin, Huygens and Bernoulli

Of great moment also are the well-known tract *De natura acidorum* (No 387), in which Newton develops striking considerations about the constitution of matter, and the correspondence with Bentley concerning the teleological interpretation of the peculiarities of the solar system in support of the existence of a divine providence It is not easy for the modern reader to pass fair judgment over what must appear to him a rather naive way of appealing to finalistic arguments, in this respect it is instructive to observe, however, that even such a bold and comparatively unprejudiced thinker as Huygens was moved to adopt a deistic opinion by the consideration of the apparently teleological organization of life In any case, the evidence we have over Newton's own philosophical thinking, scanty as it is, compellingly suggests that the conciliation of his belief in a personal God with the exigencies of the rationalistic conception of natural philosophy was not reached without a mental struggle of really tragic proportions It can scarcely be doubted, in particular, that his virtual retreat from scientific pursuits, and the steps he took to seek a public office, precisely at the time when he had apparently won his greatest triumph in science, is at least partly motivated by a sense of dissatisfaction, or even frustration, at his inability to achieve harmony in a comprehensive view of the universe That he in those years underwent great mental strain is shown by the temporary breakdown he suffered in September 1693, evidence of which appears in two letters to Pepys and Locke (Nos 420, 421), which have given rise, both at the time and later to much rumour and unfounded speculation

Although the real cause must lie deeper, it appears that Newton's breakdown was prompted by his disappointment in not being able to secure a position which would have allowed him to escape from the seclusion of his college In 1688 he had been elected, together with Sir Robert Sawyer, to represent the University of Cambridge in the Parliament assembled on the accession of William and Mary Of his not very conspicuous activity in this function the main testimony is a correspondence, duly reprinted in this volume, with John Covel, the vice-chancellor, about the confirmation of the statutes of the University, some other minor information on Newton s election may be found in the study by M B Rex, *University Representation in England 1604–1690* (Allen and Unwin, 1954) He was not re-elected in 1690 and his endeavours in the ensuing years to obtain a suitable office did not go without humiliations which—one gathers from hints in the correspondence (see Nos 377, 384, 420, 423)—he seems to have acutely resented

Newton's interest in public affairs cannot be separated from his earnest and deep-going studies of ancient history and especially of the early history of Christianity, in which he sought the foundation of political law and order in the same spirit as he tried to derive from the analysis of natural phenomena the law and order which God had designed for his creation In this volume we are given the first correct text of one of his most considerable works in this field, together with two previously unpublished additions to it (Nos 358, 359, 360), and are thus enabled to form an opinion of the true character and value of this little-known and often misunderstood aspect of his thought After reading the long "historical account of two notable corruptions of Scripture, in a letter to a Friend", I do not hesitate to put this pamphlet on a par with the *Provinciales* Certainly, there is nothing in it of the fiery Frenchman's passionate eloquence and biting sarcasm, but the same merciless exposure of fraud and delusion is here as effectively achieved by restrained statement and cool logic This does not mean that Newton's composition is artless he knew how to use rhetorical devices, and it requires great skill to display such erudition without becoming tedious, to develop intricate arguments with such unfaltering clarity and to formulate conclusions with such forceful concision But the greatest beauty of the epistle lies in its candour One sentence in it might be the motto of every scientist "There cannot be better service done to the truth than to purge it of things spurious" L ROSENFELD

PROBABILITY AND INDUCTION

Theory of Probability

By Prof Harold Jeffreys Third edition (The International Series of Monographs on Physics) Pp viii+447 (Oxford Clarendon Press, London Oxford University Press, 1961) 84*s* net

THE appearance of a third edition of this book is an indication of its permanent place in the literature on probability Its avowed object, quoted from the preface to the first edition, "is to provide a method of drawing inference from observational data that will be self-consistent and can also be used in practice" It is thus essentially a book on the use of probability in induction A substantial part of the book is occupied with discussions of statistical testing and estimation procedures, although recent developments in the analysis of time series and other stochastic processes are not seriously considered, so that the discussion of statistical procedures should presumably be regarded as illustrative primarily of the author's approach

In this new edition one or two additions and rearrangements of material have been made; for example, there is some amplification of the discussion of the problem of finding self-consistent rules for assigning values to prior probabilities Such rules, while they tend to make the theory more epistemological and conventional in character, in contrast to inverse probability procedures based on personal prior probability assessments, help to make this work one of the most impressive attempts so far to formulate an explicit induction procedure founded on probability However, I am far from alone in thinking that the procedure still has serious disadvantages as well as advantages, so that the optimum degree of formalization of the induction problem remains a matter for legitimate debate The formal method of assigning prior probabilities to theories and hypotheses not only involves the task of numerical assessment of these probabilities but also first an enumeration of all the hypotheses and an acceptance that one of them is true (compare Einstein's remark that every scientific proposition, being man-made, is false)

Probability can alternatively be studied as a purely mathematical subject, or in relation to observed statistical regularities Statisticians are largely concerned with the use of probability in this last sense, it is important to recognize this, and not slur over it, as advocates of inverse probability have a tendency to do The author's thesis is not helped by such remarks as (in the new preface) "Adherents of frequency definitions of probability have naturally objected to the whole system But they carefully avoid mentioning my criticisms of frequency definitions, which any competent mathematician can see to be unanswerable" This remark is provocative and misleading It ignores the dependence of modern probability theory, as formulated, for example by A N Kolmogorov, on frequency (cf *Foundations of the Theory of Probability*, English translation, p 3 New York, 1950) Jeffreys, in fact, makes no reference to Kolmogorov, and is also in other ways curiously uneven and cryptic in his references to relevant literature Writers such as R B Braithwaite and R F Harrod, who scarcely see eye to eye with Jeffreys, are not listed in the index Even L J Savage and I J Good authors of books advocating inverse probability methods, but with the use of prior probabilities more personal than those of Jeffreys, get no mention One feels that writers on induction have a particular obligation to appraise other points of view fairly and comprehensively, as part of the evidence before them on the merits and demerits of their own case M S BARTLETT

HISTORICAL AND PSYCHOLOGICAL ORIGINS OF SCIENCE

The Origins of Science

An Inquiry into the Foundations of Western Thought
By Ernest H. Hutten Pp. 241. (London George Allen and Unwin, Ltd , 1962) 28*s* net

"THE aim of this book is to explain science from its historical and psychological origins " The drive behind Dr Hutten's enterprise is dissatisfaction with the alleged failure of philosophers to re-fashion the categories of criticism within which the Greeks, through their relative ignorance of science, were compelled to work, his cure for this is to recognize a much less sharp boundary between inner and outer experience, reason and emotion, than has traditionally been the custom, his method, the reinterpretation of concepts in the light of depth-psychology. Thus, scientists may be shocked to learn that the line between a paranoid system and a scientific theory is perhaps very narrow (p 189), similarly natural philosophers, that Thales unconsciously took as the prime stuff the milk in the mother's breast; and mathematical logicians, that the same object served positively as the generator of the idea of the mathematical continuum and negatively as that of the principle of contradiction. To the majority of scientists these notions might be taken as a timely warning to 'keep off' this book, but to do so would, in my opinion, be a grave mistake. For not only has Dr Hutten started many speculative hares well worth chasing, despite the sometimes fantastical country through which they run, but also the relevance of his general thesis and the cogency of his reasoning are largely independent of the adequacy or 'truth' of the sexual objects that from time to time, and almost incidentally, he points to as the prime movers. Once this is accepted, the book can be—must be—read right through with growing appreciation of the importance of the author's message that if, as he believes, the scientific method is the safeguard for the criterion of truth and reality, it can be stripped of its concomitant perils only by being reinterpreted in terms of its human origins in which infantile fantasies play a not inconsiderable part. Moreover, it is in the later pages that the author's former concern with the *Language of Physics* enables him to demonstrate that his thesis rests on no merely transitory psychological fashions.

The exacting reader must, however, be prepared to be charitable in regard to Dr Hutten's loose use of prestige-words like 'the Renaissance' and derogatory words like 'metaphysics'—it is not clear, for example, that an Earth-centred cosmos is necessarily more 'metaphysical' than a breast-centred one ! The book is at times tendentious, often repetitive, sometimes exasperating; to the accusation that it is lacking in order Dr. Hutten would reply that 'a little chaos is always needed for creation'. But whatever its faults, it is never dull, which is more than can be said of many specimens of more 'scientific' writing.

W P. D WIGHTMAN

RADIATION PHYSICS AND HEALTH

Radiation and Health

By Dr Katharine Williams, Dr C L Smith and Dr H D Chalke. Pp ix+206+7 plates (London Longmans, Green and Co , Ltd , 1962) 28*s* net

THIS book was written primarily for medical readers, having its origin in a symposium of medical officers of health, held in 1958. It is rather more than an edited version of the proceedings, but has still inherited some of the common defects of such a publication, particularly in that there is a certain lack of continuity and some repetition, though the editors admit the latter and believe that it may help to emphasize some of the more important points.

The editors have, between them, contributed only three of the eleven chapters, but are backed-up by a good team, some of whom are international authorities in their fields.

Dr Burch and Mr Burns have written very good reviews of their subjects, dose-rates from natural background and radioactive waste disposal. Dr Rundo has covered the difficult problem of biological monitoring in a very able manner, though his mathematical arguments could well have been omitted for this audience, medical officers are generally the first to admit their own limitations in this direction. Dr Williams has written a general survey of public health problems and Dr Chalke has contributed a chapter on legislation and education, the latter is somewhat of a catalogue, but is a useful summary of relevant Acts. In the main, Mr Haybittle's chapter on measuring and monitoring radiation provides a good background, though his statement that photomultiplier dynodes are usually made of beryllium–copper alloy is open to criticism, this material can be used, but antimony–cæsium is nowadays much more commonly used for secondary-emitting surfaces.

Four appendixes cover maximum permissible levels of radiation, maximum permissible concentrations of some radionuclides in air and water, precautions to be taken when working with radioactive materials, and simple first-aid action to be taken in accidents involving radioactive contamination. Of these, only the first is really required in a book of this type. The second is simply a long list of information, which is virtually useless to any but the specialist and incidentally uses an out-of-date grading of radioisotopes in terms of radiotoxicity, it would have been much better to have given references to the appropriate publications. The material contained in the third and fourth appendixes is certainly useful to the user of radioactive materials, but could have been better presented in a chapter devoted to safe methods of handling radioisotopes, an expansion, perhaps, of Mr Dunster's contribution on the use and occupational hazards of radiation, which is rather on the brief side.

While the health physicist will find less to interest him than the title and the authors' names suggest, the book will certainly appeal to many scientists and doctors who wish to learn some of the background, and it should also be comprehensible to the interested layman.

HUGH D EVANS

PROGRESS IN SPACE SCIENCE

Space Research II

Proceedings of the Second International Space Science Symposium, Florence, April 10–14, 1961. Edited by H C van de Hulst, C de Jager and A F Moore. (Organized by the Committee on Space Research—COSPAR, and Consiglio Nazionale delle Ricerche.) Pp xv+1241 (Amsterdam North-Holland Publishing Company, New York Interscience Publishers, Inc , 1961) 200*s*

THIS massive book is a record of the papers presented at the second International Space Science Symposium at Florence in April 1961. During recent years, especially in new and rapidly expanding subjects, like space science, delays in publication have formed a major obstacle to scientific advance, and have been especially serious to workers remote from the main centres of activity. It is therefore very gratifying that this book was published in 1961, within eight months of the conference. The North-Holland Publishing Co , together with Prof Van de Hulst and his co-editors, are to be congratulated on this quick publication, which is all the more creditable because the book runs to more than 1,200 pages, includes abstracts in Russian of every paper, and is handsomely produced, with few errors or misprints. Furthermore, the papers have been edited in an admirably uniform fashion: for example, all the references are numbered and the clumsy names-and-year system is avoided, though no doubt many of the authors originally used the latter method.

The Florence Symposium was limited to certain special topics in space research, which were specified in advance, with the aim of keeping the number of papers within reasonable limits. In practice, however, most potential authors managed to convince themselves that their papers came under one of the specified headings, so almost all aspects of space research are covered. The coverage is, however, not balanced, and prospective purchasers should be warned that there is little about biology and not much about deep-space topics—the Sun, the Moon and the Planets, and the interplanetary medium.

The 105 papers which compose the book are divided into nine parts. Part 1 is on optical tracking, and the 17 papers include reports on experience in optical tracking in the U.S.S.R. by A. G. Massevitch and in the United States by G. Veis and F. L. Whipple. Part 2 contains ten papers on radio tracking. Part 3 is entitled "Dynamics of Satellite Motion", but also includes the first papers on determining the ellipticity of the Earth's equator from satellite orbits, by I. G. Izsak and W. M. Kaula. Part 4 consists of 7 papers on telemetry and data recovery. Part 5 is entitled "Recent Results from Instrumented Satellites and Spacecraft": the 13 papers include two by K. I. Gringauz on the charged particles in the upper ionosphere and at great distances from the Earth, largely derived from measurements by *Luniks* 1–3. Part 6 (five papers) deals with the World Magnetic Survey. Part 7 is entitled "Special Events", and refers primarily to unusual outbursts of solar activity and their consequences (although a special event of a different kind took place while this session was in progress—the orbital flight of Major Gagarin). Most of the 17 papers in this section are concerned with the solar flares of November 1960, and they include L. G. Jacchia's elegant analysis of the large changes in upper-air density during these events. Part 8, with 11 papers, has the title "Reference Atmosphere", and provides the scientific background for the International Reference Atmosphere of the Committee on Space Research (CIRA 1961), which was published later in 1961. Finally, in Part 9, there are 18 papers on research by means of sounding rockets.

Although the book costs £10, it cannot be called unduly expensive and is certainly more rewarding than any space-research experiment which could be conducted at a similar cost. Every library with any pretensions to cover space research should acquire the book.

D. G. King-Hele

CURRENT CANCER TOPICS

Advances in Cancer Research

Vol. 6. Edited by Prof. Alexander Haddow and Sidney Weinhouse. Pp. ix+524. (New York: Academic Press, Inc.; London: Academic Press, Inc., London, Ltd., 1961.) 86*s*.

THIS publication has the same high standard of writing and production as the first five volumes of the series edited by Dr. J. P. Greenstein and Prof. A. Haddow. The series began in 1953, and, except for 1957, volumes appeared annually until 1958. In 1959, Dr. Greenstein died. Now, after a lapse of three years, the latest volume has appeared with Dr. S. Weinhouse as co-editor.

There are eight interesting topical chapters in the present volume. Bodansky discusses changes which occur in enzymes of serum and blood elements in the presence of neoplasia. He notes the unique position of the leukæmic leucocyte for studies of changes in a readily available cancer cell uncontaminated with connective tissue. He also points to the possible use of studies of isozymes or enzyme variants in the detection of specific disease processes in particular tissues. Braun and Wood present a short review chapter on problems relating to the origin and metabolism of plant tumours. This topic has received scant attention in journals dealing with cancer research. Nevertheless, it is pointed out that at the cellular level there are no fundamental differences between the plant and animal kingdoms. The authors end on the hopeful note that malignant tumour cells in general may not be irreversibly altered cells and that it may be possible to bring about the return of such cells to normality.

In systemic therapy with drugs, such as HN2, severe toxic effects may be encountered. In order to minimize these effects, techniques have been evolved for the isolation of tumour areas from the rest of the circulation and for their perfusion with anti-cancer drugs. These interesting new methods are described by Creech and Krementz and the results of treatment of 242 cancer patients are given. Gross deals with the viral ætiology of mouse leukæmias and in a subsequent review chapter Miller discusses factors other than viruses which also elicit murine leukæmia. The interesting finding that thymectomy reduces the susceptibility of mice to virus leukæmia is discussed.

The topic of radiation chimeras is examined by Koller, Davies and Doak. Animals may sometimes be protected from the serious effects of X-irradiation by transplantation of spleen or bone marrow of their own or of some other species. Possible applications in the treatment of human leukæmia and of radiation accidents are described. Timmis discusses the anti-tumour action of purine, pyrimidine and folic acid antagonists and directs attention to a possible relationship between the mode of action of alkylating agents and the purine antimetabolites.

In the last chapter, Weber describes the behaviour of liver enzymes and metabolites during hepatocarcinogenesis. He presents a useful comparison of the rapidly growing Novikoff transplantable hepatoma with the slow-growing Morris hepatoma No. 5123 which is at present receiving a good deal of attention. Among other things, it is noted that these transplantable hepatomas resemble each other in that they fail to respond to injections of substrates which are able to bring about marked enzyme induction in normal liver tissue. Finally, with regard to the difficult question of the essentiality or otherwise of a particular biochemical lesion for neoplasia, Weber notes that this might be assessed by determining whether the re-establishment of a corresponding normal tissue-level leads to inhibition of tumour growth.

The present volume is a valuable stimulating contribution to the literature of cancer research and it is to be hoped that *Advances* will continue to appear regularly in future.

W. J. P. Neish

Proceedings of the Symposium on Algology

Edited by D Raghavan and P Kachroo. (Organized jointly by the Indian Council of Agricultural Research and Unesco South Asia Science Co-operation Office, New Delhi, December, 1959) Pp viii+406 (New Delhi Indian Council of Agricultural Research) n p

THE importance of algae as actual or potential parts of the food supply for the vast populations of Asia (and elsewhere) and as a source of industrial or agricultural products is now well known This symposium was organized to discuss present knowledge and stimulate further work, the latter aspect being the subject of a series of recommendations made by an international committee About thirty papers were given Prof M O P Iyengar, the doyen of Indian phycologists, described several new or interesting green algae in one of the two 'popular' lectures In the other Prof I Tamiya, who has done so much to further the controlled mass culture of algae, reviewed the present use of algae as food and the problems which must be solved before the agricultural or industrial utilization of forms such as *Chlorella* is an economic proposition The quality of the other published papers is rather variable There are accounts of original research work, valuable assessments of present knowledge, accounts of subjects previously reviewed elsewhere and curiosities such as the alleged activation of photosynthesis by sound waves from an electric bell Nevertheless, even if a few parts of the published accounts are not very interesting, the meeting together of so many phycologists to report and discuss matters of mutual interest will have been of great value This symposium is a further welcome example of the practical interest in algae shown by the Indian Council of Agricultural Research J W G Lund

Essay on Atomism

From Democritus to 1960 By Lancelot Law Whyte Pp vii+108 (Edinburgh and London Thomas Nelson and Sons, Ltd , 1961) 16*s*

MR WHYTE clearly regards the present state of thought in fundamental physics as an uneasy stage of lull before the storm His essay is an attempt to analyse the history of atomism for clues to the next series of developments The search does not, of course, result in any new theories nor does it present any startling new evidence It does, however, present existing evidence in a digestible form

The average scientist would associate the origin of the atomic concept of matter with Democritus, but is uneasily aware of a discontinuity in the story before it begins again with Dalton The chronological table and commentary, which occupy nearly half this volume, conveniently plug this gap in our knowledge and also summarize the story after A D 1800 The increasing density of entries in the post-1945 section naturally reflects both the increasing effort in physics and also the impossibility of picking out the really important developments immediately they occur

In his analysis the author reasonably suggests that further major progress will probably involve the re-examination of some tenaciously held article of faith as with the case of parity conservation This is, of course, nothing more than anyone with a broad awareness of the history of science will already have concluded However, Mr Whyte does indicate also what he considers to be the specific points of weakness in present theory and even the requirements of an 'ideal atomic theory' Few would probably agree with him in detail, but his thoughts should serve as a useful basis for fireside discussions among amateur fundamental theorists The book is firmly recommended for this purpose though scarcely to the complete layman J W Leech

Nuclear Energy Simplified

By John Ernest Radford Pp ix+141 (London Macdonald and Co (Publishers), Ltd , 1961) 28*s* net

THIS book is another attempt to make nuclear power and associated topics intelligible to the general reader It does so by simple descriptions and diagrams, avoiding the use of mathematics

The first half of the book outlines the development of the atom, nuclear fission and radioactivity The material is quite well presented but the style lacks clarity and some sections are difficult to follow In particular, the part dealing with the applications of radioisotopes is done in a perfunctory manner and suffers from loose phraseology (for example, p 53, "radiation of low energy intensity") A description of several nuclear reactors and their fuels is followed by a chapter on the design and operation of Calder Hall Possible future developments in the field of nuclear energy are explored, and here the section on thermonuclear processes suffers in that reference is made almost solely to *Zeta*, other machines being ignored The final chapter discusses health hazards from radiations, but unfortunately is misleading and out of date

The overall impression is that too many topics (from atomic structure to waste disposal) have been included in the book with a resulting loss of coherence Basically, however, the book is sound and could be useful background reading for anyone with a little knowledge in this field It is well produced with clear diagrams, a good index and few errors

D I Page

Nuclear Reactor Instrumentation

By M W Jervis (Nuclear Engineering Monographs, No 13) Pp vii+74 (London Temple Press, 1961) 12*s* 6*d* net

"NUCLEAR Engineering Monographs" are intended to give readers a broad outline of particular subjects in return for a modest outlay This latest book in the series certainly achieves this objective with considerable success, the author having covered a great deal of ground in a compact and readable volume The scope is not as wide as the title suggests, the accent being almost entirely on reactors of the gas-cooled graphite-moderated type with only occasional references to other reactor systems

In writing a treatise occupying only 74 pages a major difficulty is to decide what to omit Thus, while the choice of material included is generally good, some of the subject-matter is inadequately presented In the introduction, for example, the simplified treatment of reactor kinetics could have included with advantage the effects of delayed neutrons, the case for wide-range neutron flux instrumentation could have been made more convincingly

Despite these shortcomings, which are ameliorated by the provision of an ample bibliography, the book outlines principles and discusses the construction and application of those instruments which are peculiar to nuclear reactor control and to the safety of plant and personnel The author has justifiably avoided instrumentation commonly encountered in other engineering fields Ian Wilson

INTERNATIONAL CO-OPERATION IN SPACE RESEARCH (I)*

By EDWARD R DYER, jun
National Academy of Sciences, Washington

THE Committee for Space Research (COSPAR) of the International Council of Scientific Unions (ICSU) held its fifth plenary meeting and associated sessions of its Working Groups in Washington, D C, during April 30–May 9 Five days were given over to the Third Annual International Space Science Symposium In addition to this Symposium, COSPAR acted as co-sponsor of two other special three-day Symposia held in Washington during the week before, these were the International Symposium on Rocket and Satellite Meteorology (with the International Association of Geomagnetism and Aeronomy and the World Meteorological Organization) and the International Symposium on the Use of Artificial Satellites for Geodesy (with the International Association of Geodesy of the IUGG) The host organization was the Space Science Board of the U S National Academy of Sciences, headquarters for the meetings and the Space Science Symposium were in the Academy building, but some of the larger meetings were held in the auditorium of the New State Department Building nearby

Prof Maurice Roy, professor of mechanics at the École Polytechnique (Paris), was elected president of COSPAR to serve the remaining year of the term of office of Prof H C van de Hulst of the University of Leyden Other items of business transacted during the working sessions were

(1) The laying of plans for a co-operative international programme for space research during the International Year of the Quiet Sun, which include plans for rocket measurements in the auroral zones and the polar caps (including the southern hemisphere), for a synoptic rocket study of high-altitude winds, with special emphasis on experiments during the days immediately following two chosen dates, and a study of the *D* and *E* regions of the ionosphere during the solar minimum

(2) Acknowledgment of the announcement by the United States of her intention to launch a geodetic satellite with a flashing light, and the encouragement of potential observers with suitable optical equipment to photograph it with the assistance of orbital predictions to be provided by the U S National Aeronautics and Space Administration (NASA)

(3) The adoption of revised and unified codes for the transmission and exchange of satellite orbital data and other measures designed to expedite this process

(4) The extension and clarification of agreements to exchange space research data through the medium of the World Data Centres, as set forth in a revised *Guide to Rocket and Satellite Data Exchange*

(5) The adoption of a number of steps designed to standardize nomenclature, terminology, measurements, etc

(6) Review of work during the past year on the COSPAR International Reference Atmosphere, and plans to extend work toward further refinement of the Reference Atmosphere under the chairmanship of Dr W Priester during the coming year

(7) The establishment of a six-man Consultative Group on Potentially Harmful Effects of Space Experiments, with the membership drawn from such diverse fields as astronomy, radiation physics, atmospheric physics and chemistry, communications, meteorite penetration, and microbiology This group was set up in response to a request from ICSU, and will serve as the nucleus of a body for investigating the possible undesirable consequences of certain space experiments for the environment or for scientific research, for which purpose it will call to its assistance specialists with competence in particular problems on an *ad hoc* basis

(8) Re-confirmation of its earlier offer to assist the United Nations Committee on the Peaceful Uses of Outer Space, especially in the supplying of information on technical questions or in the arrangement of conferences on scientific topics.

(9) Acceptance of invitation of the Academy of Sciences of Poland to meet in Warsaw in June 1963

During the meetings a number of excursions was arranged, namely, to Cape Canaveral, to the NASA rocket launching facility at Wallops Island, Virginia, and to the NASA Goddard Space Flight Center, where the new laboratories in which artificial satellites are being built and tested were open to the scientific visitors Social functions included a tea-reception by the National Academy of Sciences, an Academy dinner for officers and national delegates to COSPAR, a reception at the Department of State by the Hon Lyndon B Johnson, vice-president of the United States, and Mrs Johnson, and a reception at the Embassy of the U S S R

The programme for the opening general assembly on May 2 comprised, besides ceremonial speeches of welcome and greetings (including a message from President Kennedy delivered by his scientific adviser), the oral presentation of summary annual reports from the 18 countries adhering to COSPAR on their respective programmes in space science during the previous year and, in most instances, a note on plans for the immediate future As one might expect, the reports of the U S S R and the United States were considerably more extensive than the others, but it is most interesting to see how much can be, and is being, done by those nations without satellite programmes of their own These programmes follow several patterns the launching of high-altitude balloons and rockets for the investigation of cosmic rays, the upper atmosphere and ionosphere, the deduction of useful results from the motion of artificial satellites on the gravitational field of the Earth or on the density of the upper atmosphere and time variations thereof, the building and operation of tracking facilities, both optical and radio, and a considerable amount of theoretical, analytical, design, and developmental work The United Kingdom and the United States announced the launching of *Ariel*, a satellite carrying instruments designed and built by British scientists to measure various properties of the ionosphere and launched under a bilateral international agreement by the United States (see *Nature*, 194, 432, 1962) The Canadians described the topside ionosphere sounder satellite which they have built, and which will be launched by NASA later this year Both these programmes originally received some impetus from COSPAR recommendations on international co-operative programmes

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* Part II will be published in *Nature* of August 11

The Soviet Union's report, presented by Academician A A Blagonravov, who is one of the vice-presidents of COSPAR, summarized results from 115 sounding rocket flights carried out from Franz Josef Land, from the European part of the U S S R , and from expeditionary ships in the Pacific, for the measurement of upper-atmospheric temperatures, pressures, winds, state of ionization, and composition, these last-named revealing the presence of helium in appreciable quantities at heights above several hundred kilometres Some of the firings were conducted during the solar eclipse of February 15, 1961, to study the radiation from the solar corona and its interaction with the upper atmosphere during totality The U S S R report also referred in some detail to the instrumentation and performance of the Soviet Venus probe of February 12, 1961, including pressures, temperatures, magnetic fields and variation thereof in the range from several gammas to several tens of gammas, cosmic rays, interplanetary gas, and solar corpuscular streams with flux densities up to 10^9 particles cm^{-2} sec^{-1} The report also described the epoch-making manned orbital flights of Gagarin and Titov which Titov covered in greater detail at the session on Life Sciences Further results from measurements made with instruments on the *Spacecrafts I* and *III* were reported on the energy spectra of groups of nuclei by atomic weight, the composition of primary cosmic rays, the space and time distribution of particle fluxes at heights of several hundred kilometres, and the quick increases in flux connected with bursts of solar activity Information on the Soviet visual and optical tracking net was presented, together with a statistical summary of observations

Dr Richard W Porter, who is the other vice-president of COSPAR, presented the report for the United States A statistical summary covered 70-odd large sounding-rockets which carried scientific payloads to altitudes ranging from 130 to 7,000 km, and 866 smaller meteorological rockets for measurements of pressure, temperature and winds in altitude regions up to 60–100 km, more than 300 high-altitude balloons, and the successful launchings of 31 instrumented satellites and space probes From the point of view of quantity, work with balloons has been chiefly concerned with cosmic ray and upper-atmosphere measurements, but the report also remarked on the publication of final results from the very high quality direct photographs of the Sun obtained with Princeton University's *Stratoscope I*, and on physiological measurements and space suit tests made with the U S Navy's *Stratolab V* The systematic firing of small meteorological rockets has made possible the making of synoptic maps of the 30–60 km regions, a level very important as a transition region through which some of the immediate effects of solar radiations are propagated from the top downwards. Other rocket measurements in ionospheric physics, ultra-violet, X-ray and γ-ray astronomy will be treated more fully in Part II

The review of American satellite programmes for 1961 and early 1962 included a synopsis of cloud-mapping and infra-red radiation balance results from the *Tiros* weather satellites, the analysis of the effects of the periodic daily and seasonal variations in air density at altitude from the drag on satellite motions, especially *Echo I* and *Explorer IX* (4-metre plastic sphere), ionospheric electron temperatures and ion density profiles from *Explorer VIII*, ionospheric structure deduced from propagation effects of radio-frequency radiation in the range 18 kc/s–40 Mc/s, especially with *Lofti* and satellites of the *Discoverer* series, the mapping of circumterrestrial plasmas and magnetic fields with *Explorer X*, γ-ray mapping of the sky with *Explorer XI*, solar radiation measurements with the Solar Radiation Satellite and with the Orbiting Solar Observatory, launched in March 1962; the determination of the particle energy spectrum and associated magnetic fields out to about 13 Earth-radii carried out with *Explorer XII*, and cosmic ray experiments on some of the *Discoverer* satellites The American man-in-space programme and a few important ground-based experiments related to the space programme were mentioned, especially work in meteorite analysis and in methods of sterilizing payloads that are to be landed on the Moon or planets Dr Porter concluded with a very brief outline of plans for the immediate future and a plea for even greater and more intimate degree of international co-operation, and promised that the United States would continue, or possibly improve on, its already good record in this regard

Third International Space Science Symposium

The Space Science Symposium was attended by more than 500 registered participants and observers from 30 countries Scientists from 14 of these presented an impressive total of 141 papers, distributed into sessions under the following headings (the figures in parentheses indicate the number of papers in each session)

I Upper Atmosphere and Exosphere of the Earth and the Relationship to Solar Disturbances (54)
II The Sun and the Interplanetary Medium (31)
III The Moon and Planets (22)
IV Galactic and Extragalactic Astronomy (8)
V Life Sciences (9)
VI Technologies of Space Research Payload Instrumentation (14)

The apparently somewhat unbalanced distribution among the foregoing subjects reflects the influence of two factors In view of the fact that space research activities are growing at an ever-increasing pace, the programme committee had earlier concluded that in the future it will probably be impossible to cover the whole field of space research every year, and had tentatively agreed to limit the session to a number of selected topics (as indicated by the session headings) Some of these topics would be covered almost exclusively by a small number of invited papers, others by an introductory review paper together with shorter reports on really recent results The other factor was that most of the recent results, it so happened, fitted best into category I The papers had undergone a fairly strict process of selection, a fact which was reflected in their exceptionally high average quality About half the papers were presented by American authors, the U S S R , with 31 papers, made a very strong presentation, while the next largest contributions came from Japan, the United Kingdom, France, Germany and Sweden Authors from Argentina, Belgium, Canada, Czechoslovakia, Greece, The Netherlands, and Poland also presented papers

With such a large ground to cover, this report must limit itself to a few selected topics for more detailed treatment. Omission of a particular paper should not be interpreted as being less important or less representative than those included, but only because I am less familiar with what was reported.

Session I. The Upper Atmosphere and Exosphere

This session began with an invited address on "The Dynamics of the Upper Atmosphere" by Prof. J. Blamont (Observatoire de Meudon, near Paris), who is chairman of the Panel on Synoptic Rocket Soundings of COSPAR'S Working Group II (originally on Scientific Experiments, but now renamed W.G. for the International Quiet Sun Year (IQSY)). This paper discussed the structure of the upper atmosphere, as tentatively deduced from data obtained with grenades and drifting chaff (covering the region up to 90 km), the drift of natural meteor trails observed either visually or with radar (80–100 km), and the behaviour of sodium vapour trails in the rocket experiments at Wallops Island, Woomera, Sardinia, and North Africa (up to about 130 km). Certain regularities begin to appear. There is a decided horizontal stratification, such that successive layers have rather different properties. Below 85 km, the trail disappears quickly. In the region from 85 to 101 or 102 km the cumulus-like development of the trail bespeaks a turbulent mixing régime. Above 102 km, the spreading of the trail is very smooth, implying diffusive equilibrium at that level. The boundary between the two regions appears to be no more than 100-m thick. Regularities in the velocity and direction of the upper atmosphere winds as a function of height and time, and correlations between the wind patterns at two different places are now becoming clearer.

In general, there are no large shears above 130 km, but there are wide variations in velocity and direction in the 90–130 km region, which show a correlation with similar variations at nearby localities that fade gradually to zero at about 400 km. But the pattern of these oscillations is rather similar for points at the same latitude half a world apart. The wind vector above 90 km has rotational components with 12- and 24-h periods, and the direction of the rotation and the vector amplitude between 90 and 130 km correspond to the values predicted from the tidal model. Above 130 km, the wind vector rotates in the opposite sense, which implies some other (perhaps hydromagnetic) mechanism. There is some evidence that the very thin ionized sheets of sporadic *E* found by rockets several times within a kilometre or so of 101 km may result from the concentration there of ions moving with the wind through the geomagnetic field, but in opposite directions above and below this level, in such a sense as to be driven upward from below and downward from above.

Papers immediately following, given by G. V. Groves (Great Britain) on Australian rocket results up to above 100 km and by G. I. Golyshev *et al.* (U.S.S.R.) on stratosphere results obtained from rockets launched from the ship *Ob* in the extreme south-eastern Pacific, gave detailed data related to these same questions.

K. Y. Kondratyev (U.S.S.R.) presented two papers, one of which was a series of detailed calculations of the angular distribution of outgoing thermal radiation as a function of wave-length, based on a model using the latest infra-red absorption data for the atmosphere.

A number of papers dealt with chemical composition of the upper atmosphere as it varies with height. A paper by V. G. Istomin and A. S. Pokhunkov (U.S.S.R.) reported on the abundance of the ions NO$^+$, O$_2^+$, O$^+$, N$_2^+$ and N$^+$ that they derived from rocket-borne mass-spectrometer data. They found a diurnal variation for the first three in the 100–200 km region, and the constant absolute concentration of N$^+$ which they found over the range 300–500 km they attribute tentatively to the high temperature at which they may be formed. In a fairly narrowly limited region at 101–105 km, Istomin found abnormal concentrations of Mg$^+$, Fe$^+$ and Ca$^+$, at concentrations of 10^{-4} cm^{-3}, which is about equal to the average night *E*-layer electron concentration, both the altitude and the identity of the ions strongly suggest a meteoric origin, and the ion concentration suggests that meteors play the principal part in maintaining the night *E*-layer.

A. E. Mikirov (U.S.S.R.) reported on sky-brightness measurements from 80 km and higher which can best be explained by the presence of aerosols of the order of 1μ in diameter with a concentration of about 10^{-3} cm^{-3}.

Gordon Newkirk and J. A. Eddy (High Altitude Observatory, Colorado) analysed their photometric measurements of the solar aureole obtained with a stabilized balloon-borne solar coronagraph at heights up to 25 km, they calculated the distribution in size and concentration of aerosols and conclude that since the concentration in the diameter-range 0.2–5 microns far exceeds what could be borne aloft by air currents and diffusion the aerosols must be micrometeoritic infall.

Aono and Hirao (Radio Research Laboratories, Japan) discussed their findings from a variety of different ionospheric probes carried on nine different rocket flights to heights up to 350 km, the most striking feature of their results was an excess of ions over electrons in the 100–115 km region. Bourdeau and Bauer (Goddard Space Flight Center, NASA), combining rocket measurements from above the *F*2 maximum with *Explorer VIII* results, show that the 1,000-km region is nearly isothermal, but the temperature varies with local solar time and activity, more important, they showed the existence of a region in which He$^+$ is the predominant ion, above the O$^+$ layer and below the protonosphere. Pokhunkov gave results from rocket-borne mass-spectrometer measurements which showed evidence of gravitational separation of several elements and molecules.

Several other papers reported on ionization densities deduced from propagation phenomena. for example, the steady night-time value of 3 × 10^4 electrons/cm^3 above 600 km, with an occasional peak in the auroral zone up to 2 × 10^5 (Ulwick and Pfister, United States, using 7.2 Mc/s *Discoverer* data), three absorption maxima per day recorded at Athens with a 58 Mc/s riometer (Anastassiades), a thorough analysis of Faraday rotation effects (Argence, France, and Rawer, Germany), and an electron profile from 100 km to 5,000 km obtained with incoherent scatter radar in Peru, giving a concentration of 10^5 at 600 km, 10^4 at 100 km, and 10^3 at 4,000 km, with evidence from the scatter spectrum that the protonosphere begins at about 2,000 km (K. Bowles, U.S. National Bureau of Standards). These papers were followed by several others which examined the fine structure of the ionosphere: radar studies of aurora-related structures, showing evidence of alignment with the geomagnetic field, and having r.m.s. velocities of about 100–200 m/s deduced from fading frequencies (Egeland, Ortner, and Hultqvist, Kiruna, Sweden); evidence for ionospheric irregularities from thousands of km down to 1 km in diameter, deduced from the time constant and magnitude of the angular refraction of 20 Mc/s signals from *Sputniks I–III* and *Explorer VII* (Paetzold and Zschorner, Germany); the existence of ducted modes of propagation due to parallel field-aligned irregularities spaced several km apart in the 300–1,000 km region, detected with a rocket-borne topside sounder (Van Zandt *et al.*, NASA).

A thorough and interesting theoretical study by Y. L. Alpert *et al.* (U.S.S.R.) described the wake produced by a satellite moving through a plasma and a magnetic field with a velocity much greater than the thermal velocity of the plasma particles, taking into account the electric field resulting from the charge on the satellite. The scattering cross-section of the disturbance is much greater for a body moving parallel to the field lines than across them. They programmed a computer with their formulæ to calculate the effects to be expected for altitudes in the range 300–700 km.

Papers by Groves and McDermott (University College, London), L. G. Jacchia (Smithsonian Astrophysical Laboratory), D. G. King-Hele (Royal Aircraft Establishment, England), O'Sullivan, Coffee and Keating (NASA, Langley Space Flight Center), H. K. Paetzold (University of Cologne), and Harris and Priester (NASA, Goddard Space Flight Center) all examined various aspects of the relationship between solar radiation or disturbances and the heating of the upper atmosphere, as shown by variations in atmospheric drag on satellites. Groves and McDermott deduced a close correlation between air density derived from the motion of *Discoverer XVIII* and the geomagnetic K_p index.

Jacchia, using the motions during 1958–61 of eight different satellites, with perigees of 350–750 km, finds that Nicolet's 1961 model atmosphere, based on diffusive equilibrium in which helium plays an important part, is consistent with his results, that the 27-day density oscillations previously discovered correspond to a temperature variation of 2·5° times the F_{10} flux index of 10·7-cm solar radiation, that diurnal sub-solar bulge lags 30° behind the sub-solar point on the average, and that both Paetzold's semi-annual effect and the secular decrease in density since solar maximum should probably be attributed to the varying level of corpuscular flux and the heating it produces. Similarly, Paetzold has with considerable success separated the effects that are correlated respectively with solar decimetre radiation, solar altitude, and the aspect that the Earth presents to the moving interplanetary plasma, the measure of his success being that he feels that he can predict the drag with an error of less than 15 per cent, although the temperature in the exosphere varies between extremes of 600° and 3,000° K and the density by factors up to 100. O'Sullivan, Coffee and Keating's results, based on the motion of *Explorer IX* (4-metre plastic sphere), also bear out the foregoing results; they found a decrease in density at 700 km by a factor of 10 between 1958 and 1961.

King-Hele carried out analysis of the variation in scale height in the 200–500 km region during the period 1958–61, using the decrease in perigee height and period of 14 satellites with nearly circular orbits; at 275 km the scale height decreased from 60 to 40 km during this time. (King-Hele also gave one of the three invited evening lectures, in which he explained these results in a slightly more popular way, adding by way of context a very lucid and masterly summary of practically everything that has been learned about the upper atmosphere during the past several years.) Finally, Harris and Priester described a model of atmospheric heat balance which takes into account heating by solar extreme ultraviolet radiation and by corpuscular radiation, showed tables of physical parameters as function of local time and height calculated on this model, and compared them with the observations.

The next group of papers was concerned with the complicated relationships between reaction-rates, chemical composition, incident and emitted radiation, differential escape-rate, and so on, as they affect the state of the upper atmosphere. V. I. Krassovsky's paper provided a comprehensive introductory review. A. D. Danilov's paper derived ionization and recombination co-efficients in the 100–400 km region from the day-to-night variation of electron densities, and concluded that the large cross-section demanded suggests that the incident flux responsible for ionization is composed of soft electrons. Both Hanson (United States) and Ivanov-Kholodny (U.S.S.R.) dealt with a reconsideration of earlier models in the light of new data. Nicolet (with Kockarts, both of Belgium) described a model atmosphere which took into account the production of ^{3}He and ^{4}He, the diffusion of these and H through the atmosphere, and their rate of escape, all as a function of temperature, leading to vertical profiles for these elements. The vertical distribution of O, OH and other elements contributing to the line emission spectrum of the airglow as deduced from photometric measurements was discussed by Miss Tarasova; of particular interest is the evidence for concentration of OH in a layer at 73–100 km.

NATURAL RESOURCES OF THE MOON

By Dr. JOHN W. SALISBURY
Lunar-Planetary Exploration Branch, Geophysics Research Directorate, Air Force Cambridge Research Laboratories, Bedford, Mass.

THE importance of lunar natural resources for the future of space exploration can scarcely be exaggerated. Lunar resources will not only play an important part in the establishment of a lunar base by providing life support materials and vehicle fuels but will also be an important, and perhaps a limiting, factor in the logistics of interplanetary space exploration. Certainly, only the most cursory exploration of the solar system could be conducted using either existing or planned propulsion systems so long as the rocket vehicles must lift all their fuel from the surface of the Earth. A lunar fuel source, on the other hand, would provide an extremely convenient low-gravity refuelling station in space.

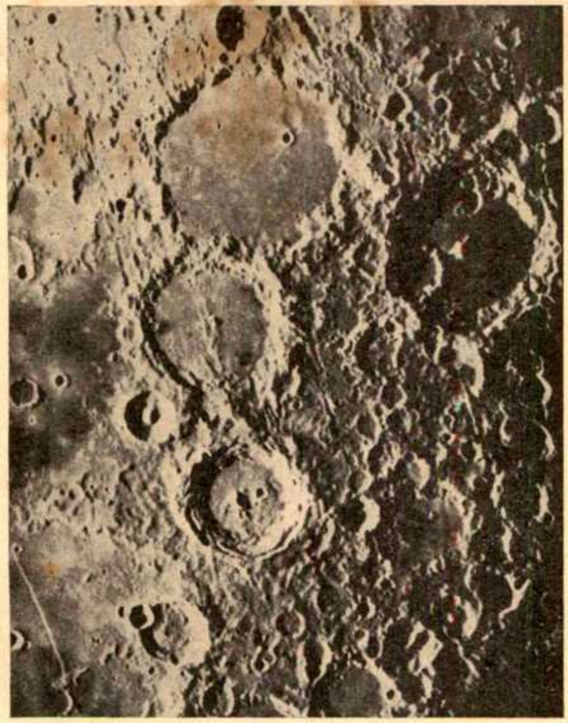

Fig. 1. The crater Alphonsus (slightly left of centre), where Kozyrev reported a cloud of luminescent gas associated with the central peak. Alphonsus is 120 km. in diameter. (*Photo: Mt. Wilson Observatory*)

Despite its smaller size, it is even less practical to make a random search for resources on the Moon than it would be to do so on Earth because of the immensely greater expense involved. It is necessary, therefore, to develop and test reliable theories of lunar mineral deposition which will predict the location of valuable mineral deposits, so that the search for lunar resources may be directed to the most promising locations.

It should be noted that, although limited amounts of useful materials, such as water, should be present in the solid state in permanently shadowed zones on the lunar surface[1-3], such mineral deposits are not considered here to be significant as lunar resources. Though relatively common, they would be small, scattered and, except at the poles, more or less random in their location. Thus, mining such deposits would not generally be economical, and they would probably correspond in usefulness to pegmatite deposits on Earth.

To be actually useful in lunar and interplanetary exploration, mineral deposits must be large, centralized, and predictable in their location. It is the purpose of this article to attempt to predict the nature and location of such deposits, and to speculate on the major processes acting in their formation.

In an investigation of the nature of lunar resources, it is necessary to define what would comprise a mineral deposit on the Moon. Certainly the richest vein of gold could not be economically mined, nor could most of the minerals which are typically of high value here on Earth. Water, on the other hand, would be extremely valuable, for several reasons. After hydrolysis, the oxygen obtained from water would also be a critical supporter of life, and the hydrogen and oxygen could provide a base reserve power supply stored in fuel cells. It appears, therefore, that at least during the early decades of space exploration, water will be the most valuable of all substances on the Moon.

Having established that water is the most valuable resource to have on the Moon, it becomes necessary to determine what evidence exists to suggest where to find it in large centralized deposits.

The first line of evidence is Kozyrev's[4] observation in the crater Alphonsus (Fig. 1) of a gas having spectral emission lines characteristic of the C_2 molecule. The C_2 molecule has been explained by Urey[5] as forming from acetylene, which could have been produced by the action of water on calcium carbide.

A second line of evidence indicating the presence of water deposits is suggested by the lunar chain

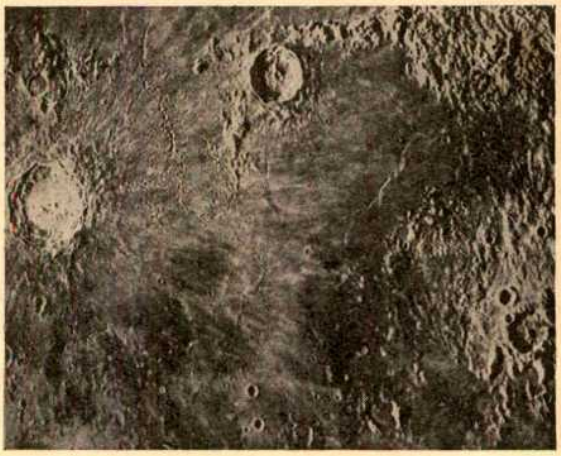

Fig. 2. Chain craters (upper left) running north–south between the craters Copernicus (extreme left) and Eratosthenes (top centre). Copernicus is 90 km. in diameter. (*Photo: Mt. Wilson Observatory*)

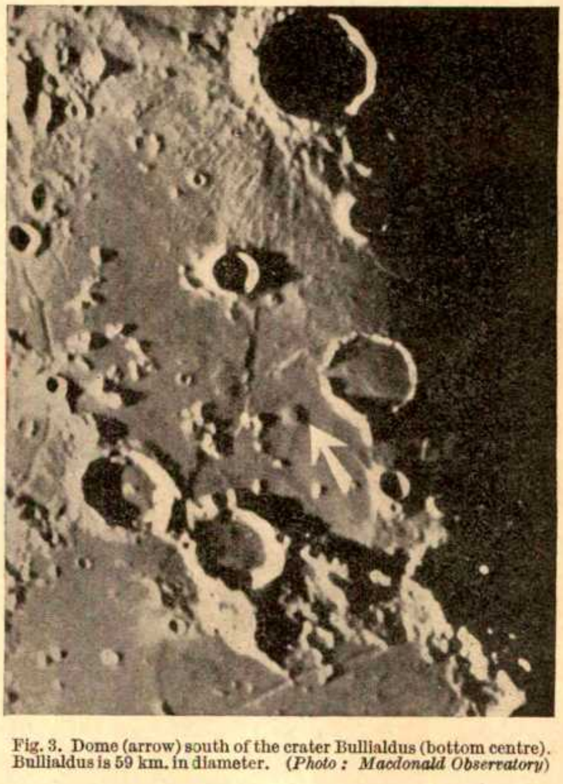

Fig. 3. Dome (arrow) south of the crater Bullialdus (bottom centre). Bullialdus is 59 km. in diameter. (*Photo: Macdonald Observatory*)

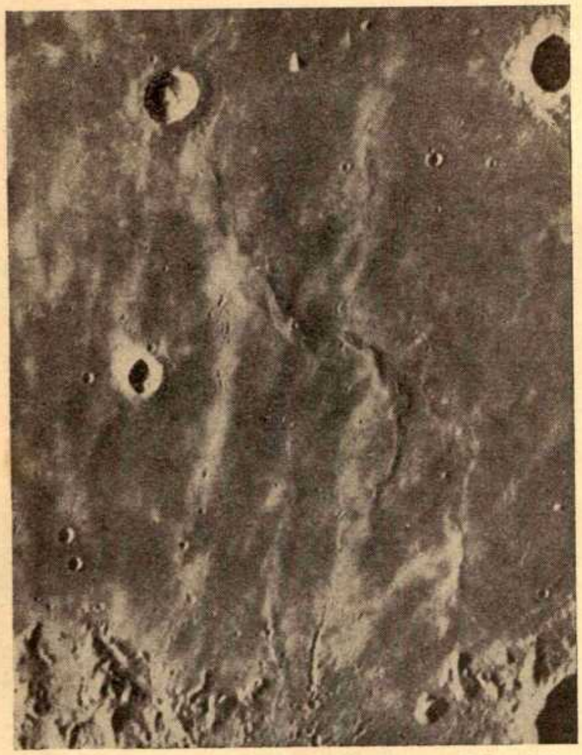

Fig. 4. Wrinkle ridges (centre) on Mare Imbrium. Ridge width varies from 1 to 10 km. (*Photo: Mt. Wilson Observatory*)

craters. Perhaps the best-known example of this phenomenon is the group of chain craters running north–south between the craters Copernicus and Eratosthenes (Fig. 2). In discussing the origin of these features, Baldwin[6] stated that chain craters rarely, if ever, show raised rims and are thus set aside as a distinct class from the majority of normal craters. He also stated that there does not seem to be any question but that they are volcanic blowholes of some kind and are directly the products of gases contained in the lunar crust. Recently, Hibbs[7] has suggested that such craters could have been formed by the impact of meteorites which were fragmented by gravitational forces as they approached the Moon, but this seems very unlikely. If, then, chain craters indicate release of gas, the question remains as to whether or not water was a major component of this gas. Here, one may fall back on a terrestrial analogy, noting that water is by far the most abundant volatile substance involved in volcanism on Earth. In addition, one may again refer to Kozyrev's[4] observation, as interpreted by Urey[5], indicating that water is involved in at least one gas release from the lunar interior. It appears a reasonable assumption, therefore, that a large amount of water was associated with the production of chain craters, and that some of this water is still available in some form at chain crater locations. Again following a terrestrial analogy, it is a reasonable supposition that the initial fumarolic phase of release of hot gas in crater formation would slowly degenerate into a hot spring phase of liquid flow, followed by a cold spring phase and then quiescence. This last phase would probably entail gradual sealing off of the liquid flow channels with deposits of dissolved minerals, most likely quartz. Considering that the present steady-state lunar subsurface temperature is between −23 and −50° C. (refs. 8–10), there might possibly be large deposits of ice sealed off beneath the surface during this process, depending on past and present lunar thermal gradients. Certainly considerable amounts of hydrated minerals suitable for simple water extraction would be expected.

A final line of evidence suggesting the presence of large amounts of water beneath the lunar surface is supplied by the maria domes and wrinkly ridges. I have suggested in a previous paper[11] that the increase of volume due to serpentinization (hydration) of olivine by slow water leakage from the interior could be the cause of lunar domes. Urey (personal communication) has extended this concept to apply also to the origin of wrinkle ridges. If these hypotheses are correct, two additional lunar water deposits are identified, although here the element of doubt is much stronger than in the case of the chain craters.

Despite an element of doubt, it would seem that there is good evidence for the presence of large, centralized, sub-surface water deposits on the Moon. This being so, it seems pertinent to speculate at this time about the possible origin of the water in these deposits, and especially about any possible interrelationships between deposits.

One characteristic of chain craters which may be related to their origin is their association with rilles, which are linear depressions in the lunar surface. Each chain crater depicted in Fig. 2, for example, is connected by a rille. At the northern end of the line the individual craters begin to merge until they almost take on the appearance of a normal rille extending out into Mare Imbrium (Fig. 4). Many other examples can be cited of rilles with similar craters spaced along their length or at their beginning and end (Fig. 5).

Rilles appear to bear a relationship to lunar crustal fractures, as well as to crater chains. Fig. 6 demonstrates that rilles commonly parallel the lunar conjugate fracture system, which has been mapped by Bulow[12], Fielder[13], Hackman[14] and others.

According to Baldwin[6], not only the orientation but also the location of rilles is important He pointed out that the rilles, though subsequent to the maria, are so clearly associated with their margins that some characteristic of the maria must have been the cause of these features. The same marginal association is also commonly true of domes and

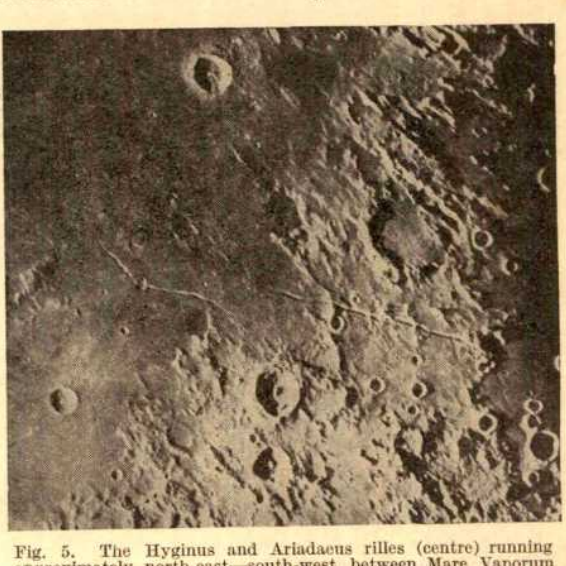

Fig. 5. The Hyginus and Ariadaeus rilles (centre) running approximately north-east—south-west between Mare Vaporum and Mare Tranquillitatis. Mean rille width approximately 8 km. (*Photo: Mt. Wilson Observatory*)

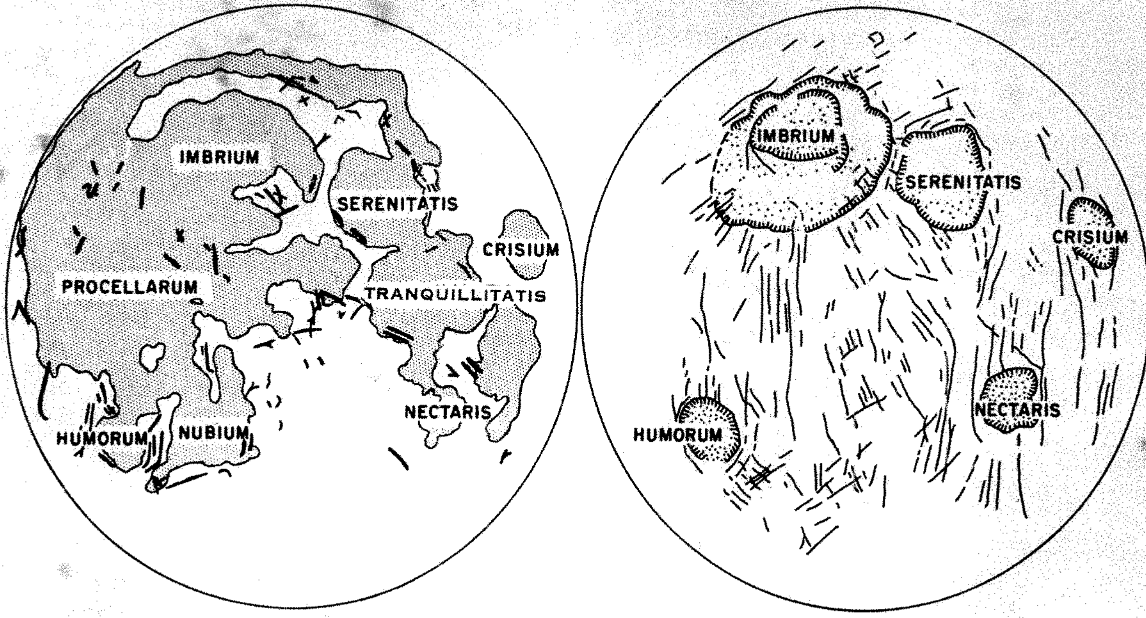

Fig. 6. Distribution of major lunar rilles (left), modified from Baldwin (ref. 6). Simplified lunar tectonic pattern (right), modified from Bulow (ref. 12)

wrinkle ridges, leading to the same conclusion as to their origin.

The origin of all the interrelated features discussed here, and the probable water deposits associated with them, can be explained as resulting from processes acting during and after the formation of the maria. All major theories of maria origin hold that they are composed largely of lava. Some disagreement remains, however, on the internal structure of maria in general, and of some maria in particular. Even so, a reasonable hypothesis is that the circular maria are not made up of many thin layers or sheets of lava, but originated as giant lava pools (ref. 6 and Lowman, 1962, unpublished).

A very idealized lava pool is followed through its cooling cycle in Fig. 7, during which the following phases might be expected to occur:

Phase 1. The lava pool is created and then forms a highly vesicular crust.

Phase 2. The vesicular crust acts as a slowly thickening insulating blanket, sealing off the pool from the surface, causing the rate of cooling to drop sharply. It would then be expected that crystallization processes would follow those of a buried magma chamber (for example, Stillwater Complex, Skaergaard Complex). Normally, this appears to involve initial formation of high-temperature minerals, their settling through the melt to the bottom of the pool, and slow differentiation of the melt as lower and lower temperature minerals are formed and withdrawn from the remaining liquid as it cools.

Phase 3. Reduction in volume with crystallization will produce sinking of the central portion of the crust. Thus, volatile constituents of the magma, which are largely excluded from the crystallized minerals, are gradually segregated near pool margins.

Subsequent to formation of maria and concentration of volatile substances near maria margins, as described here, it is suggested that lunar seismic shocks produced by meteoritic impacts or internal disturbances revitalized pre-existing fractures in the lunar crust. Such revitalized fractures were then able to tap many of these gas concentrations. Rapid escape of this gas produced circular blowouts (chain craters) along some fractures, which commonly were so closely spaced as to produce a general widening of the fracture to form a rille. Fracture widening would be aided by graben-like collapse with the release of pressure. Slow leakage of this gas, on the other hand, would produce either wrinkle ridges or domes as a result of a hydration process. Water remaining below the surface to-day might cause the release of

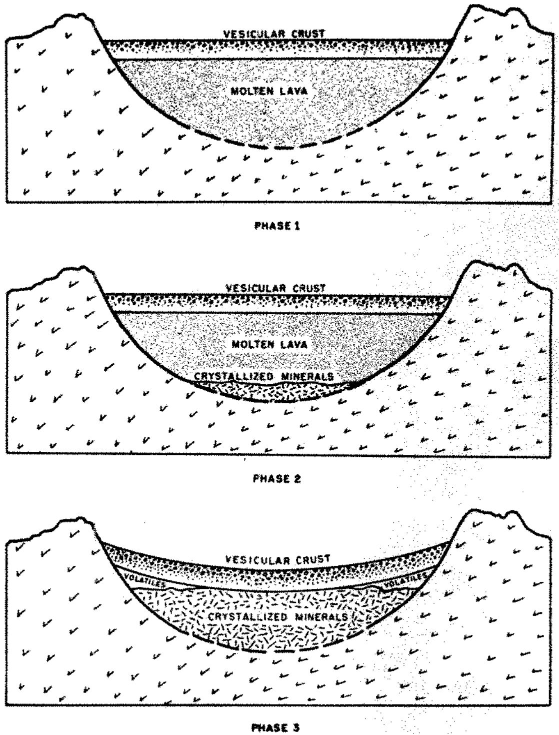

Fig. 7. Phases in the creation of volatile concentrations at maria margins. Mare is several hundred km. in diameter; vertical scale greatly exaggerated

acetylene from silicon carbide It is of interest in this regard to note that Alphonsus, site of a present-day release of gas, has associated with it both a crater chain and at least three rilles

Continuing experimentation is, of course, required to verify the speculations outlined here The lunar seismic experiment proposed by Press *et al*[15] may accomplish this, or at least give rough indications of structure It is especially important that more advanced lunar probes, such as *Surveyor*, do some of their proposed core drilling in the vicinity of rilles for more definite information Other techniques sensitive to water or hydrogen, such as neutron albedo measurements, would most appropriately be used over rilles, domes, wrinkle ridges, and chain craters

I thank Paul D Lowman, Eugene M Shoemaker, Robert C Speed and Roger A Van Tassel for helpful suggestions

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ASPECTS OF THE LIFE AND WORK OF JAMES CLERK MAXWELL*

By Sir JOHN RANDALL, F R S

King's College, University of London

THIS article, unlike others of the series, has as its object the consideration of Maxwell's own life and work rather than the consequences of his scientific genius, and for an obvious reason pays particular attention to Maxwell's association with King's College, London, during the greatest period of his scientific life

I shall begin with a minute of the College Council of July 13, 1860, which records a report of the Committee appointed to consider the applications sent in by candidates for the chair of natural philosophy There were five in number, but their names, apart from that of the successful candidate, were not recorded Committees were apparently less assiduously attended in those days, for the minute continues

> " 3 That Mr Green, the only member of the Committee who was present, saw these gentlemen and with the assistance of Professors Hall and Miller very fully considered their merits
>
> 4 That Mr Green, Professors Hall and Miller, unanimously concur in recommending Mr James Clerk Maxwell, Second Wrangler and Second Smith's Prizeman in 1854, late Fellow of Trinity College, Cambridge, and now Professor of Natural Philosophy in the Marischal College and University of Aberdeen, for the Chair of Natural Philosophy in this College "

It was resolved by the Council to elect Mr James Clerk Maxwell to the professorship of natural philosophy Maxwell was twenty-nine years of age and remained at King's College, London, for five years Nevertheless, it was during this brief period that some of his most important work was carried out It was from King's College, London, that his paper "Physical Lines of Force" (1862) and his greatest paper, "A Dynamical Theory of the Electromagnetic Field" (1864), were published This paper contains not only the famous equations of the electromagnetic field but also the substance of the prediction of the electromagnetic theory of light During the King's College period Maxwell also took part in the determination of the absolute unit of resistance, and carried out accurate colour-box measurements on the response of the normal and colour-blind eye His important investigations of the dynamical theory of gases and experimental work on the variation of viscosity of gases with temperature and pressure also belong to this period Furthermore, note-books in the possession of the College show that Maxwell planned his famous *Treatise on Electricity and Magnetism* in London (Fig 1), although it was probably only worked out in full during the period of retirement at his home, Glenlair, from 1865 until 1870 The *Treatise* was published in 1873 during Maxwell's tenure of the chair of experimental physics at Cambridge

While it is difficult in 1962 to say anything outstandingly new or original about Maxwell, I hope, perhaps by virtue of my own association with King's College, London, since 1946, to add something useful to what has already been said

Maxwell has been described by his biographers as one who has enriched the inheritance left by Newton and has consolidated the work of Faraday, a man of profound and original genius and one who was also one of the best men who ever lived

James Clerk Maxwell was born in Edinburgh on June 13, 1831, the son of rather well-to-do middle-class parents After marriage, Maxwell's parents had moved to the estate of Middlebie on the borders of Dumfriesshire and Kirkcudbrightshire Here, at Glenlair, J C Maxwell spent his early years and received his early education at the hands of a private tutor He seems to have shown considerable interest in, and curiosity about, his surroundings and was always asking questions it is difficult to know whether he showed outstanding characteristics at this age, since the official account is somewhat submerged in Victorian piety and adulation

From the age of 10 to 13 Maxwell was a pupil at the now well-known Edinburgh Academy which had been founded in 1824. He does not seem to have been happy in these surroundings, where his country ways and clothes were the object of much ridicule and where he was given the nickname of 'Dafty' While still at school he wrote a paper of a geometrical character that was accepted through Prof Forbes for reading before the Royal Society of Edinburgh During 1847–50 he attended classes at the University of Edinburgh, where he studied, among other things, certain aspects of physics, chemistry and mathematics In October 1850, Maxwell entered Peterhouse at Cambridge, but later moved to Trinity, where there were said to be greater prospects of obtaining a mathematical fellowship In 1854 he graduated as Second Wrangler in the Mathematical Tripos and was also Second Smith's Prizeman He published in 1856 the essay which won for him the Adams Prize "On the Stability of the Motion of Saturn's Rings"

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* Based on the first of the lectures delivered at King's College, London, to mark the centenary of the publication of Clerk Maxwell's papers on electromagnetic radiation It is hoped to publish these lectures in book form

In 1856, Maxwell was appointed to the chair of natural philosophy at Marischal College, Aberdeen He held this appointment for three sessions until the professorship was suppressed by the fusion of the two Colleges to form the University of Aberdeen In 1860, Maxwell applied for the chair of natural philosophy at Edinburgh but was unsuccessful His old school friend, P G Tait, was appointed But in the same year he was, as we have seen, appointed to the chair of natural philosophy at King's College, London, a post which he held until 1865 Maxwell, in his short life of forty-eight years, published more than a hundred scientific papers Excluding the geometrical juvenilia, some papers on mechanics and the Smith's Prize Essay already referred to, Maxwell's papers fall into three groups (1) colour vision and optics, (2) molecular theory (including heat and thermodynamics), (3) electricity and electromagnetic theory

Maxwell's prediction that light consists of electromagnetic waves was a consequence of his work on electromagnetic theory and not on optics as such

Colour Vision

It will not be possible here to give an extended account of Maxwell's contributions to colour vision His conceptions followed basically on those of Newton and Young Newton had demonstrated that white light was made up of all colours mixed in proportion, colour vision was essentially subjective This idea led Young, 100 years later in 1801, to suggest that the eye contains receptors for the principal colours, red, yellow and blue, only The sensation of all the intermediate colours is due to the stimulation of these receptors in varying proportions This hypothesis forms the basis of Maxwell's experiments Maxwell carried out his studies of colour vision using a colour top (originated by Young) and a colour box Hopley (thesis, University of London, 1956) considers that Maxwell should be given credit for the following important advances (1) The demonstration that the response of the human eye to coloured light may be predicted to quite a high order of accuracy (2) The development of the colour box and colour top into instruments capable of making measurements with mathematical precision (3) The discovery of the numerical laws for the evaluation of data from such measurements The method of calculation evolved by Maxwell has been used by all subsequent investigators (4) The demonstration that the sensations of all the colours of the spectrum may be simulated by suitable mixtures of three primaries (including subtraction as well as addition) (5) The application of the trichromatic theory to the reproduction of a colour photograph

In his Friday Evening Discourse to the Royal Institution of May 17, 1861, Maxwell demonstrated the three primary colours by means of three lantern projectors before which were placed troughs containing respectively ferric thiocyanate (for red), cupric chloride (for green) and ammoniated cupric sulphate (for blue) A triangle was thus illuminated so that the pure colours appeared at its angles while the rest of the triangle contained the various mixtures of the colours as in Young's triangle of colour

In the same Discourse, three photographs of a coloured ribbon taken through the three coloured solutions respectively were introduced into the projectors, giving images representing the red, green and blue parts separately When these were superimposed a coloured image was seen which, if the red and green images had been as fully photographed as the blue, would have been a truly coloured image of the ribbon

Comparison of Maxwell's colour-box measurements with those of modern workers such as W D Wright shows that Maxwell's results were surprisingly accurate

Kinetic Theory and Thermodynamics

During the year 1859 Maxwell wrote his very important paper on the kinetic theory of gases Although this was published in 1860, the year he came to King's College, London, it cannot be regarded as strictly of this period One has only to look at standard treatises on kinetic theory of the present day to realize how much the subject owes to Maxwell's clear and penetrating analysis Early attempts in this field had been made by Herapath about 1816 In his derivation of an expression for the pressure of a gas Herapath had, however, assumed that all the molecules were of equal velocity Furthermore, in attempting to relate pressure to temperature, he had assumed that the temperature of a gas is measured by the momentum of its molecules The next step in the development of the kinetic theory of gases was chronologically that of Waterston (1846), although his work was not published until the manuscript was disinterred from the Royal Society's archives by the first Lord Rayleigh in 1892 Waterston introduced the idea of groups of the assembly of molecules with the same velocity The group with velocity v_1 would contribute to the pressure an amount $1/3N_1mv_1^2$ and so on The total pressure would be given by $p = 1/3Nmv^2$, where v is no longer the actual velocity of the particles but a mean velocity given by the equation

$$Nv^2 = N_1v_1^2 + N_2v_2^2 +$$

Thus we have the conception of the mean square, v^2, of the molecular velocity

Also due to Waterston is the statement that the mean square of the kinetic energy measures the temperature This leads directly to the laws of Boyle and Gay Lussac Waterston also examined the properties of gaseous mixtures and showed that "the mean square molecular velocity is inversely proportional to the specific weights of the molecules" This was rediscovered by Maxwell fifteen years later

Although Waterston had mentioned briefly some of his ideas and conclusions in subsequent papers, it appears that it was two papers by Clausius in 1857 and 1859 that stimulated Maxwell to take up the study of kinetic theory These were concerned with the part played by rotational energy of molecules in the heat content of a substance, and with the mean free path Maxwell showed how the velocity distribution idea originally introduced by Waterston must be considered statistically and also succeeded in deducing the law of distribution The number of molecules the velocity of which lies between v and $v + dv$, where v is taken in all possible directions, is

$$N \frac{4}{\alpha^3\sqrt{\pi}} v^2 e^{-v^2/\alpha^2} dv$$

where α is related to the mean value of v^2 by the expression $3/2\ \alpha^2 = \overline{v^2}$

In a later part of his paper Maxwell determined the average number of collisions in a given time and hence, from a knowledge of their velocities, the mean free path of the molecules He then proceeded to show how by means of experiments on the viscosity of gases the mean free path could be determined experimentally

Maxwell used a value for the viscosity of air obtained by Stokes and deduced that the mean free path of molecules in air was 1/447000th part of an inch, and that the number of collisions per second experienced by each molecule was about 8×10^9 He also deduced that the viscosity should be independent of pressure This was the origin of his London experiments on viscosity of gases, and of its variation with temperature (Bakerian Lecture, 1865) These were, however, not very accurate

Maxwell published his "Thermodynamic Relations" in the fifth edition of his text-book, *The Theory of Heat* (1877) Hopley points out that he used an effective geometrical method of demonstrating these results, but it is not clear that their derivation was achieved in this way

As Glazebrook and others have pointed out, Maxwell's work on the subject of heat and kinetic theory would in itself have been sufficient to secure for Maxwell a distinguished place among those who have advanced physical knowledge His greatest work, however, his theory of the electromagnetic field, was still to come

Electricity and Electromagnetic Theory

Maxwell's first contribution to the study of electricity was made at the age of twenty-three in a paper on "Faraday's Lines of Force", read to the Cambridge Philosophical Society on December 10, 1855 We find him writing to Thomson (better known by his later title of Lord Kelvin) on February 20, 1854, for advice on the reading of papers on electricity Maxwell corresponded continually with Thomson and Tait throughout his scientific life, and the former in particular had a most important influence on his ideas At a much later date, 1873, Maxwell stated in the introduction to his *Treatise on Electricity and Magnetism* "Before I began the study of electricity I resolved to read no mathematics on the subject till I had first read through 'Faraday's Researches on Electricity'" Although the lives of Faraday and Maxwell overlapped by some 36 years, for only 13 of these could Maxwell be said to be concerned actively with electrical phenomena

It is of great interest to recall the state of the subjects of electricity and magnetism at the time Maxwell wrote his first paper on 'lines of force' Mathematicians had had great success in applying analytical methods to problems of distribution of charge on bodies of various shapes (for example, the work of Laplace and Poisson) Ampère laid the foundation of his theory in a study of the forces existing between circuits carrying current The essence of all this work was embodied in the conception of 'action at a distance' The space outside an electric charge or magnetic pole did not differ on this view from 'empty' space A quite different standpoint was taken by Faraday, who had been making fundamental experimental discoveries on electricity for some twenty years Faraday originated the hypothesis that lines of force spread out in all directions from a point electric charge or magnetic pole In this sense the space surrounding such charged or magnetic bodies differed from other space To Faraday the lines of force had physical properties, they were not just convenient mathematical abstractions They were in a state of tension which was thought to be responsible for the attractive force between charges of opposite sign The energy of the field was also supposed to exist in the space around the lines of force

Faraday was not mathematically minded and there is not an algebraic symbol to be found in his *Experimental Researches* J J Thomson has pointed out that the mathematicians could not understand him and were rather contemptuous of his ideas

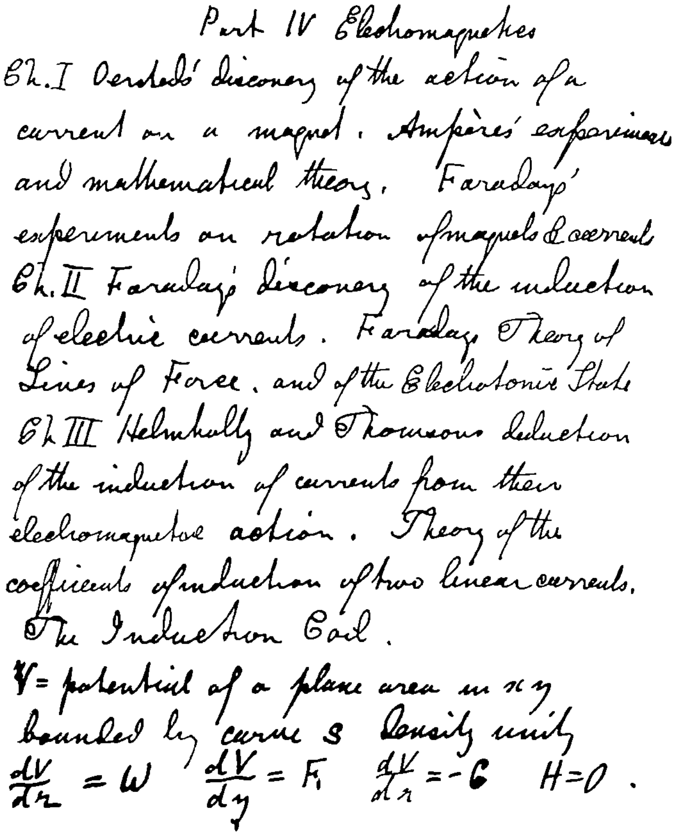
Maxwell's first important contribution in 1855 was to show that for the types of problem so far studied Faraday's views led to exactly the same results as the theory of action at a distance It is a sobering thought to ask which side we would have been on if presented with the same alternatives

Maxwell was a very capable mathematician, but the mathematics was always the servant of the physics He was always keen on the idea of an analogy or a model, and the introduction to his paper on 'lines of force' is very prophetic in this respect

Faraday's outstanding discovery of electromagnetic induction had shown that space occupied by lines of force was different from empty space A circuit rotated in space through which no lines of magnetic force pass will not register an electric current It was thus that Faraday arrived at his idea that space containing lines of magnetic force is in a special state, the 'electro-tonic state'

In his second paper on electrical phenomena, "Physical Lines of Force", published in 1862 during his professorship at King's College, Maxwell laid the foundations of his greatest contribution to electrical theory and devised a model to illustrate Faraday's law of electromagnetic induction In this work Maxwell made use of the analogy between lines of force in the electromagnetic field and vortex filaments in a liquid with finite fixed boundaries In Maxwell's model the lines of magnetic force were represented by cylinders rotating around these lines as axes The magnitude of the force was represented by the velocity of rotation and its direction by that of the axis of rotation

It would take us too far from our present purpose to examine this model in detail Indeed, in the *Treatise* this model, 'the scaffolding of the Theory', as it has been called, is discarded Suffice it to say that to represent a uniform magnetic field Maxwell's cylinders must all rotate in the same direction But cylinders in contact would rotate in opposite directions To achieve his purpose Maxwell introduced into his model small spheres to act as 'ball-bearings'


Part IV Electromagnetics

Ch. I Oersted's discovery of the action of a current on a magnet. Ampère's experiments and mathematical theory. Faraday's experiments on rotation of magnets & currents

Ch. II Faraday's discovery of the induction of electric currents. Faraday's Theory of Lines of Force. and of the Electrotonic State

Ch. III Helmholtz and Thomson's deduction of the induction of currents from their electromagnetic action. Theory of the coefficients of induction of two linear currents. The Induction Coil.

V = potential of a plane area in xy bounded by curve s density unity

$\frac{dV}{dx} = W \quad \frac{dV}{dy} = F_1 \quad \frac{dV}{dx} = -G \quad H = 0$.

Fig. 1. Part of an early draft of the contents of Maxwell's *Treatise on Electricity and Magnetism* taken from his note-books. It is believed to have been written some time between 1860 and 1865 and well before the publication of the *Treatise* in 1873

The ball-bearings were supposed to represent particles of electricity, and their motion an electric current. When the cylinders have the same velocity the ball-bearing has no translatory motion and there is no current. If the speed of one of the cylinders is changed the centre of the ball between them will begin to move. The motion of the ball indicates an electric current. The model thus illustrates Faraday's discovery of the production of electric currents by changes in magnetic force.

J. J. Thomson has stated that this is a very striking illustration of the advantage of using a model. Maxwell designed his model to illustrate Faraday's discovery that changes in a magnetic force produce an electrical force. When he came to use the model he found that it suggested that changes in the electric force would produce a magnetic force. The introduction and development of this idea was one of Maxwell's outstanding contributions to physics. The importance of the theory lies in the implication that all changes in electric and magnetic fields caused waves to be propagated in space. On previous theories waves could not exist. The paper on "Physical Lines of Force" contains a form of the well-known Maxwell equations. In the later paper, "The Dynamics of the Electromagnetic Field", the model is dropped and the Maxwell equations are in effect developed. Both these papers were written while Maxwell was at King's College, and his work in London is therefore unquestionably the most important of his life. Few ideas can be said, however, to belong to a particular period except in a very broad sense. Maxwell was already thinking about lines of force when inspired by Faraday's experimental researches ten years before he wrote his famous paper.

Maxwell's ultimate account of his theory is given in the *Treatise*, published in 1873. Note-books in the possession of the College show that Maxwell was already planning this work when in London, and it is of interest to compare the titles of the chapters as originally planned and the chapter headings of the published work (Fig. 1). It is particularly interesting that the contents at this stage betray no mention of his own electromagnetic theory.

Maxwell's theory was not very enthusiastically received either in Great Britain or on the Continent. This was due in part to the extremely obscure presentation and in part to the earlier success of the action-at-a-distance theory. One of the most controversial ideas embedded in the theory is what Maxwell called a 'displacement of electricity', and he was so guarded in defining what he meant by electricity that his idea was much misunderstood. This obscurity was in Hertz's mind when he said that Maxwell's theory was Maxwell's equations. Helmholtz said he would be puzzled to explain what an electric charge was on Maxwell's theory, beyond its being the recipient of a symbol. A physical interpretation of Maxwell's theory lies in Faraday's lines of force. J. J. Thomson has written that if what Maxwell termed electricity is interpreted as 'lines of force' and what he termed displacement as the 'density' of these, we are able to satisfy the two definite statements in his work: (*a*) wherever there is 'electric force' there is 'electric displacement', (*b*) electricity behaves like an incompressible fluid.

Faraday's law of electromagnetic induction states that the rate of alteration in the number of lines of magnetic induction passing through a circuit is equal to the work done in taking an electric charge round the circuit. Maxwell's law states that the rate of alteration in the number of lines of electric force passing through a circuit is equal to the work done in taking a unit magnetic pole round it. The expression of these two laws in mathematical form gives Maxwell's equations. The two laws just stated are symmetrical.

Maxwell's theory predicts the extremely important idea that electrical disturbances are propagated as transverse waves of electric and magnetic force. The velocity of propagation depends on quantities which can be determined by purely electrical measurements and should be equal to the velocity of light. In a paper published in 1868 Maxwell reported his results on the determination of the ratio of the electrostatic to the electromagnetic unit of electricity (with air as the dielectric). The principle of his method was to balance the attraction between two electrified disks by the repulsion between two coils of wire in which currents were flowing in opposite directions. One of the disks and one coil were placed at one end of the beam of a torsion balance while the other pair remained fixed. A third coil was used to eliminate the effect of the Earth's magnetic field

The result was a value of 2 88 $\times 10^{10}$ cm sec $^{-1}$ compared with that of Kohlrausch and Weber of 3 11 $\times 10^{10}$ The accuracy of Maxwell's result is dependent on that of the British Association unit of resistance—the velocity being represented by 28 8 ohms This work, of course, did nothing to establish the electromagnetic nature of light which was a matter of vital importance for this theory

Not until some years after his death was it experimentally possible to both produce and detect electrical waves when the method of producing them by the oscillatory discharge of a condenser or induction coil became available It fell to Hertz, a pupil of Helmholtz, to show that they could be detected by means of very small electric sparks passing between two metal balls placed close together, Hertz himself believed that in some respects von Bezold had prior claim in his publication of 1870 Simultaneously with Hertz, Oliver Lodge in Liverpool investigated the theory of the lightning-conductor and carried out a series of experiments on the discharge of small condensers which led him to observe oscillations and waves in wires Hertz says of Lodge's experiments "Inasmuch as he entirely accepted Maxwell's views, and eagerly strove to verify them, there can scarcely be any doubt that if I had not anticipated him he would also have succeeded in observing waves in air and thus also proving the propagation with time of electric force"

G F Fitzgerald of Dublin gave two papers of significance in this field at the British Association meeting at Southport in 1883, four years before Hertz's publications appeared The first of these suggested a method of producing electromagnetic disturbances of comparatively short wave-lengths (10 m or less) which involved the discharge of an accumulator through a small resistance Hertz later used this method without knowing of Fitzgerald's experiments and obtained waves of 24 cm length. Fitzgerald's second paper was entitled, "On the Energy Lost by Radiation from Alternating Circular Currents"

By a curious chance my own work has had a connexion with that of Hertz When more than fifty years later it became necessary to produce short electromagnetic waves, not in a broad spectrum but with a precise wave-length and of great intensity, it was to Hertz's resonators that Boot and I turned A single resonator of the cavity magnetron is but a cylindrical extension of a Hertzian dipole

Measurement of Electrical Resistance

No account of Maxwell's work in London would be complete without some mention of experiments he carried out for a Committee of the British Association, Maxwell ultimately became a member of the Committee, the report made by Maxwell and Fleeming Jenkin is published in the *British Association Report* for 1863 To us a century later this work may seem rather small beer But one has only to read this extremely clear account of the fundamentals of electricity to realize that it formed the basis of most of the text-books of the subject for many years to come It had its importance, too, in the establishment of standards and it is of interest to King's College that the measurement of the standard of resistance was carried out in its own buildings in the Strand

Surely enough has been said to give us the impression of a scientific leader of the first order, someone who would come in most physicists' rating very close to Newton and Faraday Einstein has said that the change in the conception of reality brought about by Maxwell is the most profound and the most fruitful that physics had experienced since the time of Newton Maxwell's description of reality is represented in his double system of partial differential equations in which the electric and magnetic fields appear as dependent variables Since Maxwell's time physical reality has been thought of as represented by continuous fields governed by partial differential equations and not capable of any mechanical interpretation The advent of quantum mechanics, rather than the theory of relativity, has produced a situation in which we have to deal with amounts of energy concentrated in small spaces and this is in direct contradiction to Maxwell's equations and to the Newtonian system of mechanics The quantities that appear in the laws of quantum mechanics make no claim to describe physical reality as such, but only the probabilities of the appearance of a particularly physical reality

Whatever the present difficulties and whatever the future may hold, Maxwell's theory will always remain a step of outstanding importance in the development of physical ideas His work has been so important and lasting because it was based on experiment

I wish to return for a brief space to further consideration of Maxwell's position in the study of electromagnetism It is clear, of course, that he did more than anyone else of his time to advance the subject, and this was achieved as a consequence of his recognition (1) that a changing electric field constitutes a current and is accompanied by a magnetic field, (2) that light consists of electromagnetic waves propagated with a velocity of 3 $\times 10^{10}$ cm /sec

Although Ludwig Lorenz in 1867 derived equations essentially equivalent to Maxwell's without any primary hypotheses about the medium, this has largely beeen ignored by the bulk of physicists This is strange because it is clear that the device of a displacement current is thereby rendered unnecessary. Mathematically, it is simply a way of transforming an equation of the Poisson type to one of wave-motion J J Thomson explained Maxwell's displacement current in terms of lines of force But we must recognize that the idea of lines of force came from Faraday and is also hypothetical Thomson thus appears to have fallen into the trap of explaining one hypothesis in terms of another No one seriously denies that the conception of lines and tubes of force is an extremely useful one to the physicist and engineer in the laboratory It was Maxwell's explicit objective to put into mathematical form Faraday's ideas

The work of the German and other Continental physicists contained no hypotheses about lines of force Lorenz introduced the idea of retarded potentials, thus following an earlier (1848) suggestion of Riemann His theory provides for the propagation of electric waves with a finite velocity without the necessity of introducing the idea of a material medium and a displacement current Lorenz, however, failed to make the vital link between the velocity c and that of light It is perhaps for this reason more than any other that his work has been neglected and almost forgotten Maxwell himself in his *Treatise* acknowledges Lorenz's work as being very close to his own It was apparently published without knowledge of Maxwell's two papers Maxwell's theory was not very well understood on the Continent because physicists there were fully attuned to the action-at-a-distance theory and slow to accept the Maxwellian implications of Faraday's lines-of-force hypothesis Lorenz's ideas were, however, the logical development of the Gauss–Weber–Neumann school Yet neither Lorenz's work nor Maxwell's led to an experimental search for electromagnetic waves until 1888, when Hertz carried out his experiments

It is curious that physicists as a whole should have consistently clung to the trappings of Maxwell's theory As a consequence a good deal of confusion in the subject persists to this day It is often said that the object of a theory is to do no more than provide ideas for new experiments This is certainly one extremely important object In Maxwell's case it led to the superb experiments of Hertz However, would not the subject of electromagnetism have benefited enormously if physicists had at any time in the past fifty years taken a fresh look at the premises and hypotheses on which the subject rests ? Considerations of this kind go to the heart of the accepted presentation of electromagnetic theory

Perhaps no better tribute to Maxwell could be made at this time than to re-examine the foundations of classical electricity in terms of the physics of to-day and to state them and teach them in a self-consistent manner

Maxwell as a Teacher

In thinking of any mid-nineteenth-century physicist as a teacher, we have to bear in mind three aspects of significance and importance

(1) *The importance of the laboratory* Laboratories for the teaching of experimental physics, as distinct from the applied mathematics of natural philosophy, were rare In consequence any scientist who wished to teach experimental physics had to provide first of all the laboratory In King's College it appears that some at least of the physics teaching was carried out in the workshop Fees were paid by the students for the use of the workshop The *College Calendar* of the day states that there was a curator of the College Laboratory, which appeared to serve all subjects It seems probable that Maxwell made use both of the College Laboratory and of its workshop for the teaching of experimental physics Although Wheatstone had been appointed professor of experimental philosophy in King's College in 1834, and was given a grant of £50 to cover the cost of apparatus used in his lectures, it seems clear that he did not give courses of lectures for more than a very few sessions Maxwell was certainly teaching experimental physics to evening students at King's College in the session 1864–65 This was nine years before the Cavendish Laboratory was founded under his leadership, and it is not unreasonable to infer that his experience at this time had some influence on his later plans for laboratory design In Scotland, both in the time of Forbes at Edinburgh and Kelvin at Glasgow, students assisted the professor in his experimental work, but this scarcely constituted organized class teaching as we know it While it seems unlikely that Maxwell initiated any new features of laboratory design at King's College, we know from his Cambridge period that he did so there In so far as a good laboratory is essential to good teaching, Maxwell's standard was very high

(2) *The course of study*, especially at a time of change, gives some measure of the mind of the lecturer The course in natural philosophy given in King's College in 1859, a year before Maxwell took up his appointment, was, apart from some experimental lectures on light, entirely concerned with the properties of matter, a little of heat and of elementary astronomy Maxwell's evening class in experimental physics for the session 1864–65 dealt with magnetism and electricity A separate course in statics, dynamics, sound and optics was given by T A Cock Maxwell's course is worthy of a little attention since its content reflects much of what is taught of electricity and magnetism in schools to this day The subject-matter of the syllabus was as follows

I Preliminary mechanical ideas , the three standard measures of length, time and mass , different ways of measuring forces , the gravitation system , the absolute system

II Magnetism , the loadstone , properties of soft iron and steel , action between two magnets , lines of magnetic force , determination of the magnetic axis of a magnet and of its magnetic moment

III Magnetism of the Earth , determination of its direction and intensity at different places , the declinometer , magnetic charts , the work of a magnetic observatory , photographic representation of the magnetic elements , magnetic storms and sudden disturbances , the diurnal and annual variation , the secular variation

IV Magnetism of iron ships , errors of the compass , methods of correction , magnetic induction

V Electrical attractions and repulsions , transference of electricity , conductors and insulators , measurement of quantities of electricity by the statical method

VI Electrical induction , the Leyden jar , electricity produced by work , the electrophorus , Varley's condenser , electric potential , electric capacity , electroscopes and electrometers , atmospheric electricity

VII Electricity in motion , the electric current , relation to statical electricity, to chemical action, to magnetism , Oersted's discovery of the mechanical action between a current and a magnet , Ampère's laws of the mutual action between currents , Faraday's discovery of the production of currents by magnets and by other currents

VIII On the electromagnetic system of measurement , measurement of currents , tangent galvanometer , Weber's electro-dynamometer

IX Ohm's law for the strength of currents , derived currents , Wheatstone's bridge , comparison of resistances by the electrical balance , method of finding the position of a fault in a submarine telegraph cable Siemen's thermometer for deep-sea sounding

X Determination of resistance and other electrical qualities in measures founded on those of length, time and mass The standard resistance unit of the British Association founded on experiments at King's College

XI Heat produced by electric currents , calculation of the mechanical equivalent of heat from Dr Joule's experiments , electric currents in unequally heated circuits , thermo-electric pile

XII The electromagnetic field and its lines of force , exploration of the field by means of magnets , shape of the lines of force , what happens to a magnet, a piece of iron, a piece of bismuth, a conductor carrying a current, a moving conductor, and a beam of plane-polarized light in the electromagnetic field ? effects of a disturbance of the electromagnetic field

XIII Conservation of energy in electromagnetic phenomena , hypotheses about the nature of electromagnetic action

XIV On electric telegraphs

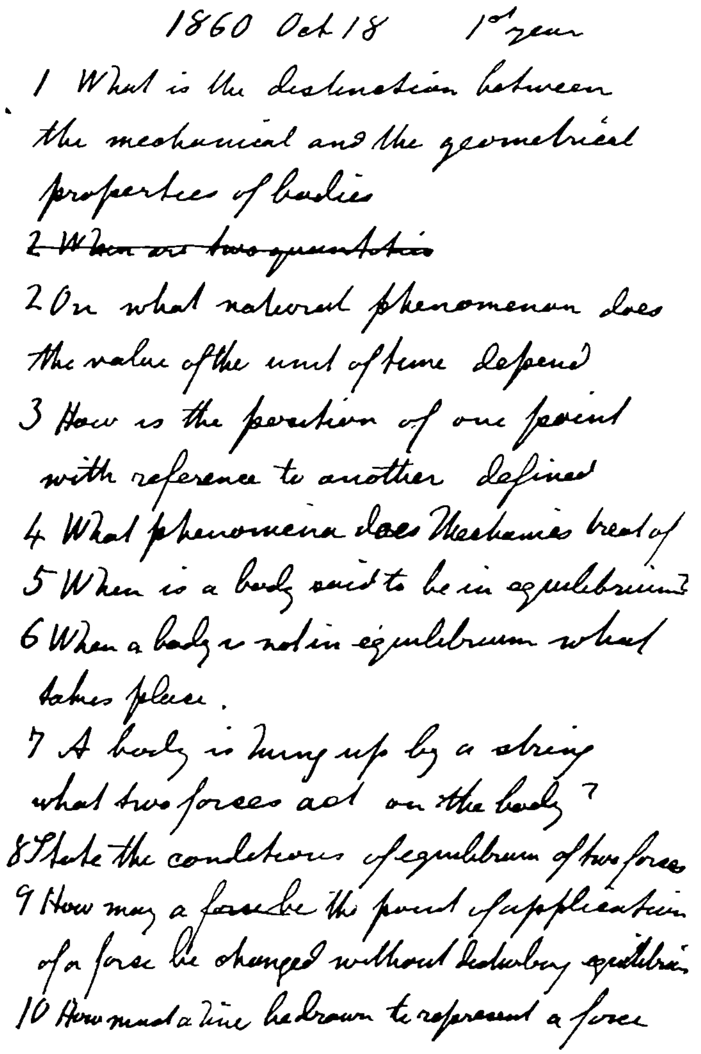
It is immediately apparent that this was and still is in many ways a very good course It is fundamental in outlook, was ahead of its time, and contains for the most part subjects that are still important to-day Maxwell was a close friend of Thomson (Kelvin) and we do not know to what extent the teaching of physics was discussed between these two men. Maxwell was clearly able to design a good course in magnetism and electricity. Fig 2 is taken from a set of the earliest questions set by Maxwell to his elementary students at King's College

(3) *Maxwell's ability as a lecturer* Doubts about this have often been expressed and derive in part from statements made in the *Centenary History of King's College, London,* by F J C Hearnshaw.

> "As a teacher of raw youths he did not prove to be a success He was, says one who knew him, a quiet and rather silent man and it seems not unlikely that the students were too much for him "

The Rev Canon R Abbay, who was appointed a lecturer and demonstrator in physics at King's College in July 1868, wrote to the centenary historian

> "It was difficult to keep order in the class in those days, especially when the blackboard was much used, but probably the disorder led to the greatest scientific discovery of the century It was believed by scientific men that the Governors asked Clerk Maxwell to resign his professorship of physics because he could not keep order; that then he went back to Cambridge and quietly worked out his theory of the æther "



1860 Oct 18 1st year

1 What is the distinction between
the mechanical and the geometrical
properties of bodies
~~2 When are two quantities~~
2 On what natural phenomenon does
the value of the unit of time depend
3 How is the position of one point
with reference to another defined
4 What phenomena does Mechanics treat of
5 When is a body said to be in equilibrium?
6 When a body is not in equilibrium what
takes place.
7 A body is hung up by a string
what two forces act on the body ?
8 State the conditions of equilibrium of two forces
9 How may a force be the point of application
of a force be changed without disturbing equilibrium
10 How must a line be drawn to represent a force

Fig 2 Probably the first questions set by Maxwell to his first-year students in natural philosophy at King's College, London, in October 1860 Taken from a Maxwell note-book in the possession of the College

In a later letter Canon Abbay added

> "It was Professor Clifton who told me—I think in 1869 —that he had heard that Clerk Maxwell had been asked to resign because of the disorder at his lectures "

and he went on to point out that Clifton was in a position to know

Dr G R Wilkinson and I have searched the College minute books and archives for further evidence on this point, but without success Others, we suspect, have been before us, for the boxes for 1860 and 1865 are not to be found

Not all speak of Maxwell as a poor lecturer, he is, for example, praised by Horace Lamb This would only go to suggest, however, that Maxwell could be appreciated by the brilliant student rather than by the less-gifted Stone of Piccadilly (never, so far as I know, a practising scientist) told Prof Derek Price that Maxwell was a bad lecturer

As a teacher of research students there can be no doubt that Maxwell was remarkable for the inspiration he instilled in the course of an hour's discussion

It is unfortunate that the official historian of King's College has left the impression, without adequate evidence, that in 1865 Maxwell was asked to resign his chair on account of his alleged lack of teaching ability and power to keep order in class There is certainly no record even hinting at such action in the minute books of the College Council Hearnshaw, however, accepts the word of Abbay and remarks that Maxwell's departure was thus indirectly of great benefit to science Since Maxwell could equally well have been invited to Cambridge from either London or Glenlair, this point also is of dubious value

Maxwell, as we have seen, had published much of his outstanding work from London The period of retirement at Glenlair was largely occupied with the completion of the *Treatise on Electricity and Magnetism*

Maxwell and the Cavendish Laboratory

The chair of experimental physics was founded in 1871 in circumstances now so well known that I shall not repeat them here Kelvin turned down the offer of the chair and Maxwell, apparently somewhat reluctantly, accepted In a letter written at this time Maxwell points out that he has no experience of teaching experimental physics But we have seen that he did in fact have a course at King's College during the session 1864–65 There seems to be little doubt that Maxwell's ideas on laboratory design owe a great deal to the interest in building matters derived from his father and particularly from what he saw in Glasgow and Oxford, as well as from his own experience After all, he had used apparatus since his undergraduate days with Forbes in Edinburgh The design and construction of the Cavendish Laboratory went on until 1874, when in June of that year it was first used for experiments

Both in design and in the principles of experiment and measurement that Maxwell set out to achieve, the Laboratory set standards which were unexcelled elsewhere for many years Part of this was due to the inspiration and genius of Maxwell himself, part to the coincidence of the unexampled brilliance of his successors, and part to the fact that the time was indubitably ripe for such developments A comparison of the merits and achievements of Maxwell and Kelvin is beyond our present scope, but it may be noted that Kelvin had shown the way to the success of physical measurement and design in the improvement of industry as well as of natural knowledge

It has been remarked that Maxwell showed superb political astuteness in bringing the Cambridge of the day to approve of his plans But is this really so ? Cambridge had founded the chair and had the munificent interest of its Chancellor in the objective before Maxwell ever arrived there Both Maxwell and Kelvin had been gradually steering the Mathematical Tripos examination towards more physical subjects for some years Political manœuvre seems far removed from the recorded impressions of Maxwell's character One is left with the impression that the establishment of the Cavendish Laboratory on a firm basis followed smoothly from the natural and quiet expression of Maxwell's firm convictions It is the great achievement of British science of the period, and was accompanied in Maxwell's inaugural lecture by a set of precepts and principles which have never been matched in their prescience and acuity

Maxwell the Man

So much has been written about Maxwell the physicist that we are left with a rather blurred picture of the man and particularly it seems difficult to place him in relation to the times in which he lived In some senses he was a prophetic genius who was of the nineteenth century but not in it In this he differed profoundly from Kelvin, who began his career with deeply fundamental investigations (such as that which led Maxwell to the discovery of the electromagnetic theory of light) and ended it a very rich man deeply involved in industry Maxwell's genius, like that of Newton and Faraday, was timeless, Kelvin's was of his day Maxwell could always see beyond the investigation of the moment to the implications for the future, in the deepest sense Kelvin could not Maxwell predicted the electromagnetic theory of radiation and thus set in train a series of events that still profoundly affects our daily lives In his famous paper "On the Dynamical Theory of the Electromagnetic Field" he discusses the problem of the gravitational field and thus began the long and as yet unsuccessful attempt to derive a unified field theory of matter

But again, what of the man ? Physically he was of middle height and classic features with deeply penetrating eyes set off by black hair and beard He had a quiet ironic sense of humour which is clearly evident from his well-known occasional verses But above all Maxwell was a deeply religious man who seems to have accepted the tenets of the Christian faith from an early age Many of the letters from Maxwell to his wife that appear in the official *Life* are in effect sermons discussing various aspects of the *New Testament* It is unfortunate that there are virtually no letters from Mrs Maxwell on record, either none was preserved or they were thought to have been irrelevant to the major theme We are left in ignorance, and so we cannot know Maxwell cared deeply for his wife, who had indifferent health for many years and who surprisingly survived him Even as he lay dying he gave orders for her comfort Although Maxwell was so religious there is not much evidence that he was interested in the philosophy of his day, indeed, interest in the one was his philosophy and precluded the other As J G Crowther has pointed out, this probably led to a greater concentration on scientific matters

Religion was a deep and driving interest to many Victorians It was an age characterized by tremendous industrial development and consequent economic circumstance The middle classes were confirmed in their status and the mild feudal atmosphere in which Maxwell was brought up received no jolts There were no deep scars of war to distract an intellectual from his chosen task Both at Dalbeattie, at Cambridge and in London Maxwell had time to think, and thought to some purpose Maxwell liked to lecture to working men, and this he did both in London and in Cambridge But we are left with no account of his views of the working man Was he just a good Christian dispensing culture or was he deeply moved and concerned with the ordinary man's estate ? We do not know Maxwell was born the year before Russell's Reform Bill in 1832 and died a few years after the Acts passed by Gladstone and Disraeli to give trades unions some status It was only in 1870 that Forster's Education Act provided primary education for all These events were the background to Maxwell's life, but it does not appear that he was affected by them in any profound way

We are told that Maxwell was a reader of the works of Dryden, Swift and Butler So far so good, but he lived in one of the richest periods of English literature Shelley, Keats, Lamb and Scott were not long dead, and Dickens, Tennyson, Thackeray, Mathew Arnold, Macaulay, Mrs Gaskell, Trollope, George Eliot and Meredith were all writing, and many were at the height of their powers in his lifetime Maxwell has been described as a civilized man, in a narrow sense, yes I would say rather that he was a man of concentrated scientific genius, devoted to his home and family and little influenced by, or interested in, the comfortable broad middle-class culture of his day This picture may be unjust in that his biographers have completely failed to paint in the general background to his life We do not particularly wish to know what a great scientist eats for his breakfast but we would like to know something of his relationship to the times in which he lived Maxwell's culture was in truth a spiritual one, not a material one like Kelvin's, and it is for this reason perhaps that we have such an imperfect picture of Maxwell the man

In the preparation of the lecture on which this article is based I have of course, made much use of Maxwell's collected works and of the well-known *Life* by Campbell and Garnett In addition to these and other standard sources, I am particularly indebted to Dr I B Hopley's University of London thesis (1956) I also thank Dr G R Wilkinson of King's College, London, for his help in searching what remain of the King's College archives and for much valuable discussion

OBITUARIES

Sir William Mitchell, K C M.G

Sir William Mitchell, emeritus professor of philosophy, former vice-chancellor and chancellor of the University of Adelaide, died in Adelaide on June 24 at the age of 101

Sir William was born at Inveravon, Banffshire, Scotland, in March 1861 He received his early education at Elgin and later entered the University of Edinburgh, where he graduated M A and D Sc in philosophy

His first appointment was as lecturer in ethics in the University of Edinburgh, and from there he went to University College, London, as lecturer in education Later, he was appointed examiner in philosophy at Edinburgh and in the theory of education at the University of Cambridge

In 1894 he joined the University of Adelaide as Hughes professor of English language and literature and mental and moral philosophy From the beginning of his professorship, he took an active part in the government of the University, and in 1896 was elected to its Council Sir William became vice-chancellor in 1916 and held this office continuously until 1942 In 1927, he was created a Knight Commander of the Order of St Michael and St George On the death of Sir George Murray in 1942, he was elected chancellor of the University He relinquished that office on January 31, 1948

Sir William was counted among the distinguished philosophers of his day In 1924, he was invited to deliver the Gifford Lectures in the University of Aberdeen, and in 1934 he delivered the philosophical address of the year to the British Academy But perhaps his greatest contribution to scholarship was his work as a teacher He had a singular and powerful gift for enabling those whom he taught to discover where their deepest interests lay and to develop themselves with confidence and vigour

He was also remarkable for the natural and most acceptable leadership which he exercised throughout his long service as a member of the governing body and as vice-chancellor of the University of Adelaide He bore his years lightly, and his spare, vigorous figure was often to be seen in the University after he had passed his eightieth birthday Indeed, he enjoyed remarkable health and mental vigour until a serious illness beset him in 1958 From this he recovered, but was thereafter much restricted in his activities He kept until the end of his long life a graciousness and serenity which made and preserved for him many friends

He is survived by a daughter, Mrs J R Thomson, and a son, Sir Mark Mitchell, professor of biochemistry and general physiology and deputy vice-chancellor of the University of Adelaide H B Basten

Prof Granville Poole

Prof Granville Poole, emeritus professor of mining engineering in King's College, Newcastle upon Tyne (University of Durham), died on May 26, aged seventy-six

Prof Poole was a man of outstanding character who commanded respect not only by his ability as a mining engineer of wide experience, but also by his sincerity and deep interest in all matters relating to safety in mines He was a tall, straight, distinguished man, identified by his wing collar and bow tie, and as we know him, with a gentle manner and a commanding personality.

His students at Armstrong College (later King's College), Newcastle upon Tyne, where he held the Milburn chair of mining engineering from 1923 until 1951, will always remember his custom of sitting with them in the front row of the lecture theatre and illustrating his lectures by recalling incidents in his earlier career in the Inspectorate and at the Ministry of Mines

His experience was unique in the coal-mining industry in that he was associated, as private secretary to H M Chief Inspector of Mines during 1911–13, with the passing of the 1911 Coal Mines Act and again in 1954 as an adviser to the National Union of Mineworkers Parliamentary Committee when the 1954 Coal Mines Act was being formulated.

His career belies present-day beliefs, in that as a young graduate from the University of Birmingham he was given responsibility at the early age of twenty-four as one of H M Inspectors of Mines in 1909 He gained promotion in the Inspectorate to senior rank in 1917 His appointment to the chair of mining at the University of Leeds in 1919 when only thirty-three must also make him one of the youngest mining engineers of his era ever to hold a chair He moved to Newcastle upon Tyne and the Milburn chair of mining at Armstrong College in 1923, following the retirement of Prof Henry Louis

It was at Armstrong College, which became King's College in 1929, that Prof Poole was responsible for securing the funds for building the present Department of Mining Engineering He was a justifiably proud and happy man in 1929 when the Prince of Wales formally opened the new building This Mining Engineering Department was unique and well equipped and established a permanent building in a College which had taught mining engineering subjects as long ago as 1865

Prof Poole was an active member of the Institution of Mining Engineers from 1908, first as a member of the South Staffordshire Institute and later of the North of England Institute of Mining and Mechanical Engineers He was a member of Council from 1935, a vice-president from 1945 and president of the North of England Institute during 1950–52 It was particularly appropriate and was a great personal honour and pleasure that he was the president during the Centenary Celebrations of this Institute in 1952

He was widely consulted in mining matters and retained his links with the Staffordshire Coalfield as a consultant to the Shelton Iron and Steel Co

Prof Poole will, however, always be remembered by his students as a man who set the highest example in integrity and loyalty and for his continual encouragement to those who passed from the University into the industry E L J Potts

NEWS and VIEWS

Physics at Chelsea College of Science and Technology :

Dr. W H George

Dr W H George, who has been head of the Department of Physics in Chelsea College of Science and Technology since 1938, is retiring at the end of the present session Dr George was Royal Society Sorby Research Fellow in the University of Sheffield, then lecturer in the University of Southampton before his appointment at Chelsea, his research has been mainly in X-ray crystallography and acoustics He has greatly stimulated the provision of post-graduate degree courses, particularly since the War

Dr K. W Keohane

Dr K W. Keohane has been appointed to succeed Dr George He served during the War in the Radar Branch of the Royal Air Force and graduated with first-class honours in physics in the University of Bristol in 1947 After work on reflecting microscopy with C. R Burch, Dr. Keohane's interest turned to biophysics and he afterwards joined Prof J M Yoffey, first as a research associate and then as lecturer He was awarded a Ph D for the application of absorption spectrometry to single cells and is well known for his work in collaboration with W K Metcalf on the physical properties of erythrocytes and lymphocytes He was appointed reader in physics at Chelsea in 1959 and has developed research in biophysics at the College, particularly in the field of electrical properties of cell membranes

Geology at Liverpool :

Prof W S. Pitcher

Dr W S Pitcher, reader in geology in King's College, London, has been appointed to the George Herdman chair of geology in the University of Liverpool, rendered vacant by the resignation of Prof R M Shackleton, who has been appointed to the second chair in the Department of Geology in the University of Leeds (*Nature*, 193, 1233, 1962) Dr Pitcher, who is forty-three years of age, was awarded the degree of B Sc at the University of London in 1947, and the degree of Ph D in 1951 He served with H M Forces during the Second World War and on demobilization he became a student at the Chelsea College of Science and Technology In 1947 he was appointed demonstrator at the Imperial College of Science and Technology, London, afterwards being promoted assistant lecturer, and later lecturer, and in 1955 he was appointed reader in geology in King's College, London In 1948 he served as assistant secretary to the International Geological Congress Dr Pitcher's special field of research has been the geology of the County of Donegal, where he has made a special study of the mode of emplacement of the Caledonian granites In recognition of his researches he was awarded the Lyell Fund of the Geological Society of London in 1956 His other interests are the ring complexes of Northern Nigeria and certain aspects of the Tertiary geology of south-east England

Personal Chair of Biochemistry at Leeds :

Prof S Dagley

Dr S Dagley was awarded an open scholarship in natural science at Keble College, Oxford, in 1934 and graduated in 1937 with honours in chemistry He obtained the degree of B Sc in 1938 for a thesis on "The Chemical Kinetics of Bacterial Growth" under the supervision of Sir Cyril Hinshelwood During the Second World War he was employed as a chemist in the Ministry of Supply, and in 1944 he was appointed chemist in charge at the Royal Ordnance Factory, Pembrey, Carmarthenshire From 1945 until 1947 he was a lecturer in chemistry at the Sir John Cass College, Aldgate, where he continued his research work on the kinetics of bacterial growth In 1948 he was awarded the degree of M Sc at the University of London and in 1956 the degree of D Sc Dr Dagley was appointed a lecturer in the Department of Biochemistry in the University of Leeds in 1947, and in 1952 the title of reader in physiochemical aspects of biochemistry was conferred on him His research at Leeds has been concerned chiefly with the biochemistry of bacteria His research team has studied new ways by which bacteria open the benzene nucleus, the reactions by which they synthesize their cell constituents from simple chemicals and also the effect of antibiotics on certain structures inside the bacterial cell He has contributed to symposia organized by the Faraday Society, the Society for General Microbiology and the Chemical Society

New Research Synchrotron for Northern England

Lord Hailsham, Lord President of the Council and Minister for Science, has announced the approval of the proposal of the National Institute for Research in Nuclear Science to establish a second laboratory, which will contain a 4-GeV electron synchrotron The new laboratory will be in the north of England and will be particularly associated with the Universities of Liverpool, Manchester and Glasgow, but scientists from other universities and institutions, as well as scientists on the Institute's own staff, will also carry out research there The estimated capital cost of the laboratory and the synchrotron is about £3½ million The first director of the laboratory will be Prof A W Merrison, who is being released from administrative responsibilities at the University of Liverpool for a period of five years so that he may take up this post (for details of Prof Merrison's career see *Nature*, 185, 284, 1960) Like *Nimrod* at Harwell, the new synchrotron will be used for research into the nature of the fundamental particles of matter, but as it will produce high-energy electrons rather than high-energy protons, the experiments carried out will be quite different The new accelerator will also be complementary to *Nimrod* as regards the universities to which it will be conveniently accessible The Physics Departments at Liverpool, Manchester and Glasgow are particularly interested in this field of physics The 4-GeV electron synchrotron was selected after a careful study carried out by the National Institute for Research in Nuclear Science in 1960 and the first half of 1961 The proposal to build it was announced in the Institute's annual report for 1960–61 A similar accelerator is in operation jointly by Harvard and the Massachusetts Institute of Technology in the United States, and two others are under construction, at Hamburg and in the U S S R

The Biohistorical Institute of the University of Utrecht

The new Biohistorical Institute of the University of Utrecht was formally dedicated on May 30 Among the speakers were Prof J Lanjouw (director of the Utrecht Institute for Systematic Botany), who made the original plans for the Institute, and Prof G A Lindeboom (Free University of Amsterdam), who spoke on the importance of the humanistic aspects of biology and early medicine in general Prof F Verdoorn, the Institute's director, outlined the concept of biohistory, that is, all areas where the pure and applied life sciences (including early medicine), on one hand, and cultural history, literary and art history, and sundry other branches of the humanities, on the other, overlap He also described how an effort is being made to develop the Utrecht Biohistorical Institute simultaneously as a research, as an educational, as a documentation and (in conjunction with the International Biohistorical Bureau) as an international institution In the annals of the University of Utrecht, the Biohistorical Institute is unique by being housed in one of the oldest University buildings (which served for more than two centuries as the official residence of the director of the *Hortus Academicus*), while the contents (the former *Chronica Botanica* Library and Archives) were chiefly built up in the United States

As to the organization of the Biohistorical Institute, there are three divisions of a primary nature, each constituting a 'triple unity' and consisting of (*a*) books, (*b*) extensive card indexes of the relevant world literature, and (*c*) supplementary archivalia (such as engravings, autographs, manuscripts, portraits), the historical and bibliographical, the biographical, and the topographical division Other units are the literary and art historical division, the reading and periodicals room, etc In connexion with the dedication of the Utrecht Biohistorical Institute, a number of selective exhibits from the Institute's own holdings were organized, including (1) garden history and landscape architecture in the past and present, (2) Nature prints, (3) works by and about Carolus Linnaeus, mainly of a general biological and medical interest, (4) literature and art in biology and early medicine, (5) works edited by Fr and J G Verdoorn (1935–62), (6) recent acquisitions of the Institute's Library, (7) *Leeuwenhoeckiana* (a survey of the work of the Leeuwenhoek Commission of the Royal Netherlands Academy of Sciences, which has its headquarters at the Utrecht Biohistorical Institute)

The British National Committee on Surface Active Agents

The British National Committee on Surface Active Agents has recently been inaugurated Membership is open to all *bona fide* manufacturers of surface active agents in the United Kingdom The Committee has been formed primarily to foster British interests in the field of surface activity on an international basis Its aims and objects have been defined as (*a*) to seek membership of the Comité International de la Détergence, (*b*) to consider all questions of a technical character relating to the work of the Comité International de la Détergence, its technical Commissions and International Standards Organization Committees on Surface Activity, (*c*) to maintain liaison with organizations and individuals in the United Kingdom having interests in the field of surface activity, (*d*) to co-ordinate participation by the United Kingdom in International Congresses on Detergency The seventeen founder members include all the major manufacturers of surface active agents in the United Kingdom The officers are Mr R C. Tarring, Shell Chemical Co , Ltd (president), Dr. A F Kertess, Associated Chemical Companies, Ltd. (treasurer), Mr H W Vallender (secretary-general). Further information concerning membership, etc , can be obtained from the Secretary-General at 86 Strand, London, W C 2

John Alexander Reina Newlands

Dr F W Gibbs, of the Royal Institute of Chemistry, has recently provided the editor of *Nature* with the following information "John Alexander Reina Newlands, propounder of the 'Law of Octaves', died on July 29, 1898, aged sixty (according to 'W S ', *J Soc Chem Indust*, 743, 1898) or sixty-one ('W A T ', *Nature*, 58, 395, 1898) The year of his birth has thus been stated by some as 1838 and by others as 1837 Comprehensive registration of births began in the summer of 1837, and a search at Somerset House has revealed the existence of a birth certificate It transpires that Newlands was born at the family address, 19 West Square, Southwark, on November 26, 1837 The birth was registered on December 26 (Bank holidays were introduced by the Act of 1871) His father, William Newlands, was a Presbyterian minister and his mother was formerly Mary Sarah Reina"

Radiation and Health

In October 1960, the International Atomic Energy Agency and the World Health Organization sponsored a scientific meeting on the diagnosis and treatment of acute radiation injury (*Diagnosis and Treatment of Acute Radiation Injury* Pp 425 Geneva World Health Organization, 1961 £2) The proceedings were reviewed together with other recent developments in radiation medicine in a special number of the *WHO Chronicle* published in December 1961, and have recently been reprinted as a brochure (*Radiation and Health* Pp. 44 Geneva World Health Organization, 1962 1*s* 9*d*) In addition to three general sections on radiation protection, there are short reviews on health hazards from nuclear-powered merchant ships, accidental radiation injury, management of radiation casualties, therapeutic uses of radiation injury, and statistics in genetic and radiation studies

Radioactivity in Diet

The seventh report of the Agricultural Research Council's Radiobiological Laboratory (H M S O , *ARCL* 7) records measurements carried out between December 1961 and April 1962 The resumption of nuclear weapons trials in September 1961 lends particular interest to this interim report Iodine-131 reached a maximum concentration in milk about the end of October 1961 (see *ARCL* 6) and thereafter fell rapidly to levels below the limits of detection by the beginning of 1962 The country-wide average in milk over the period of 32 weeks following the resumption of nuclear weapon trials was 17 per cent of that which would give rise to the annual average of 130 picocuries per litre which the Medical Research Council has stated would not cause the acceptable radiation dose to be exceeded for any age group of the population The strontium-90 levels in milk rose by about 20 per cent between October and December 1961 as compared with the corresponding levels the previous year although the country-wide figure calculated over the whole year was a little lower than that for 1960. These figures are reassuring, but the full effects of the weapons trials on strontium-90 levels in milk are not expected to be apparent until the results for the summer of 1962 are available. For the average mixed diet of the whole population, the strontium-90 level during 1961 was about 6.1 picocuries of strontium-90 per gram of calcium. This value is similar to that of 1960 and lower than that of 1959.

The Lace Research Association

The thirteenth annual report of the Lace Research Association covers the year 1961 (Pp. 35. Bilborough, Nottingham: The Lace Research Association, 1962). In the account of the progress of research, it is noted that production of cellular cotton blankets for hospital use has now reached an advanced stage of development, and it is considered that a similar blanket structure made from an acrylic yarn might be more suitable for the domestic market. A successful technique has been devised for producing tubular nets on the furnishings machine, while the problem of winding an increased length of usable yarn into the Leavers machine, in which the brass bobbins carrying the binding thread of the lace structure are spread between 8 and 30 per inch, is being tackled. This is being done by investigating the use of special nylon yarns of fine count and extra strength, by radically improving the machinery for winding the yarn into the bobbins and by examining the processes of heating and pressing the bobbins after winding. The new 'Lacra' bobbin winders have fulfilled in production all the claims made for them, fundamental work on the heating and pressing of brass bobbins continues. The new beam thread-break indicator has also given satisfactory results on a production scale. The Association has assessed the potentialities of the new high-speed lace machine designed by Dr. McGallion and Prof. Pope of the University of Nottingham. Two of the major projects of the year were concerned with the mechanical removal of floats from lace and the treatment of lace to give low flammability.

The Rubber Research Institute of Ceylon

The rubber industry of Ceylon, though small compared with that of Malaya, is nevertheless of vital importance to the country's economy, as it was to Great Britain and her allies during a critical phase of the Second World War. It is interesting to recall that it was in Ceylon in 1876 that the plantation industry was born—from plants nurtured at Kew—though it was not until the present century that plantation rubber began to oust native Brazilian rubber as the primary commodity of commerce. The place of science in the subsequent development of the industry is recognized in a booklet issued on the occasion of the fiftieth anniversary of the foundation of the Rubber Research Scheme—the forerunner of the present Rubber Research Institute of Ceylon—to which leading members of the Government and of the industry have contributed (*Rubber Research Institute of Ceylon: Golden Jubilee, 1910–1960.* Pp. xviii+14. Dartonfield, Agalawatta: Rubber Research Institute of Ceylon, 1961). In the face of intense competition from the synthetics, the plantation industry depends for its survival on the reduction of costs and improvement of quality which can only be brought about by the fullest exploitation of scientific knowledge and scientific methods. Factors of major importance are the replacement of older and inefficient trees with newly developed high-yielding types, the effective control of disease, and good husbandry and estate management. In all these aspects the Institute's scientific and advisory staff, working within a frame-work of Government assistance, has played an indispensable part, particularly in relation to the small-holders.

Research into Urban 'Decay'

The June *Newsletter* of the Manchester Joint Research Council Technical Information Centre, besides notes on some inquiries handled in May, includes a note by Mr. F. Medhurst, director of the Research Group of the North West Civic Trust, on research into urban 'decay', which touches on some aspects of his task. The Research Group, which hopes to discover the obstacles to improving the general environment of the mixed areas around city centres, will study land and property values, investment-levels, public transport services, changes in land uses and obsolescence factors for clues to the causes of urban decay. The study is planned to continue over three years, and an endeavour will be made actively to improve a selected area as an experiment in methods and techniques. The Research Group will welcome the suggestions and co-operation of any who are interested in the problem.

Ancient Monuments Boards

The eighth annual reports of the Ancient Monuments Boards for England, Scotland and Wales contain much of interest in addition to the usual formal information (Pp. 19+4 plates. London: H.M.S.O., 1962. 2*s*. net). The difficulties encountered in draining the land at Stonehenge are discussed, but as yet no solution has been found. It is stressed that it is desirable to retain the turf which provides such an admirable setting for the stones. It is now recommended to the Minister that, because of irresponsible behaviour, there should be an absolute ban on the solstice ceremonies on Midsummer Eve. It is proposed that the long barrow known as Wayland's Smithy in Berkshire should be examined by modern techniques, and it is reported that the Hull Museum is willing to take over and re-display the important pavement at the Roman villa at Rudstone. It is hoped that means will be found to preserve the somewhat unusual seventeenth century water garden at Westbury-on-Severn, Gloucestershire, and the Board welcomes the action of the Council for British Archæology in their endeavours to preserve and record early industrial buildings. Reference is made to emergency excavations including that of the Saxon and medieval palace at Cheddar, and recommendations for scheduling are made in respect of 340 monuments. The report for Scotland refers to the continual quarrying at Traprain Law and the great interest of the Orkney monuments. The Board in Wales have recommended the scheduling of a further 31 monuments, several of which have only been discovered by aerial photography.

The Museums Association

The sixty-eighth annual conference of the Museums Association was held in London and Brussels during June 25–29 under the presidency of Dr. Mary Woodall. The presidential address and the annual general meeting were held in London, and the delegates then travelled to Brussels for the rest of the meeting. In her address, Dr. Mary Woodall dealt with present-day matters in the business of the Association and made especial reference to the importance of the forthcoming report which is to be presented to H M Government by the Standing Commission on Museums and Art Galleries It is anticipated that the report will be available in the late autumn and that it will be intimately associated with the recently established Area Museum Councils Reference was also made to the continued generosity and interest of the Carnegie U K Trust, new regulations for the training of curators and technicians, and the new format of the *Museums Journal* Diplomas and technical certificates were presented to the successful candidates in the recent examinations, and Dr D A Allan, Sir George Dyson and Dr F J North were elected to Honorary Fellowship Alderman H Barks and Dr Barnett Stross were elected as institutional councillors, and Miss Anne Buck, Mr A A Cumming, Mr T H Scrutton and Mr H R Singleton were elected as professional councillors Sir Trenchard Cox was elected president for 1962–63, and the invitation of the University and City to hold the 1963 meeting at Newcastle upon Tyne at the end of July was accepted with thanks In Belgium, visits were made to the museums and art galleries in Antwerp, Limburg, Brussels, Tornai, Halle, Ghent, Bruges and Liège Opportunity was also taken to visit various historical buildings in the same places The annual dinner of the Association was held at the Hotel Metropole in Brussels

The Institute of Applied Science of Victoria

THE report of activities of the Institute of Applied Science of Victoria for the year ended June 30, 1961, comprises the report of the Trustees and the report of the director, Dr C M Focker, both of which refer to a donation for the establishment of a planetarium (Pp. 24 Melbourne Institute of Applied Science of Victoria. 1962) The Radiocarbon Dating Laboratory was opened in May 1961, some seven age determinations have been completed and the equipment has functioned well Completion of the new wing offered a major increase in display and working space and visitors are expected to exceed 400,000 annually when the planetarium is operating Long-term displays noted by the director include some on decimal currency, Edwin Land's sodium mirror, solar batteries, radioactivity and the Trevithick high-pressure steam engine

Microdoc

Microdoc, the journal of the Council on Microphotography and Document Reproduction, the first number of which is commendably concise and to the point, is intended to keep members abreast of progress and events, as well as provide a forum for the research article, the review, the query and the complaint (1, No 1 Edited by Mark Patterson Pp 16 London The Library Association, 1962 Subscription (non-members) 30*s* per annum) In this number, Mr L L Ardern writes briefly on the opaque versus the translucent screen and on the Dagmer Super Reader as well as contributing a review of Mr G U Davison's *Microtext Review of Progress in 1960* Mr E F Patterson describes the new 'V C Executive Reader', and Mr L Corbett the 'AO Micro Opaque Reader', and Dr L A Sayce's address at the meeting in October 1960 which voted in favour of establishing an advisory council on microphotography and document reproduction is reproduced Altogether, the first issue of this new venture gives a very pleasing impression of purpose and performance

British Chemicals and their Manufacturers

THE Association of British Chemical Manufacturers has recently issued the 1962 Supplement to the 1961 edition of the Association's Directory, *British Chemicals and their Manufacturers* (see *Nature*, 192, 1025, 1961) The Directory is published at two-yearly intervals, and during this period there are inevitably many changes and additions in the list of products manufactured This Supplement provides full details of these changes and will ensure that users of the 1961 Directory have up-to-date information regarding manufacturers of chemicals in the United Kingdom In order to assist users, a new lay-out and format have been adopted so that the information contained therein may be incorporated in the main Directory if so desired As in the case of the Directory, the Supplement is available gratis to all firms and persons interested in the purchase of chemicals and allied products (Pp 29 London Association of British Chemical Manufacturers, 1962)

Guide for Families of those in Mental Hospitals

To most people the realization that a relative or close friend is mentally ill and needs hospital treatment comes as a grief and a shock To help people faced with this situation, the National Association for Mental Health has produced a booklet of simple explanation and practical advice (price 2*s*) It replaces a publication issued some years ago before the new Mental Health Act made it possible for mental illness to be treated with as little formality in most cases as physical illness The booklet explains what kinds of help are available to patients and their families from the family doctor to the staffs of clinics and hospitals and workers in the local authority services. A brief summary of legal provisions is added as an appendix, with a glossary of some terms used in connexion with mental illness, a reading list and a list of local voluntary mental health associations existing in different areas

Chemistry and Mental Disease

A SYMPOSIUM on "Chemistry and Mental Disease", arranged by the Royal Institute of Chemistry, the Chemical Society and the Society of Chemical Industry, will be held in the University of Manchester on September 26 The programme will include biochemical processes in relation to mental disease (Dr A Todrick), inborn metabolic errors and mental disease (Dr L I Woolf), pharmacological assessment of psychotropic activity (Dr M W Parkes), tranquillizers—structure and activity relationships (Dr A F Crowther), psychostimulants—structure and activity (Dr E H P Young), use of modern drugs in psychiatric practice (Dr. F A Jenner) Further information can be obtained from Mr H H Armstrong, Science Department, Stockport College for Further Education, Wellington Road South, Stockport

The Jane Coffin Childs Memorial Fund for Medical Research

THE Jane Coffin Childs Memorial Fund for Medical Research has recently announced that during the past year twenty-eight grants-in-aid and twenty-three postdoctoral fellowships were awarded, totalling 382,459 85 dollars The Fund supports investigations in the fundamental aspects of neoplastic growth, and awards postdoctoral fellowships to candidates of outstanding potential in the same field Applications for grants-in-aid are reviewed by the Board of

Scientific Advisers and the Board of Managers of the Fund three times a year, in the autumn, winter and spring Final dates for receipt of applications are September 1, November 1 and March 1 Applications for fellowships are reviewed at the winter meeting of the Fund, the final date for applications being October 1 of each year Grants-in-aid and fellowships are not restricted to United States subjects Information can be obtained from the Office of the Director, 333 Cedar Street, New Haven 11, Connecticut

University News.

Edinburgh

THE following grants have been received by the University of Edinburgh £69,150 from the Nuffield Foundation, in support of the fibroblast research programme being carried out in the Department of Zoology, 5,000 dollars from the Rockefeller Foundation, for the expenses of a symposium on "Genetics of Colonizing Species" in the Department of Animal Genetics, £950 from the Department of Scientific and Industrial Research, for a research project on the rearing of a rare spawning of 'non-pigmented' toad eggs in the Department of Zoology, £2,500 from the United Kingdom Atomic Energy Authority, for a two-year investigation on unsupported oxide layers to be carried out in the Department of Chemistry, £50,000 from the Wellcome Trust for the cost of erecting a building to accommodate the new Medical Research Council Research Group on Epigenetics in the Department of Animal Genetics

London

THE following appointments have been announced Dr Roland Hill, lecturer in animal nutrition at the University of Reading, to the readership in animal husbandry and hygiene tenable at the Royal Veterinary College, Dr A A Johnson, lecturer at the Imperial College of Science and Technology, to the readership in physical metallurgy tenable at that College. The title of reader in civil engineering analysis has been conferred on Dr R E Gibson in respect of his post at the Imperial College of Science and Technology

The title of professor emeritus has been conferred on the following Prof J W S Blacklock (professor of pathology, St Bartholomew's Hospital Medical College), Prof J H Birkinshaw (professor of biochemistry, London School of Hygiene and Tropical Medicine), Prof G P Crowden (professor of applied physiology, London School of Hygiene and Tropical Medicine), Prof J McCunn (professor of veterinary anatomy, Royal Veterinary College), Prof L A Reid (professor of philosophy of education, Institute of Education), Prof W R Spurrell (professor of physiology, Guy's Hospital Medical School) The title of professor of theoretical mechanics has been conferred on Dr R S Scorer, in respect of his post at the Imperial College of Science and Technology and that of reader in psychopharmacology on Dr Hannah Steinberg, in respect of her post at University College

Nottingham

THE title of reader has been conferred on Dr C A M King (geography), Dr T J King (organic chemistry), Dr H Fessler (mechanical engineering)

Announcements

MR B D HANKIN has been appointed director of the City and Guilds of London Institute with effect from October 1 Mr. Hankin is at present a principal scientific officer with the Ministry of Transport As director, he fills a vacancy created by the Institute in its reorganization in which Major-General C. Lloyd was raised to the status of director-general

THE next award of the Meldola Medal, the gift of the Society of Maccabæans, will be made early in 1963 to the chemist who, being a British subject and less than thirty years of age on December 31, 1962, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Institute of Chemistry before December 31, 1962 Further information can be obtained from the Secretary, the Royal Institute of Chemistry, 30 Russell Square, London, W C 1, the envelope being marked 'Meldola Medal'

A SYMPOSIUM on "Measurements in Hydrology", organized by the Water Research Association, will be held in the University of Reading during September 18–20 Further information can be obtained from the Water Research Association, Medmenham, Marlow, Bucks.

THE sixth International Congress on High-Speed Photography will be held in Scheveningen during September 17–22 Further information can be obtained from Dr J G A de Graaf, Secretariat of the Sixth International Congress on High-Speed Photography, 14 Burgemeester de Monchyplein, The Hague

AN informal discussion on "Nuclear Chemistry, Fission and other Low Energy Nuclear Processes" will be held in the University of Oxford during September 18–20 Further information can be obtained from Mr G N Walton, Scientific Administration Office, Atomic Energy Research Establishment, Harwell, Didcot, Berks

A SYMPOSIUM on the latest developments and techniques in auto-analysis and also the introduction of several new devices, organized by Technicon Instruments, Ltd , of Chertsey, will be held at the National Film Theatre during September 17–18 Further information can be obtained from Mr James Evans, Technicon Instruments, Ltd , Hanworth Lane, Chertsey

A SUMMER school on "Interaction between Atmosphere and Ocean", supported by the Scientific Affairs Division of the North Atlantic Treaty Organization, will be held in London during September 3–14 Further information can be obtained from Mr H Charnock, Viale San Bartolomeo 92, La Spezia, Italy

THE first European malacological congress, organized by a joint committee of the Conchological Society of Great Britain and Ireland and the Malacological Society of London, will be held in London during September 17–21 Further information can be obtained from Mr J F Peake, Department of Zoology, British Museum (Natural History), Cromwell Road, London, S W 7

AN international course on "Chromatographic Methods in Lipid Research", sponsored by the Institute of Pharmacology and Therapy of the University of Milan and the Italian Society for the Study of Oils and Fats, will be held in the University of Milan during September 17–29 Further information can be obtained from Prof R Paoletti, Institute of Pharmacology, University of Milan, Via Andrea del Sarto 21, Milan

GOVERNMENT EXPENDITURE ON SCIENTIFIC RESEARCH, 1961

THE report of the Council for Scientific and Industrial Research for the year 1961*, which this year is mainly concerned with assisting industry to apply the results of research, records gross expenditure in the year ended March 31, 1961, of £14,467,278, compared with £12,008,536 in the previous year, and reduced to £12,759,180 by various receipts from industry and other sources for services rendered, of which £607,910 was from other Government departments and £819,255 from industry, £249,325 being received by the National Physical Laboratory, the net increase on 1960 was £2,416,489. Grants to students amounted to £1,105,415 (£870,289 in 1960), and 1,102 new research studentships, with 1,607 continuing from previous years, brought the total current on October 1, 1961, to 2,709. Besides these, there were 339 advanced course studentships, 66 first year studentships and 88 research fellowships, these figures include 154 NATO studentships and 35 fellowships.

Of the research students, 356 were in biology and biochemistry, 190 in chemical engineering and metallurgy, 833 in chemistry, 99 in electrical engineering and 161 in other engineering, 173 in geology, 46 in the human sciences, 261 in mathematics and 590 in physics. The advanced course studentships were distributed mainly in physics (70), mathematics (63), engineering (other than electrical) 53, with 35 in chemical engineering and metallurgy, 32 in geology, and 30 in the human sciences. All the first year studentships were in chemistry, and of the research fellows, as in previous years, chemistry (30), physics (27) and biology and biochemistry (18) claimed the most.

Grants for special researches totalled 696, of which 332 were new, amounting to £7,937,910 in the year ended March 31, 1961, and of this total 72 in nuclear physics amounted to £3,196,947, only in technology (113 grants totalling £804,593), chemistry (141, £771,801) and low-temperatures and solid-state physics (81, £710,117) did the total exceed £500,000, though this figure was approached by 100 grants in biology (£491,569) and 66 in geology and geophysics (£494,514), 31 grants in space research totalled £395,300. There were 41 grants, totalling £316,910, in the human sciences. During the academic year 1960–61, the Research Grants Committee considered 447 applications for grants, other than those for very expensive capital projects, with a total value of £3,156,000, compared with 422 valued at £3,444,000 in the previous year, and made 362 grants with a total value of £1,961,000, compared with 315 amounting to £1,712,000 in 1959–60. In addition, a capital grant of £803,516 was made to the University of Oxford to establish a nuclear structure group under Prof. D. H. Wilkinson, and grants totalling £167,355 for the maintenance of large nuclear physics machines at the Universities of Birmingham, Cambridge, Glasgow, Liverpool and Oxford, and for completion of the linear accelerator at Manchester.

With the exception of fire research, which decreased to £156,130 from £158,441 in 1959–60, and radio research, which at £239,957 was practically unchanged, the increased expenditure was distributed fairly generally over all branches of the Department's work. The major increases are in the expenditure of the National Physical Laboratory (£1,787,542 gross, £1,438,776 in 1959–60), Road Research Laboratory (£872,805, £718,058), National Engineering Laboratory (£811,164, £686,156), Warren Spring Laboratory (£453,985, £402,854), Building Research Laboratory (£785,413; £656,975), Geological Survey and Museum (£586,640, £490,014), and the National Chemical Laboratory (£384,073, £316,080). Expenditure by the Government Chemist now under the Department's vote increased from £290,237 to £460,257. Expenditure on headquarters administration also increased, from £623,206 to £758,126, and annual grants to research associations from £1,761,368 to £1,849,572, with £31,677 in special grants. Grants for special researches amounted to £1,495,469, compared with £912,944 the previous year, while contributions to the European Organization for Nuclear Research rose to £1,330,000 and to NATO scientific schemes to £122,534. There was a record net increase in staff of 544, bringing the total to 6,224, and the 52 research associations have a total expenditure of more than £8 million.

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* Department of Scientific and Industrial Research. Report of the Research Council for the year 1961. Pp. 60. (Cmnd. 1734.) (London: H.M. Stationery Office, 1962.) 4s. net.

NITROGEN HETEROCYCLIC CHEMISTRY

IN 1955, a symposium on heterocyclic chemistry, organized by the Chemical Society, was held at the University of Exeter. Its success was noted by the publication of the papers presented there[1]. A second related symposium was held in the Australian National University at Canberra in 1957. It attracted equal interest, though for geographical reasons largely from a different audience, once again the papers and discussions are accessible[2].

It is a tribute to the importance of the subject, to its rapid development, and to the active interest of teachers and workers in the field, that the Hatfield College of Technology, with the support of the Chemical Society, was able to arrange, in attractive quarters and surroundings, during May 10–11, a third, equally successful, symposium. The title was "Nitrogen Heterocyclic Chemistry". A wide field of chemistry was covered in nine papers delivered in four sessions, one of which was chaired by Prof. A. Albert, of the Australian National University, who played an active part in each of the two previous symposia.

The symposium was opened by Prof. W. Baker. A remarkable standard of material and presentation was set in the first paper, by Dr. J. W. Cornforth of the National Institute for Medical Research. He described an elegant and imaginative approach to the synthesis of vitamin B_{12}, which is of interest as an important factor in metabolism and as a coenzyme in some remarkable rearrangements of carbon chains *in vivo* Considered as a heterocyclic compound it poses unusual problems, a macrocyclic ring containing nine asymmetric carbon atoms and a combination of conjugated C=C and C=N bonds The route described involved separate syntheses of structures, each containing some of the asymmetric centres, in such a way that subsequent combination should be possible Some novel methods had to be devised One of them, a synthesis of 1,3-diketones, depends on the chemical reduction of pyridinium enol betaines, and an interesting discussion on this point developed at the conclusion of the lecture

In the following paper, Prof P B D de la Mare of Bedford College (University of London) discussed electrophilic substitution in nitrogen heterocycles by reference to analogies from the chemistry of non-heterocyclic systems It was pointed out that electrophilic attack on compounds containing unsaturated nitrogen often occurs at nitrogen rather than at carbon The fact that the different species thus produced may have different relative reactivities in subsequent steps was illustrated by discussion of the extreme cases of quinoline-N-oxide, the quinolinium cation, and the N-nitroquinolinium cation, and of derivatives of aniline and carbazole. The importance of the identity of any nucleophile which may participate in an addition-elimination sequence, and of details of the structure of any intermediate thus produced, was mentioned in connexion with the similarities and differences between the halogenation of phenanthrene, naphthalene and quinoline It was observed that, despite a recent publication taking a contrary view[3], the quinoline molecule itself is not known to be attacked electrophilically at carbon in any experiments yet described In the discussion which followed, misconceptions concerning the ease of addition of strong acids to C=C, C=O, C=N and N=N bonds were mentioned, it seems important to remember that, even when such additions are reversible and the consequent equilibria lie largely towards the starting materials, the adducts may, when their chemistry is appropriate, be important intermediates leading to further reactions

The third paper, by Prof Wilson Baker of the University of Bristol, described meso-ionic compounds as a varied group of nitrogen-containing heterocycles having six π-electrons associated usually with a five-membered ring In general, they are highly polar, and show the characteristic stability of aromatic compounds They are distinguished by the fact that they cannot be represented satisfactorily by a single canonical structure, but only as a resonance hybrid of a number of zwitterionic or tetrapolar canonical forms A review was given of their discovery and of the general chemistry of the various different types that have been recognized The discussion centred largely round the question of definition, terminology, and classification

In the fourth paper, on the synthesis of heterocyclic compounds by extrusion of sulphur, Dr J D Loudon of the University of Glasgow described ring-contractions in which extrusion of sulphur yields naphthalenes from benzo[*d*]thiepins, phenanthrenes from dibenzo[*b,f*]thiepins, phenanthridines from dibenzo-[*b,f*]-1,4-thiazepines, and benzoquinolizinium salts from pyrido[2,1-*b*]benzo[*f*]-1,3-thiazepinium salts The influence of the polar effects of substituents was discussed, and it was pointed out that the probable reaction course involves bond rearrangement to an episulphide, followed by extrusion of sulphur in harmony with the olefine-forming tendencies of episulphides and episulphones Other types of ring contraction are shown by the dihydrothiadiazepine which is obtained from diphenacyl sulphide and hydrazine this, like its S-dioxide, affords 3,6-diphenylpyridazine, whereaas the S-monoxide yields 3,5-diphenylpyrazole

In the fifth paper, Dr A Katritsky of the University of Cambridge described the chemistry of heteroaromatic N-oxides in terms of the capacity of the N—O bond in these compounds to act in appropriate circumstances either as a donor or an acceptor of electrons The various manifestations of this potentially two-fold character were discussed in some detail and provided interesting illustrations of the power of modern physical methods in the interpretation of structure and reactivity in organic molecules The use of proton resonance spectra in distinguishing between tautomeric structures was illustrated by reference to the benzofuroxan–*o*-dinitrosobenzene equilibrium

The evening was marked by a reception and buffet dinner given by Smith, Kline and French Laboratories, Ltd , at their Welwyn research centre, where interest in the displays of specialized equipment added to the enjoyment of the friendly hospitality shown to the participants

The second day of the symposium was opened by a paper by Prof D H Hey and his co-workers of King's College (University of London). Prof Hey described synthetic attempts to extend the Pschorr phenanthrene synthesis to the elaboration of various heterocyclic systems These experiments had revealed anomalous and new reaction-paths, including routes to a number of interesting spirodienones An interesting new intramolecular nucleophilic displacement of a nitro-group as nitrite ion was mentioned, as was a novel intramolecular cyclization involving a benzyne intermediate

Dr A H Jackson of the University of Liverpool gave a valuable account of the application of proton magnetic resonance to porphyrins, for which these spectra are of special interest because they cover such a wide range This is attributed to the large ring-current in the macrocyclic conjugated system Although effects of substituents can in some cases be interpreted on an additive basis, there are some indications that the spectra can be modified if steric effects result in local distortions in the molecule

Dr K Schofield of the University of Exeter gave an account of the stereochemistry of the simple quinolizidines. The infra-red and proton resonance spectra of the isomeric 1-,2-,3- and 4-methyl-quinolizidines were interpreted with reference to the probable conformations of the molecules It was concluded that these studies suggest (contrary to conclusions from other techniques) that the lone-pair of electrons on nitrogen is not more space-filling either than a C—H bond or than a $\equiv\overset{+}{N}$—H bond in aprotic solvents The discussion centred around the position of conformational equilibrium and its relation to protonation

The final paper, by Dr S F Mason of the University of Exeter, described the electronic spectra of related series of heterocyclic compounds in terms of a general model consisting of a conjugated system of carbon atoms from which the heterocyclic compounds are schematically derived by replacement of carbon by hetero-atoms The various types of electronic transitions responsible for visible and ultra-violet spectra were classified, and the formulation of the species responsible for the spectroscopic properties of methiodides of pyridine, quinoline and related compounds was discussed Prof D H Hey, chairman of the final session, closed the meeting with a vote of thanks to the organizers of the symposium

The widespread discussion of heterocyclic molecules in courses for Higher National Certificates in chemistry no doubt plays a part in creating an area of interest which makes it appropriate to hold a symposium of high academic standard on this subject in a college of technology It was clear to all those attending the symposium that great credit is due to Dr R F Robbins, head of the Department of Science at Hatfield College of Technology, for his imaginative forethought and efficient organization of such a successful gathering All the participants will want also to thank the officers and administration of Hatfield College of Technology for making such satisfactory arrangements Some of those present will have returned from this symposium wondering whether they might not expect the registration fees they pay for other conferences to be put as obviously as on this occasion towards the benefit of those who take part in the proceedings Others concerned elsewhere in the finance of scientific education will have been impressed by what can be achieved when good scientists are given the means wherewith to fulfil their vision There are probably some participants, and others who were unable to attend, who may regret that there is no permanent record of the scientific contributions presented at the symposium

P B D DE LA MARE

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[1] Chemical Society Special Publication No. 3

[2] *Current Trends in Heterocyclic Chemistry*, Albert and Shoppee (eds) (Butterworths, 1958)

[3] Eisch, *J Org Chem.*, **27**, 1318 (1962)

COMPARATIVE NEUROCHEMISTRY

FIFTY-FOUR scientists from many different countries met at St Wolfgang, Austria, during June 11–15 for the fifth International Neurochemical Symposium. The topic chosen for this meeting was "Comparative Neurochemistry" and the programme was designed to integrate the available data on differences in structure, chemical composition, metabolism, enzymatic activity and drug and hormone action in the nervous systems of invertebrate and vertebrate species The meeting was interdisciplinary in approach, providing an opportunity for research workers in different fields to relate biochemical and pharmacological data to the morphology and function of nervous structures The formal presentations were followed by lively and prolonged discussion in an atmosphere of informality which promoted the free exchange of information and ideas

The Symposium opened with a discussion of the structural bases for similarities and differences in neural function in different species The organization of nervous structures in invertebrates was described by Dr G A Horridge (St Andrews) The nerve-net of the jellyfish, an anastomosing syncitium of motor neurones acting as a single cell, appears to provide a simple mechanism for the production of co-ordinated, rhythmic motions of the whole body In the polychætes, segmental and whole-body movements may be differentiated by a network of neurones and interneurones with symmetrical synaptic connexions providing a system for conduction of the nerve impulse in either direction As pointed out by Prof V Hamberger (St Louis), spontaneous swimming movements of the whole body prior to individuation of the limbs is the earliest manifestation of neuromuscular activity during the development of vertebrate embryos, although this may be preceded by precocious reflex activity in the case of the mammals Sir Frances Knowles (Birmingham) summarized the present state of knowledge with respect to neurohumoral mechanisms of lower forms In the phylogenetic sequence from molluscs to arthropods, a reduction of neurosecretory elements with a concomitant increase in the complexity of neural structures and connexions appears to occur

Qualitative and quantitative comparisons of the chemical composition of vertebrate and invertebrate nervous systems were presented by several of the participants The bulk and complexity of the data do not permit ready summarization, but, in general, the basic similarities in composition of nervous tissues were more striking than the intraspecies differences, probably reflecting the fundamental unity of cellular organelles, and of membrane and myelin structures in nerve cells throughout the animal kingdom For a given region of the nervous system the chemical composition, especially with respect to lipids of the myelin sheath, nucleic acid content, and patterns of free amino-acids, is characteristic of the stage of differentiation, and is relatively insensitive to changes in the external environment Some interesting features unique to certain forms are the absence of detectable quantities of glycolipids in certain invertebrate species, and the occurrence of distinctive compounds such as threonine–ethanolamine phosphodiester in the brain of some fishes Of interest is the presence of amino-ethylphosphonic acid in the nervous system of some echinoderms

Several papers were presented concerning comparative aspects of the transport of metabolites across nerve cell membranes In a comprehensive review of blood–brain barrier systems, Dr R V Coxon (Oxford) concluded that in the cœlenterates and lower phyla no effective barrier exists between nervous tissue and the extracellular fluid The first indication of isolation of the nervous system from its surroundings occurs at the phylogenetic level of *Branchiostoma*, where a barrier to penetration of foreign substances appears at a relatively late stage of development in higher forms, and appears to be associated with increased vascularization and proliferation of glial elements The maintenance of ionic gradients in nervous tissues of various species in passing from marine to freshwater and terrestrial environments was discussed by Dr H Hillman (London) Although the degree of regulation of extracellular cations may vary widely, in all species studied, the nerve cell behaves fundamentally as a potassium electrode, and the intracellular/extracellular K^+ ratio is regulated in conformity to the Nernst equation

The metabolism of the brain in relation to hibernation received special attention. Prof A V Palladin (Kiev) described a marked reduction in the *in vivo* incorporation of labelled amino-acids into brain proteins during hibernation The biochemical basis of the dramatic increase in body temperature and metabolic rate during arousal from hibernation was discussed by Dr L Mokrasch (Waverley) Changes in the activities of oxidative and glycolytic enzymes do not appear to play a significant part, but it appears possible that activation of phosphorylase, and enzymes (adenosine triphosphatase and acyl phosphatase) capable of cleaving high-energy compounds may be implicated in the mechanism of heat production during arousal

The distribution of neurohypophyseal peptides in different classes was traced by Prof H Heller (Bristol). In the cyclostomes, fish, amphibia and birds, the principal antidiuretic peptide is 8-arginine-oxytocin (vasotocin), in most mammals, including the ruminants, arginine-vasopressin is typical, while in the pig and hippopotamus lysine-vasopressin is characteristic A basic phylogenetic division in distribution of the octapeptides may be traced, but important exceptions to the general pattern exist

Dr D B Carlisle (London) posed a provocative question for the participants in the title of his paper "What makes the Shrimp's Heart Beat Faster ?" One answer proved to be "another shrimp !" but in all, six substances (including two polypeptides, and four indole derivatives) isolated from extracts of the shrimp pericardial organ will produce the same effect The most potent compound has been identified as 5 6-dihydroxytryptamine This compound probably serves to produce instantaneous effects on the heart beat, while the polypeptides appear to account for long-term responses during prolonged stress Other papers were concerned with the distribution and functions of acetylcholine, indoles, catecholamines and other possible neurotransmitter substances in different species

In the final session, devoted to comparative neuropharmacology, the basis of species differences in the sensitivity to drugs was considered The selective toxicity of piperazine, an anthelmintic agent, was shown by Dr E Bueding (Baltimore) to be related to its ability to produce a selective neuromuscular blocking action in *Ascaris* without affecting the neuromuscular junction of the mammalian host As pointed out by Dr D Richter (Carshalton) in the final discussion, the practical value of comparative neurochemistry is evident in the exploitation of such intraspecies differences for the development of new anthelmintics and pesticides

Many excellent papers and interesting commentaries have been necessarily omitted from this brief report I would be remiss, however, if I failed to comment on the fine tissue culture experiments on *in vitro* myelin formation by Prof Margaret Murray (New York), the elegant electronmicrographs of nerve membranes by Dr J D Robertson (Waverley), the stimulating speculations concerning the macro-molecular basis of coding in the memory mechanism by Prof F O Schmitt (Boston), and the techniques used by Dr H Blaschko (Oxford) in estimating the amine oxidase activity of the Malpighian tubules in the cockroach The proceedings of the Symposium are being published in the form of a book, *Comparative Neurochemistry*, edited by Dr D Richter

During our stay, the participants were entertained at receptions and banquets by the Mayor of St Wolfgang, the Mayor of Salzburg and the Government of Upper Austria The hospitality of our Austrian hosts and the beauty and gaiety of the Salzkammergut made this Symposium an exceedingly enjoyable as well as scientifically profitable occasion W C McMurray

THE COLONIAL DEVELOPMENT CORPORATION

THE annual report and statement of accounts of the Colonial Development Corporation for the year ended December 31, 1961*, follows the pattern of the previous report However, the form of the accounts has been altered to give effect to the changes in financial arrangements which the Government announced in April 1961, deferred Government interest currently accruing in 1961 is charged for the first time in the accounts to the extent of some £800,000 The 102 approved projects at the end of the year, totalling £115,770,000, included thirteen new projects totalling some £10 million, of which the largest were the Swaziland Railway Board (£4 25 million), the Trinidad Mortgage Agency Co , Ltd (£1 97 million), the Land Development and Settlement Board, Kenya (£1 5 million) and Northern Rhodesia Africa Housing (£1 million) Four projects were abandoned and one existing project was split into two projects Of the total commitments, £16 454 million was in the Caribbean region, £20 315 million in the Far East, £22 484 million in East Africa, £22 743 million in Central Africa, £20 347 million in the High Commission territories, and £13 427 million in West Africa Functionally, 54 8 per cent of the committed capital in 1961 was for basic development, 34 4 per cent for primary production and processing, and 10 8 per cent for commerce and industry

While the British Exchequer remains the chief source of the Corporation's finance, a steadily increasing part of its investment is financed either by self-generated funds or by loans from sources other than the Exchequer It is emphasized, however, that the Corporation's operations are more immediately likely to be restricted by territories becoming independent with consequent exclusion of the Corporation, despite their continued need for its help, than by shortage of funds, and the total of new projects under serious consideration fell from 38 to 26 during the year The present terms of reference in independent territories are, however, now under review

The report indicates the type of scheme which will receive serious consideration by the Corporation once compliance is established with the Corporation's normal powers Priority is increasingly given to schemes which help the inhabitants of the country to establish themselves as members of an economically self-supporting and ordered community, for example, agricultural small-holder projects, householder mortgage finance companies and local industrial development agencies Through the small-holder schemes also, production of tea in Kenya, palm oil in Malaya, tobacco at Kasungu, Nyasaland, and sugar in Swaziland should yield an increasing volume of cash exports The success of these schemes depends in the long run on other aspects of the territorial economy reaching a certain level of development and affording such projects as power, water supplies, transport and nucleus agricultural estates, the means to investigate and establish the cultivation and management techniques appropriate to small-holder crops, and that high priority be given to production industries which generate the cash exports to service capital investment overseas The Corporation is already committed to nearly £6 million in some fourteen projects for local industrial development agencies.

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*Colonial Development Corporation Annual Report and Statement of Accounts for year ended 31st December, 1961 Pp vi+90 (London H M Stationery Office, 1961) 5s 6d net

Stress is also laid on the way the Corporation is placed to keep in touch with local circumstances and views The Corporation believes that the presence on the spot of Corporation staff, ready to share the risks of local ventures by equity investment, engenders a sense of partnership not present when development finance is provided only as block loans from abroad. The maintenance of the Corporation's offices and staffs in times of rapid political change certainly provides an essential element of economic continuity, the extent of the management organization implied in the fact that the Corporation itself has at least some responsibility fot the management of more than half the 102 continuing projects is seldom realized Although there is less reference in this report to this training function, the importance of making every effort to train local men for executive posts of responsibility is fully recognized Some success in this direction is reported in the Far East, in the Caribbean and West Africa, while in other parts of Africa the Corporation is concentrating, as a first step, on providing scholarships at agricultural and technical colleges and on project training schemes

MINERAL TECHNOLOGY AT THE IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

IN 1952 the University of London established at the Royal School of Mines a course in mineral technology leading to a B Sc degree, the first undergraduate course of its kind in the Commonwealth and one of the first in the world In 1961 a new chair was founded (see *Nature*, 190, 1153, 1961) and the inaugural lecture of Prof M G Fleming has now been published by the Imperial College of Science and Technology*

Mineral technology, embracing as it does both the engineering and the scientific aspects of the separation of the valuable mineral constituents from their ores, has its origin in prehistoric times with man's selection and dressing of stones for his tools and weapons, and its long history is closely linked with that of civilization itself Silver from the mines at Laurium built the Greek fleet that defeated Xerxes, and copper from Falun financed Sweden's part in the Thirty Years' War During the twentieth century, more irreplaceable minerals have been consumed than in the whole previous history of the Earth From 1911 to 1959 the world production of iron ore increased from about 130 million tons to 406 million, nickel ores from 24 to 290 thousand tons and sulphur and pyrites from some three million tons to eight times that figure This expanding demand has necessitated the treatment of increasingly low-grade and complex deposits which in the past would have been considered valueless, and mineral dressing which even fifty years ago was still essentially an art has now become a branch of industrial science in its own right How important it is for Britain, not only to keep abreast of the latest developments in this field but also to play the fullest part in new research of all kinds, is evidenced by the fact that Britain no longer has control of enormous quantities of raw material which is necessary for her existence as a leading industrial nation As a result of the granting of independence to many territories much more than half the mineral production of former members of the Empire has already passed out of Britain's hands

It is in order to play its part in this that Prof. Fleming's Department has come into existence Quite apart from its undergraduate work, the investigations already under way range from the determination of heats of adsorption, a study of the mechanism of adhesion of colloidal particles and the kinetics of solvent extraction, to dense-medium separation in hydrocyclones and the beneficiation of concentrates by pelletizing, roasting and leaching This most interesting inaugural lecture concludes with an account of work on flotation in which the necessary reagents are introduced as a fine spray, the separation of ore from gangue being further improved by the application of an appropriate electrostatic charge whereby the droplets of reagent are attracted to the valuable minerals and actually repelled from the particles of gangue

F C THOMPSON

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* Mineral Technology—Progress and Problems (Inaugural Lecture, December 5, 1961) Pp 79–98 By Prof M G Fleming (London Imperial College of Science and Technology Distributed by the Modern Book Co, Ltd, 19 Praed Street, London, W 2, 1962) 6*s*

EVALUATION OF THE CARCINOGENIC HAZARDS OF FOOD ADDITIVES

THE Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives met in Geneva during December 12–19, 1960, and, in its fifth report* has recommended the following general basic principles

(1) Food additives should be used for the benefit of the consumer, they should not be permitted for any deceptive or misleading purpose

(2) The use of certain food additives is justified since the present position with respect to world food supplies makes it imperative that good techni-

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* *W H O Tech Rep Ser*, No. 220 (H M S O, 1961)

cological procedures should be developed and used to a maxima extent

(3) Food materials and all chemicals and processes at present in use for the preparation and distribution of food should be subjected to adequate examination to ensure minimum risk in use, if this has not already been done, or unless previous knowledge indicates that this is unnecessary

(4) All chemicals or processes proposed in the future should be subjected to adequate examination to minimize risk before being accepted for use

(5) The presence of carcinogenic substances in food might be a significant factor in the occurrence of what is considered to be spontaneous cancer in man and animals

(6) Since dose–response relationships have been demonstrated in the case of carcinogenic agents, the reduction of carcinogenic substances in food to the lowest practicable level may be one of the effective measures towards cancer prevention

(7) Many factors may influence dose–response in carcinogenesis Their complexities are such that it is agreed that no assuredly safe level for carcinogens in human food can be determined from experimental findings at the present time

(8) The elimination, or at least the reduction to a minimum, of all proved carcinogenic substances in the diet of man and of animals used as human food is a worth-while objective

In recent years a number of food additives or food contaminants have been suspected of being carcinogenic As a practical approach to the problems arising in this field, the Committee has reviewed the evidence available and the action taken with regard to a number of these substances Many points of interest were revealed, of which the most important are

(*a*) The apparent lack of information on the toxicity or potential carcinogenity of many food additives

(*b*) The inadequacy of the design, execution and interpretation of some experiments or of reported information in some of the publications in this field and the frequent lack of corroborative evidence

(*c*) The need for detailed pathological evaluation of any lesion observed in experimental studies

(*d*) The difficulties arising in interpretation of local sarcoma formation at the site of the injection

(*e*) The difficulty of completely excluding, at the present time, carcinogenic contaminants from food, from processes used in the preparation of food, and from substances coming into contact with food

(*f*) The possibility that some natural constituents of the diet or even an essential nutrient, such as selenium, may constitute a carcinogenic risk Clearly, these substances cannot be completely excluded from the diet

(*g*) The difficulty of carrying out and interpreting epidemiological studies

(*h*) The wide variety of food colours, many of which do not appear to have been adequately tested, including the 'permitted' lists of different countries

(*i*) The necessity for separate assessment of the carcinogenic risk for each individual substance

EARLY FOSSIL RODENTS AND THEIR EVOLUTION

THE literature of science overflows with immature works, but at rare intervals a *magnum opus*, the seasoned fruit of many years patient study, is offered for our digestion Such is *The Early Tertiary Rodents of the Family Paramyidae*, by Albert E Wood, published in the *Trans Amer Phil Soc* (New Series, 52, part 1 Pp. 261 Philadelphia American Philosophical Society, 1962 6 dollars) Behind the innocuous title nestles a major contribution to the study of fossil rodents, a veritable landmark in mammalian palæontology, and a wealth of detailed anatomical, taxonomic and stratigraphic observations for the student of evolution

The paramyids comprise the earliest known rodents of the Upper Palæocene and Eocene When last reviewed by W D Matthew in 1910 [1] he was able to deal exhaustively with the group in thirty pages The size of the present work is a measure of the vast quantity of additional material available for study half a century later Wood begins with a review of the history of the classification of the paramyids, discussing the views of earlier writers on the importance of the paramyids in the evolution of rodents

All but 30 pages of this great work are devoted to a systematic treatise of the family paramyidae Wood states he has examined approximately a thousand specimens, referred to 22 genera and 78 species, ranging from the Upper Palæocene to Upper Oligocene, mainly from North America but including European forms In several instances nearly complete skeletons were available Where material in a sample was adequate he has made variation studies, incisor teeth have been carefully examined and found to be of considerable systematic value Each species is monographed, its probable affinities discussed and where possible the functional interpretation of its characteristics are suggested These details enabled Wood, for example, to reconstruct *Paramys delicatus* as a rat-like animal, rather than the squirrel-like reconstruction of Matthew In two species Wood has been able to recognize a series of sub-species—material for so detailed a study of gens or *rassenkreis* rarely obtains in mammalian palæontology The illustrations are admirable, clearly drawn and well printed it is unfortunate that the phylogenetic tree could not have been three-dimensional

Following the systematic treatise, Wood discusses the interrelationships of paramyids, evolutionary trends within the family, relationships to later rodents, rodents subordinal classification, evolutionary rates in paramyids and the origin of rodents Several of the opinions and interpretations set out here have already appeared in print in earlier writings of the author, nevertheless the summary accompanying the monograph is useful in restating Wood's case alongside his evidence

In discussing evolutionary trends Wood distinguishes three series First, those that can be followed in all or nearly all lines within the family, representing results of selective pressures for increased rodent adaptation, these include continuously growing incisors and reduction of pre-molar dentition. Secondly, those which represent divergent trends characteristic of one or more sub-families as a result of its adaptive radiation; these affect shape of incisors, crest pattern on molars and degree of reduction of last molar. Finally, those which show no significant pattern as in the erratic evolution of the third upper pre-molars. Wood has calculated the rates of evolutionary change which at generic level within the paramyids he found to be similar to those of horses, but in some lines much faster.

Regarding classification, relationships and origin of rodents in general and of paramyids in particular, Wood gives a very fair summary of the views of Stehlin and Schaub[2], his chief rivals, though it becomes patently clear to the reader that the two schools are still very distinct. On the evidence made available in this monograph, together with his interpretation of cusp homologies and the significance of parallelisms, Wood's thesis that "the Paramyidae are ancestral to all other known rodents" seems rather more probable than Schaub's view, based on a different interpretation of cusp homologies, which places the ancestry of the rodents farther back in the Palæocene with the paramyids as specialized descendants and the Oligocene squirrels as structurally the most primitive known rodents. Both views and their consequent implications are plausible analyses of the present evidence; only new evidence can produce an approximation nearer the truth. Wood's monograph enables us to ask more questions about rodent evolution; this is the surest way of unravelling the enigma.

R. J. G. SAVAGE

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[1] Matthew, W. D., *Bull. Amer. Mus. Nat. Hist.*, **28**, 43 (1910).

[2] Stehlin, H. G., and Schaub, S., *Schweiz. palaont. Abh.*, **67**, 1 (1951).

A SUGGESTED RECONSTRUCTION OF THE LAND MASSES OF THE EARTH AS A COMPLETE CRUST

WEGENER'S theory of continental drift is by no means universally accepted by geophysicists[1]. However, as Carey[2] has pointed out, the degree of congruence between Africa and South America, compared at the 2,000-m. isobath, is so great that pure chance can scarcely be the explanation for the fit. Moreover, this theory satisfactorily accounts for the fact that the Earth's granite is collected in the continents, the remaining three-quarters of the Earth's surface, forming the ocean bed, almost certainly consisting of basalt.

An alternative hypothesis is that the Earth was formerly much smaller and was covered by a complete crust of granite, subsequent expansion causing the splitting of the crust and the filling of the cracks by upward movement of the underlying molten basalt. If this hypothesis were valid it would be possible to fit together the outlines of the continents on a small globe, thus representing the Earth in its early state. I attempted to do so before becoming aware of the evidence adduced by Egyed[3] and Heezen[4] that this type of expansion may in fact have occurred, and the outcome provides collateral evidence in support of their views.

It was estimated that the representation of the continents, together with the continental shelf extending to the 1,000-fathom isobath, on a globe 4½ in. in diameter, had a total area which corresponded to somewhat less than the surface area of a 3-in. sphere. The outline of each continent was transferred from such a globe to thin sheets of rubber and cut out. The separate pieces of rubber were then placed on a wooden ball 3 in. in diameter and their outlines marked on the wood, to represent the appearance of the Earth soon after the hypothetical splitting of the crust. Despite the crude method used, the land masses fitted together reasonably well, though there was some distortion of certain regions and there was a triangular gap in the North Atlantic ocean (Fig. 1). It is to be noted that post-palæozoic geology need not be identical in masses which would appear to have been adjacent, as the movements envisaged possibly originated at a much earlier phase. It is difficult to

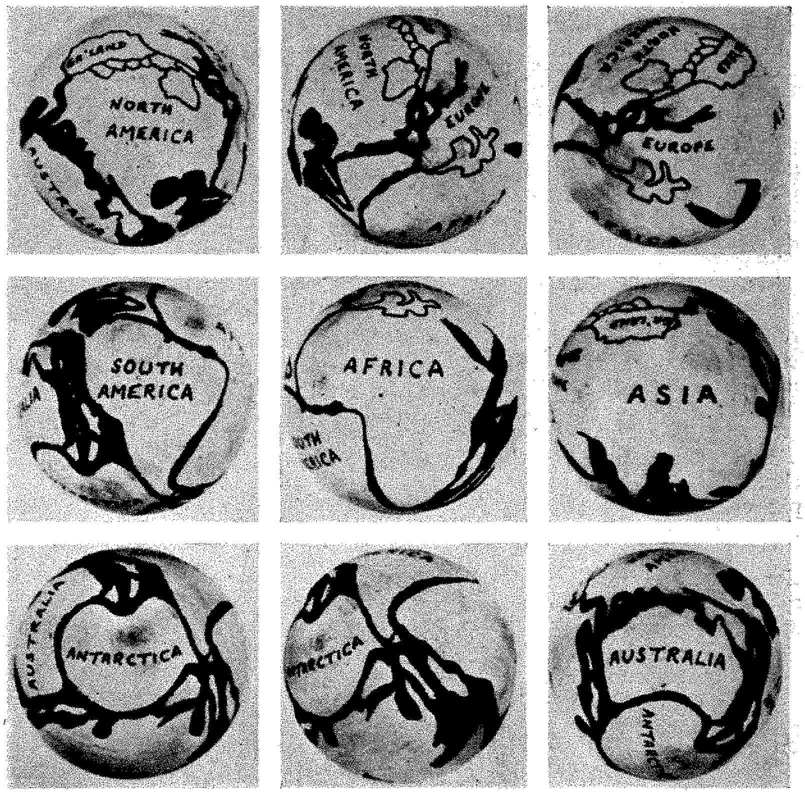


Fig. 1. Nine photographs of the wooden sphere representing the suggested pattern of the continents soon after splitting of the crust

believe that chance alone can explain this fitting together of the continental margins.

I thank Mr. P. S. B. Digby for helpful advice.

C. H. Barnett

Department of Anatomy,
St. Thomas's Hospital Medical School,
London, S.E.1.

[1] Jeffreys, H., *The Earth*, fourth ed. (Cambridge University Press, 1959).

[2] Carey, S. W., *Geol. Mag.*, **92**, 196 (1955).

[3] Egyed, L., *Geol. Rdsch.*, **46**, 101 (1957); **50**, 251 (1960); *Geophysica, Helsinki*, **7**, 13 (1959).

[4] Heezen, B. C., *Sci. Amer.*, **203**, 98 (1960).

It is interesting to see what sort of fit can be produced in accordance with Egyed's ideas. It is better than I should have expected, and will probably give rise to much discussion. Apart from the problem of explaining how the Earth's volume can have increased three-fold, it will probably take some time for structural geologists, palæontologists, meteorologists and magneticians to agree about whether the separation of the land masses occurred soon after the formation of the Earth, in the Palæozoic, or at the end of the Cretaceous.

However, it should be pointed out that areas on a 4½-in. sphere cannot be placed on a 3-in. one without distortion; the rubber must have been stretched, and the quality of the fit must depend on the distribution of the stretching. I cannot, therefore, accept the suggestion that the only alternative to the reconstruction is chance.

I pointed out in the 1929 edition of *The Earth* that Wegener's alleged fit of South America into Africa is a misfit by about 15°. This is obvious on inspection of a globe. The east coast of South America is close to a pair of great circles meeting at 90°; Africa from Liberia to the Cape near a pair meeting at 105°–110°. Nevertheless, drawings have been published until recently showing a good fit. This cannot be produced without considerable distortion, which has disappeared in Barnett's reconstruction owing to the extra curvature downwards in fitting to the smaller sphere.

Dr. Barnett is wrong in claiming that Wegener was the first to explain the differences between continents and oceans. The notion of a light upper layer was due to Airy, based on deflexions of the plumb-line in India, and was extended to the difference between continents and oceans by Suess.

Harold Jeffreys

St. John's College,
Cambridge.

RELEASE OF IODINE-131 FROM IRRADIATED URANIUM OXIDIZING IN AIR AND IN CARBON DIOXIDE

By W. J. MEGAW and J. E. BRIDGES

Health Physics and Medical Division, Atomic Energy Research Establishment, Harwell

We have recently published[1] the results of an investigation into the release of iodine-131 from irradiated uranium. In those experiments the irradiated uranium slug was heated in a vertical channel by a radio-frequency heater and the oxide particles were removed from the heated zone by gravity, or by the circulating gas, as soon as they were formed. We therefore made a distinction between: (*a*) iodine-131 which was released from the uranium as elemental iodine in the gas phase; (*b*) iodine-131 which was released contained in or adsorbed on particles of uranium oxide.

It was apparent from the results that the quantity of iodine-131 in category (*a*) which was released when the oxidation occurred in air, depended to some extent on the rate and quantity of oxidation and had a maximum value of about 30 per cent of the iodine-131 content of the oxidized uranium. In carbon dioxide, however the releases were very much less than this even when, at temperatures above the β–γ phase-transition temperature of 771° C., the oxidation-rates were much greater than in air.

We have therefore made a more detailed examination of the connexion between oxidation and iodine release, at a single temperature 800° C., using the apparatus shown in Fig. 1, which enabled both the oxidation and release of iodine-131 to be followed continuously throughout an experiment of several hours duration. By suitable choice of operating conditions we restricted ourselves to the iodine of category (*a*), that is, elemental iodine in the gas phase.

The uranium disk, about 6 gm. in weight, was supported in a platinum crucible which was suspended in the furnace at *A* from one arm of a balance. In each experiment the uranium was brought up to the working temperature of 800° C. in an atmosphere of argon, and the preheated air or carbon dioxide was then passed in at the top of the reaction vessel. The flow of gas used was 0·2 l./min., which was sufficiently low to ensure the retention of the oxide particles within the crucible. The temperature of the uranium was measured by means of a thermocouple inserted in a small hole drilled in the uranium disk, the difficulty of weighing the specimen continuously

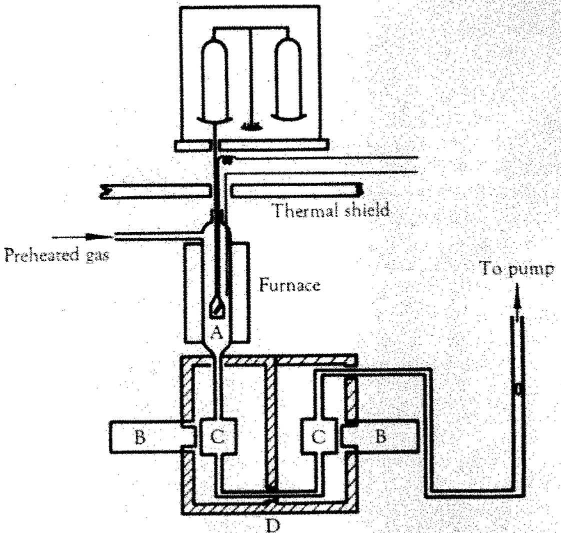


Fig. 1. Experimental arrangement. *A*, irradiated uranium disk; *B*, γ-counters; *C*, iodine samplers; *D*, lead shield

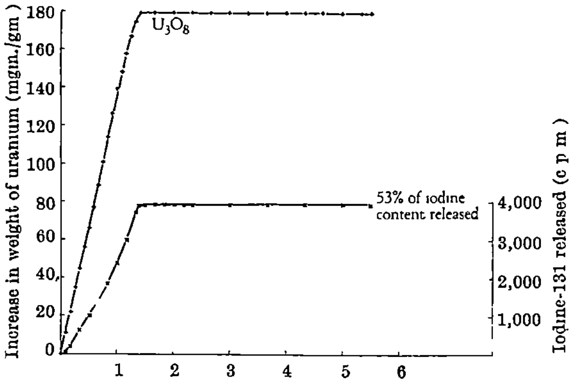


Fig 2. Oxidation of uranium and release of iodine-131 in air at 800° C +, oxidation, ×, release of iodine

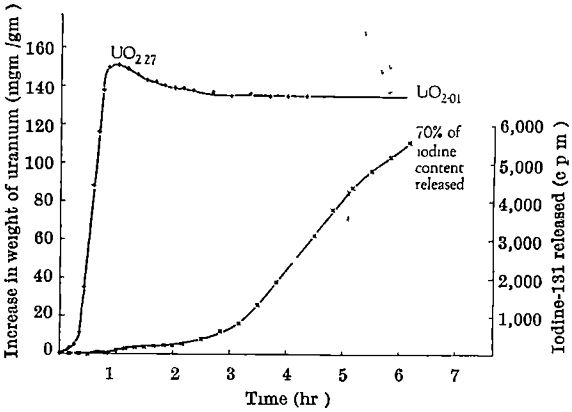


Fig 3 Oxidation of uranium and release of iodine-131 in carbon dioxide at 800° C +, oxidation, ×, release of iodine

while it was carrying a thermocouple being overcome by inserting flexible sections of fine copper wire in the thermocouple leads Parallel check measurements with a conventional thermocouple showed that the addition of the flexible link made no significant difference to the accuracy of temperature measurement.

The gas was passed out of the reaction vessel through a series of iodine samplers which contained in succession two acid-etched copper gauzes, an *AA* grade 'Millipore' filter paper, a charcoal-loaded filter paper and a bed containing 20 gm. of granulated activated charcoal Samplers of this type have been found to be highly efficient for all known forms of airborne iodine and are described more fully by Megaw and May[2] As the experiment proceeded the build-up in the sampler of iodine released from the oxidizing uranium was followed by means of a γ-scintillation counter

The experiments were continued for several hours after oxidation was complete, in order that any subsequent release of iodine or changes in weight of the uranium could be followed After each experiment radiochemical analyses for iodine were performed on uranium wires, which had been irradiated in holes in the uranium disk, and on samples of the oxide so that the percentage of the iodine originally present in the uranium disk which was released during the experiment could be determined The analytical method used was that given earlier by us[1]

Typical results are given in Figs 2 and 3, in which the increase in weight of the uranium disk and activity of iodine-131 in the samplers are plotted against time for oxidation in air and carbon dioxide respectively In Fig 2 it is seen that in air the uranium oxidizes to a weight which corresponds to a composition of U_3O_8, and that the iodine is released during the period of oxidation The release of iodine ceases when oxidation is complete and about 50 per cent of the iodine content of the uranium has been released. Fig 3 shows that in carbon dioxide the uranium oxidizes initially to a weight which corresponds to a composition of $UO_{2\cdot3}$ and during the period of oxidation virtually no iodine is released As heating continues, the uranium oxide loses oxygen and releases iodine, and after several hours has a weight corresponding to a composition of $UO_{2\cdot0}$ By then about 70 per cent of its initial content of iodine-131 has been released Substantial releases of iodine only begin when the oxide has practically reverted to UO_2.

The reason for this behaviour is not understood According to Katz and Rabinowitz[3], the oxides of uranium in the range $UO_{2\cdot0}$ to U_3O_8 are solid solutions of oxygen in uranium dioxide It is possible that the iodine atoms, which are formed at random in the uranium, are held in the uranium dioxide lattice in the same manner as the dissolved oxygen atoms and released with them when the oxide reverts to uranium dioxide

[1] Megaw, W J., Chadwick, R C, Wells, A C., and Bridges, J E, *J Nuclear Energy*, A/B 15, 176 (1961)

[2] Megaw, W. J, and May, F G, *U K Atomic Energy Auth AERE-R 3781* (1961)

[3] Katz, J J, and Rabinowitz, E, *The Chemistry of Uranium* Pt 1 (McGraw-Hill, 1951)

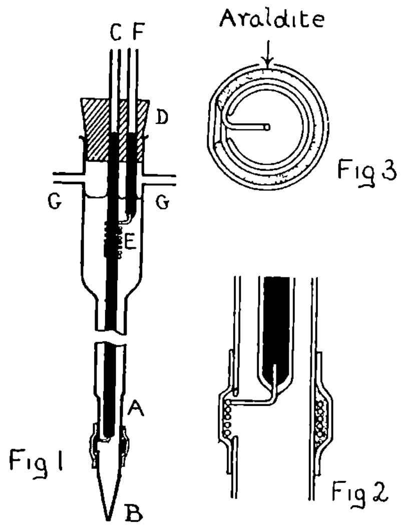
A DIFFUSION-CONTROLLED CATHODE FOR THE MEASUREMENT OF OXYGEN TENSIONS

By Dr R W PITTMAN

Department of Chemistry, Birkbeck College, London

A NUMBER of diffusion-controlled electrodes for the estimation of oxygen tensions by cathodic reduction have been described[1,2] However, in general, they lack robustness, and this is a grave disadvantage in many practical usages such as in biological fermentations where sterilization is essential The electrode described here was designed to overcome this fault and was rendered possible by the particular properties of silicone rubber

The body of the electrode (Fig 1) was made of 'Pyrex The dimensions, which are really defined by the apparatus to which the electrode is ancillary, are not critical, except for the lower end *A B* which carries the cathode and diffusion membrane In the prototype electrode this end was made from a length of 'Pyrex' tubing of 6 5 mm external diameter, fused at the top end *A* to the main body of the cell and drawn off gradually to a rounded point at the lower



Figs. 1–3

end *B*. A hole, 2 mm. in diameter, with smooth edges, was blown in the wall of this tube, approximately equidistant between *A* and *B* to allow the wire forming the cathode to pass from the inside to the outside of the cell. The cathode consisted of a pure platinum wire, 0·014 in. in diameter, of sufficient length to give five complete turns on *A B*, after being sealed into the soft glass tube *C* which acted as a mercury contact tube and which was supported at the other end by the rubber bung *D*. Before the final positioning of the cathode the anode *E* was fitted. This comprised approximately 9 in. of silver wire, 0·028 in. in diameter, joined by fusion to a short length of platinum wire for sealing into the contact tube *F*, and after this tube was inserted into the bung *D*, the silver wire was wound in a close spiral around the contact tube of the cathode. The bung carrying these electrodes was then inserted into the cell so that the end of the contact tube of the cathode came as close as possible to the hole in *A B* through which the platinum wire passed. Five turns of this wire were then wound tightly around the outside of the tube so as to cover the hole (Figs. 2 and 3) and the free end sealed down with 'Araldite'. When this had set, the whole of the wire, except that immediately adjacent to the hole, was thinly coated with 'Araldite', so as to leave an area of 6–8 mm.^{2} of bare wire covering the hole. When this insulating layer was completely hardened, the diffusion membrane, which consisted of a length of about 2 cm. of a proprietary silicone rubber ('Escorubber') tubing of 1·5 mm. bore and 0·5 mm. wall, was fitted. This was accomplished by lubricating the tube *A B* and the cathode assembly with a few drops of a 20 per cent aqueous solution of 'Teepol' and then carefully sliding the silicone rubber tubing over the tapering end and along the tube until the cathode was completely shrouded (Fig. 2).

During this operation the wall of the silicone tube became considerably stretched and much thinner. Electrolyte, 0·5 *M* aqueous ammonium chloride, was then added to the cell until the level was such that the silver anode was completely submerged. Ammonium chloride was used instead of the more conventional carrier electrolyte, potassium chloride, because of the adverse effect on the silicone rubber sheath of the high alkalinity developed around the cathode, in solutions of this salt, on prolonged working of the cell. This frequently resulted in rupture of the sheath.

Before measurements could be made it was necessary to remove dissolved oxygen from the cell, and this was done by placing the cathode bearing end in an atmosphere of nitrogen, passing a slow current of nitrogen through the anode compartment via the tubes *G G* and applying a steady potential difference of 0·5 V. across the cell in the appropriate sense. Left overnight in these circumstances the current, measured by a 0–50 microammeter in series with the cell, became zero. The response curve of the electrode, that is, the current passed by the cell at a constant applied potential difference, plotted against oxygen pressure, was linear between 0·0 and 0·2 atmospheres of oxygen. At oxygen pressures above 0·2 atmospheres the curve drooped from linearity. Thus the electrode could be calibrated for use in aerated systems simply by means of air. The electrode will operate either in a liquid or a gaseous environment and the responses are similar; nevertheless, it seems advisable to calibrate the instrument in the medium in which measurements are to be made. In liquids, vigorous stirring is essential to dissipate secondary diffusion gradients, and it is necessary, because of the considerable temperature coefficient of diffusion of oxygen in the controlling membrane, that calibration be done at the same temperature at which measurements are made.

The selection of 0·5 V. for the polarizing potential difference was not arbitrary. It was observed with a gold electrode in this cell that, at a polarization between 0·55 and 6·0 V., a change of mechanism of oxygen reduction occurred which loosely corresponded with a switch from a two to a four electron process. With platinum such a change has not yet been observed; nevertheless, over the same polarization range anomalous behaviour was noted, hence it was thought wise to limit the polarizing potential difference to 0·5 V.

Measurements with the electrode are easy, for it may be poked through a bung and into an apparatus just like a thermometer. With a polarization of 0·5 V. and a diffusion membrane made from silicone rubber tubing of 0·5 mm. wall and 1·5 mm. bore the response time was relatively small; the current became constant within 10 min. of a change of oxygen pressure from 0·0 to 0·2 atmospheres in a gaseous ambience. With a thicker membrane for which tubing of 0·8 mm. wall and 2·5 mm. bore was used a steady current reading was obtained within 30 min. for the same change of conditions.

In systems uncontaminated with catalyst poisons this electrode behaved reproducibly, but in 'dirty'

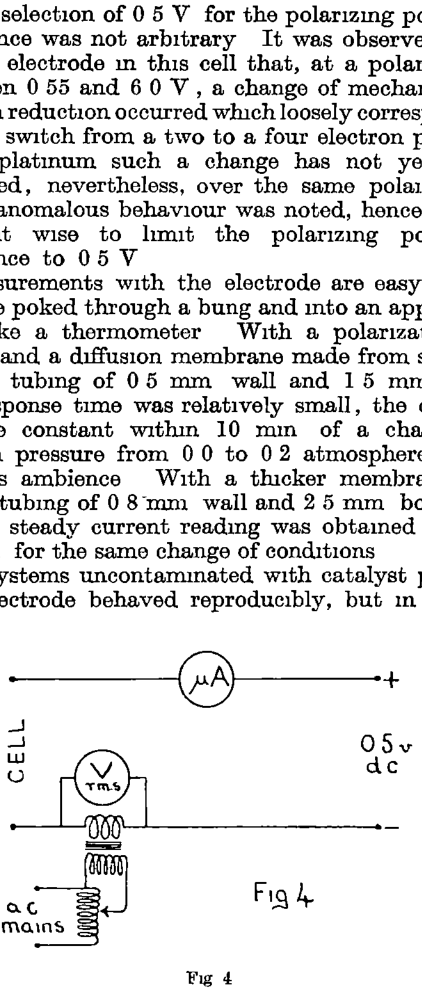


Fig. 4

systems, for example, spent media from long-term continuous bacterial cultures, poisoning was rapid and the electrode ceased to respond to changes of oxygen tension. This effect was neutralized in a simple way, namely, by imposing an alternating voltage, of the order of 1.0 V (root mean square), on the direct polarizing potential by connecting the secondary to a suitable transformer (a 6-V microscope lamp transformer, with a low impedance secondary, serves admirably) in series with the cell and the source of the direct polarizing potential difference, the primary of this transformer being connected to the a.c. mains through a Variac variable auto-transformer (Fig. 4). The magnitude of this a.c. signal was not critical. It was found to depend on the rate at which poisons reached the cathode, but in all experiments, so far, any value between 0.5 and 2.0 V (root mean square) seemed to serve. If the a.c. signal was applied from the start of a run with an initially unpoisoned electrode, poisoning did not supervene. Also, if it was imposed even on a heavily poisoned electrode, 'clean up' took place in a time dependent on the extent of the poisoning and the magnitude of the signal.

The imposition of this signal necessarily modified the technique of measurement. This arose because, in clean systems, rectification occurred, owing to the depolarizing action of oxygen, during the negative going swing, while in dirty systems rectification occurred in the positive going swing, at least some of the 'clean up' being due to oxidation. This means that the reading of the d.c. microammeter with the a.c. signal imposed was in part dependent on the concentration of poison in the system. The procedure which was adopted to deal with this was to impose both alternating and direct voltages from the beginning of an experiment and to turn off the alternating component when a reading was required, allowing a sufficient length of time, which was about 2 min., for a steady microammeter reading to be attained.

[1] Ives, D., and Janz, G., *Reference Electrodes*, 504 (Academic Press, New York, 1961).

[2] Phillips, D. H., and Johnson, M. J., *Biochemical and Microbiological Technique and Engineering*, 3, No. 3,261 (1961).

PLASMA PROTHROMBINASE

By Dr. M. J. CROSS

Department of Pharmacology, Royal College of Surgeons of England

WHEN a mixture of normal plasma, normal serum, platelets and calcium chloride is incubated at 37° C it develops the ability to accelerate the clotting of recalcified normal plasma and to convert partially purified prothrombin into thrombin[1]. If such a mixture is centrifuged at high speed and the sediment resuspended in a suitable buffer, the resuspended sediment is found to possess most of the activity of the original mixture[2–4]. It is probable that the active component of such sediments is also formed during normal blood coagulation and is responsible for the conversion of prothrombin to thrombin under physiological conditions.

Recently, I have described a method of preparing and partially purifying this active component from pig plasma, using a modification of the thromboplastin generation system of Biggs and Douglas[1]. The product appears to act directly on prothrombin, converting it to thrombin[5], and the kinetics of the conversion, which will be reported elsewhere, are as would be expected if the material were an enzyme. I therefore feel justified in describing the material as a prothrombinase, and I shall refer to it as plasma prothrombinase to distinguish it from prothrombinase derived from tissue extracts[6].

In the experiments to be described here the activity of prothrombinase was estimated by adding 0.1 ml of a suspension of prothrombinase in iminazole buffer containing 6×10^{-3} M calcium chloride to recalcified citrated plasma and measuring the clotting time. A suspension of prothrombinase which clotted the plasma in 10 sec was arbitrarily taken to contain 100 units of prothrombinase per ml.[5]

The most active suspensions of prothrombinase which I have prepared contained 300–550 units per ml. They contained 23–26 μg protein, as measured by the method of Lowry *et al.*[7], and 0.22–0.45 μg of lipid phosphorus per 100 units of prothrombinase. Lipid phosphorus was extracted by the method of Folch, Lees and Sloane Stanley[8], and estimated by a modification of the method of Berenblum and Chain[9] (Sloane Stanley, G. H., personal communication). In Table 1 these results are compared with the activities of other potent preparations of natural coagulants. In terms of lipid *P*, my plasma prothrombinase had 50–100 times more thromboplastic activity than the most active suspensions of plasma thromboplastin isolated from a similar thromboplastin generation system by Bentley and Krivit[10]. In terms of protein my plasma prothrombinase had about 2 per cent of the thromboplastic activity of the purest thrombin that has been made[11].

Table 1. COMPARISON OF THE ACTIVITY OF PROTHROMBINASE WITH A SIMILAR THROMBOPLASTIN (REF. 10) AND WITH THROMBIN (REF. 11)

Preparation	Clotting time (sec) of recalcified plasma on adding 1.0 ml sample	Protein μg/ml	Lipid P μg/ml
Partially purified prothrombinase	10	23–26	0.22–0.45
Thromboplastic sediment from thromboplastin generation test (ref. 10)	9.6–10.6	—	8–35
Thrombin (ref. 11)	*c.* 10*	0.5	—

* Calculated from the activity of this preparation of thrombin, which was 4,100 units/mg.

Suspensions of plasma prothrombinase in iminazole buffer were fairly stable at 4° C, losing about 50 per cent of their activity in one week, at *p*H 6.6–7.0, but the activity was lost much more rapidly at *p*H values below 6.1 or above 7.6. Heating to 70° C for 30 min abolished activity almost completely[5]. Even when, under these conditions, the suspensions had lost their own prothrombinase activity they could still replace platelets in the modified thromboplastin test. Since platelets can be replaced in this test by certain phospholipids[3,4], these procedures presumably denature a protein component of prothrombinase, leaving a phospholipid component intact. This confirms earlier observations with cruder preparations[2].

Table 2 Recovery of Prothrombinase Activity after Centrifugation in the Presence and Absence of Calcium

The recoveries were recorded as a percentage of the prothrombinase activity initially present

	Calcium present			Calcium absent		
Experiment	Sediment	Supernatant	Mixed sediment and supernatant	Sediment	Supernatant	Mixed sediment and supernatant
1	57	31	104	7	21	75
2	47	26	98	16	22	107
3	62	13	75	7	19	75

Plasma prothrombinase was fully active only when suspended in a buffer containing calcium[4,5] The requirement for calcium has been investigated by measuring the recovery of prothrombinase in fractions obtained by centrifuging it in the presence and absence of calcium The results of three typical experiments are shown in Table 2. When a suspension of plasma prothrombinase in iminazole buffer containing 6×10^{-3} M calcium chloride was centrifuged for 30 min at 200,000*g* and the sediment resuspended in the same buffer mixture, 47–62 per cent of the original activity was recovered in the sediment A further 13–31 per cent was recovered in the supernatant, giving a total recovery of 73–88 per cent When the sediment and supernatant fractions were mixed together again the activity was often a little greater, that is, 75–104 per cent, than the sum of their separate activities When, on the other hand, a suspension of plasma prothrombinase in iminazole buffer not containing calcium was centrifuged in the same way the original activity became distributed very differently While the supernatant still contained 19–22 per cent of the initial activity, only 7–16 per cent was now recovered in the sediment However, when the supernatant and sediment fractions were mixed together at 0° C, the activity of the mixture increased to 75–107 per cent. Therefore, after centrifuging prothrombinase in the absence of calcium, its activity was only recovered by mixing the sediment and supernatant, and the activity of this reconstituted prothrombinase was equal to that of the prothrombinase centrifuged in the presence of calcium Similar results were obtained when 5×10^{-4} M sodium ethylenediaminetetraacetate was added to the suspension of prothrombinase in buffer without calcium in order to bind any remaining calcium ions before centrifugation.

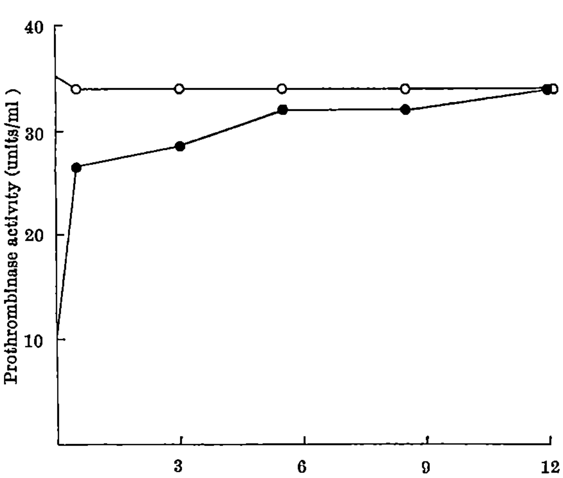


Fig 1 Changes in prothrombinase activity when sediment and supernatant obtained by centrifuging prothrombinase are mixed The two fractions were obtained by centrifugation in the presence (○) and in the absence (●) of calcium In both cases the activities shown at zero time were the sum of the activities of the two fractions tested separately

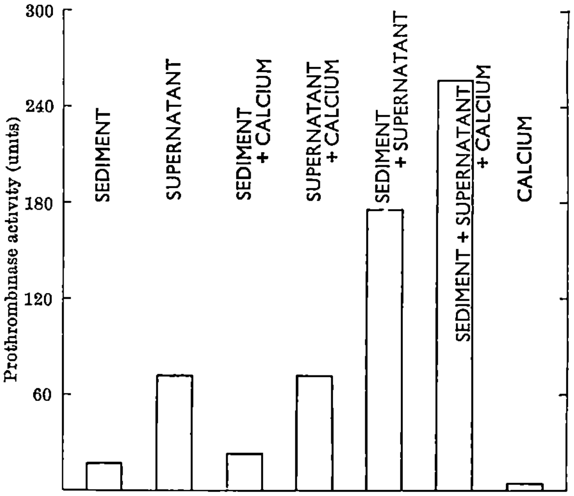


Fig 2 Effect of calcium on the activities of sediment and supernatant obtained by centrifuging prothrombinase in a medium lacking calcium

These results suggest that, in the absence of calcium, plasma prothrombinase splits into two components, one of which is largely deposited when centrifuged at 200,000*g* while the other is not If these two components are mixed in the presence of calcium the full activity of the prothrombinase is restored Fig 1 shows that, after mixing, the full prothrombinase activity took several minutes to develop, suggesting that some definite reaction must take place between the two components in order to reform plasma prothrombinase Fig 2 shows that the maximum activity only developed when the sediment, the supernatant and calcium chloride were all present

When the *sediment*, suspended in buffer containing calcium, was heated at 70° C for 30 min, it lost most of its intrinsic prothrombinase activity However, it was still able to restore some prothrombinase activity to the supernatant fraction when they were mixed in the presence of calcium (Table 3) It seems, therefore, that the component of plasma prothrombinase which is deposited on centrifugation in the absence of calcium is relatively heat-stable It is probable that this component is phospholipid because the sedimented fraction, like plasma prothrombinase itself, could replace platelets in the modified thromboplastin generation test, both before and after being heated at 70° C for 30 min In contrast, the *supernatant* fraction could not replace platelets, so that it presumably contains little phospholipid The supernatant fraction, like the sediment, lost its small intrinsic prothrombinase activity when heated at 70° C for 30 min When heat-treated supernatant was mixed with normal sediment in the presence of calcium, the mixture had little activity (Table 3) Therefore, the component of plasma prothrombinase remaining in the supernatant is heat-labile

Twenty years ago Macfarlane suggested that blood thromboplastin, the activator of prothrombin, might consist of two separate substances, one being a water-soluble enzyme and the other a lipid[12] He cited the analogy of the action of Russell's viper venom which accelerates clotting only in the presence of certain lipids More recently, a thrombokinase which is potentiated by cephalin or by platelets has been isolated from plasma[13] In 1956 Bergsagel and Hougie

Table 3. EFFECT OF HEAT ON THE FRACTIONS OF PROTHROMBINASE OBTAINED BY CENTRIFUGATION IN THE ABSENCE OF CALCIUM

Sample	Prothrombinase activity (units)
Unheated sediment	22
Unheated supernatant	30
Unheated sediment mixed with unheated supernatant	195
Heated sediment	7
Heated supernatant	8
Unheated sediment mixed with heated supernatant	40
Heated sediment mixed with unheated supernatant	145

Each fraction was heated at 70° C for 30 min in iminazole buffer containing calcium chloride.

obtained evidence that a reaction occurs between a calcium–protein complex (product 1) and platelets to yield a coagulant which can be sedimented by centrifugation[2]. This was in general accord with Macfarlane's view but suggested that prothrombinase might be a single complex of protein, lipid and calcium.

The properties of the prothrombinase which we have partially purified support this conclusion. Prothrombinase is apparently made up of two parts, one consisting of protein and the other of phospholipid, which are bound together by calcium. There were about 20 moles of protein nitrogen to 1 mole of lipid phosphorus; this ratio is of the same order as that found in the major serum lipoproteins. The complex is dissociated by reducing the concentration of calcium in the suspension medium and this process is reversible. This may account for the requirement for calcium in the formation of thrombin from prothrombin.

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USE OF DIHYDROFOLATE REDUCTASE AS A BIOCHEMICAL INDEX OF CHEMOTHERAPY

By STEWART R. HUMPHREYS, DR. LOUIS B. THOMAS, DR. MICHAEL A. CHIRIGOS and DR. ABRAHAM GOLDIN

Laboratory of Chemical Pharmacology and Pathologic Anatomy Branch, National Cancer Institute, Bethesda, Maryland

AND

ELIZABETH J. CRAWFORD and PROF. MORRIS FRIEDKIN

Department of Pharmacology, Tufts University School of Medicine, Boston, Massachusetts

IT has been demonstrated that, on continuous therapy in serial transfer of murine leukæmia, the leukæmic cells may become markedly resistant to treatment with antifolics[1,2]. Several of the resistant sub-lines of leukæmia *L*1210 established in this manner[3–5] have been shown to have a high level of dihydrofolate reductase activity[6,7]. Both the resistance to therapy with folic acid antagonists and the high level of folic reductase, once present, appear, in large measure, to be heritable[7]. The possibility was considered that resistant leukæmic cells with high levels of dihydrofolate reductase might serve as biochemical markers, to measure the efficacy of chemotherapy. This appeared feasible, since the high levels of dihydrofolate reductase could readily be measured in tissues showing any extensive degree of invasion by leukæmic cells.

There has been considerable interest in the contribution of spread of leukæmic cells in the brain and meninges, and in the inability of the drug to reach the brain site, to the ultimate failure of therapy with effective compounds[8–11]. In the present experiment, the anti-leukæmic action of *N,N,bis*-(β-chloroethyl)-*N′*, *O*-propylene phosphoric acid ester diamide ('Cytoxan', obtained from Mead Johnson and Co., through the courtesy of the Cancer Chemotherapy National Service Center) is studied from the foregoing point of view. For this purpose, the animals were inoculated intracerebrally and the 'Cytoxan' was administered subcutaneously. The following questions were asked: (*a*) Will 'Cytoxan' increase the survival-time of mice bearing an amethopterin-resistant sub-line of leukæmia, inoculated intracerebrally? (*b*) What effect does therapy with 'Cytoxan' have on the transplantability of tissues of the leukæmic mice? (*c*) Does the level of dihydrofolate reductase reflect the therapeutic effect? (*d*) What effect does therapy have on the distribution of leukæmic cells, particularly in the brain and meninges? A detailed account of the pathological anatomy is presented separately[12].

The model experiment was conducted with the *FR*-9 amethopterin-resistant sub-line of leukæmia *L*1210 (ref. 7). The history of the *FR*-9 sub-line may be summarized as follows. The sensitive leukæmia *L*1210 had been treated for ten generations with amethopterin (0.75 mgm./kgm./day) and was then maintained for ten generations without treatment. The tumour was then subjected to treatment again, using progressively increasing doses of amethopterin (from generation 21 to generation 31). It was then carried for 12 generations without treatment, at which time it was used in the present experiment. The dihydrofolate reductase activity of the *FR*-9 leukæmia sub-line ranges from values of 2,000–4,000 (mμmoles dihydrofolate reduced/mgm. protein/hr.) for infiltrated spleen or tumour. This is approximately 50 times greater than that observed for the sensitive line of *L*1210 (ref. 6).

In the model experiment the leukæmic inoculum consisted of 0.05 ml. of a 1:10 dilution in normal

Table 1 Effect of Treatment with 'Cytoxan' on the Growth of Intracranially Inoculated Amethopterin-Resistant Leukæmia (*L*1210 *FR*-9) as determined by Pathological Examination, Retransplantation of Tissues and by Enzymatic Assay*

Gp	Treatment (days)	Survival time (days)		Sac (day)	Pathology					Transplantation							Dihydrofolate Reductase activity‡	
		Med	Range		Degree of infiltration§	Spleen	Liver	Dura	Brain (arachnoid)	Conc †	Spleen Takes/Total	Spleen Day of Death Med	Spleen Day of Death Range	Brain Takes/Total	Brain Day of death Med	Brain Day of death Range	Spleen	Brain
1	None	8	8–9	—														
2	4–	15	9–17	—														
3	None	—	—	4	0 to ±	6/6	3/6	1/6	1/6	1 10	3/3	14	12–15	3/3	15	13–15	39 8	30 8
					+ to + +		3/6	1/6	1/6	1 100	3/3	14	14–15	1/3	—	17		
					+ + + to + + + +			4/6	4/6	1 1,000	3/3	16	16–18	0/3	—	—		
										1 10,000	0/3	—	—	0/3	—	—		
4	None	—	—	7	0 to ±					1 10	3/3	8	8–8	3/3	11	10–11	2,980 0	243 0
					+ to + +					1 100	3/3	10	9–11	2/2¶	11	11–11		
					+ + + to + + + +	3/3	3/3	3/3	3/3	1 1,000	3/3	12	10–13	3/3	14	13–14		
										1 10,000	3/3	14	13–14	1/3	—	16		
5	4–6	—	—	7	0 to ±	3/3	3/3	3/3		1 10	3/3	12	12–17	3/3	11	11–12	56 1	129 0
					+ to + +					1 100	0/3	—	—	3/3	14	14–14		
					+ + + to + + + +				3/3	1 1,000	0/3	—	—	2/3	19	14–24		
										1 10,000	0/3	—	—	0/2¶	—	—		
6	4–10	—	—	11	0 to ±	3/3	3/3	2/3	1/3‖	1 10	2/3	22 5	15–30	3/3	11	11–11	20 5	280 0
					+ to + +			1/3		1 100	0/3	—	—	3/3	14	12–14		
					+ + + to + + + +				2/3	1 1,000	0/3	—	—	3/3	17	16–17		
										1 10,000	0/3	—	—	1/3	—	15		
7	4–13	—	—	14	0 to ±	3/3	3/3	3/3		1 10	1/3	—	15	3/3	12	12–13	18 5	298 0
					+ to + +					1 100	0/3	—	—	3/3	12	12–14		
					+ + + to + + + +				3/3	1 1 000	0/3	—	—	3/3	16	16–16		
										1 10,000	0/3	—	—	0/3	—	—		

* Tumour inoculum heart blood from a mouse with resistant sub-line of leukaemia *L*1210 (*FR*–9) diluted 1 10 with normal saline, inoculated intracerebrally, 0 05 ml /mouse Treatment 'Cytoxan' diluted in normal saline, injected subcutaneously daily 75 mgm /kgm /day from day 4 on schedules indicated

† Pooled brain or spleen of three mice in normal saline

‡ $m\mu$moles dihydrofolate reduced per mgm. of protein per hour at a temperature of 32°

§ 0 to ± little to no leukæmic infiltration + to + + moderate leukæmic infiltration + + + to + + + + extensive leukæmic infiltration

¶ Accidental deaths not included

‖ The inoculation may have been unsuccessful for this mouse.

saline of whole blood from an *FR*-9 leukæmic mouse The inoculum was injected intracerebrally, by use of a microlitre syringe with a 25-gauge needle[9] The experiment was conducted with *DBA*/2 male mice, approximately 3–4 months of age

The 'Cytoxan' was dissolved in normal saline and administered in 0 01 ml of vehicle per gram of body weight, subcutaneously, in the axillary area The treatment was initiated on the fourth day following leukæmic inoculation and continued daily The animals were observed for survival-time, or were killed for transplantation of tissue, determination of dihydrofolate reductase activity, and for pathological examination, as indicated in Table 1

The first two groups of animals (untreated leukæmic controls, group 1, treated leukæmic animals, group 2), containing ten animals per group, were not killed, but were used for determination of survival-time The untreated leukæmic group (group 3) had 14 mice Each of the remaining groups contained eleven mice Six of the mice from group 3 and three from each of the remaining groups were killed for pathological investigations Three of the mice were killed, and pooled brain and pooled spleen mash prepared in normal saline and inoculated into *DBA*/2 mice at 1 10, 1 100, 1 1,000 and 1 10,000 dilutions Each of the recipient mice was injected subcutaneously with 0 1 ml Five of the mice were killed, and enzyme determinations made of pooled brain and pooled spleen Treated animals were killed one day following discontinuance of therapy This was done in order to permit the elimination of any transformation product of 'Cytoxan'[13] which might interfere with the assay of dihydrofolate reductase 'Cytoxan', at a final concentration of 3 2 × 10^{-3} *M*, was found not to inhibit leukæmic dihydrofolate reductase *in vitro*

For the determination of dihydrofolate reductase, the tissues were quick-frozen in beakers submerged in solid carbon dioxide and acetone The preparation of the acetone powder and the spectrophotometric assay for dihydrofolate reductase has been described previously[6] Protein was determined by the method of Lowry *et al* [14] The dihydrofolate reductase activity is expressed in $m\mu$moles of dihydrofolate reduced per mgm of protein per hour at a temperature of 32°

The results are summarized in Table 1 (ref 12) The untreated control animals with intracerebral leukæmia (group 1) showed a median survival-time of 8 days (range 8–9 days) The animals treated with 75 mgm / kgm of 'Cytoxan' daily from day 4 (group 2) showed a median survival-time of 15 days (range 9–17 days)

On the fourth day following intracerebral inoculation of the leukæmia (the day on which therapy was initiated), pathological observation of untreated controls (group 3) revealed only moderate infiltration of the spleen, liver and marrow (Table 1) At this time transplantation of a 1 10 to 1 1,000 dilution of spleen suspension did result in progressive tumour growth and death However, the degree of infiltration was apparently not very extensive since the transplants grew slowly and the animals showed rather prolonged survival-times (group 3) The untreated animals killed on the fourth day showed fairly extensive diffuse leukæmic cell infiltration of the arachnoid space and the dura Although the leukæmic cells had been inoculated directly into the brain, diffuse growth of leukæmic cells in the brain tissue was not observed The number of leukæmic cells growing in the arachnoid space was apparently less than the number of leukæmic cells growing in the spleen since brain inoculum taken on the fourth day was less potent than spleen inoculum taken from the same animals Three re-transplants from the brain succumbed with the 1 10 dilution, but only one out of three animals died with the 1 100 dilution from brain (group 3)

That the infiltration of spleen and brain was not extensive on the fourth day after leukæmic inoculation is also indicated by the low values for dihydrofolate reductase in spleen (39 8) and brain (30 8) These values would appear to be somewhat above the range for normal spleen[6] and brain, both of which have little to no measurable enzyme

On the seventh day following intracerebral inoculation of the leukæmia, there was extensive infiltration of the spleen, liver and bone marrow (group 4) The arachnoid and dura were also heavily infiltrated and there were a few leukæmic cells in the perivascular spaces of the brain. The increase in the number of cells was reflected in the results obtained on transplantation of tissue of spleen and brain. With transplant of spleen tissue, 3 out of 3 animals died at a 1 : 10,000 dilution of the inoculum. With brain, 3 out of 3 animals died at a 1 : 1,000 dilution of the inoculum. As compared with the fourth day, transplantation of both spleen and brain resulted in a reduction in the time to death. At the 1 : 10 dilution of spleen and brain the median survival-times were 8 days and 11 days respectively.

On the seventh day following leukæmic inoculation, the spleen showed a very high value for dihydrofolate reductase activity (2,980) and the brain also showed an increased value (243). As compared with the fourth day, the activity of the spleen was increased approximately seventy-fold, and the activity of the brain was increased approximately eight-fold.

The treated mice killed on day 7 (group 5) had received three injections (days 4, 5 and 6). At this time autopsy of the animals and histological study revealed a virtual disappearance of the leukæmic cells from the spleen, liver and bone marrow, and from other extracranial tissues. Also, there was almost a complete disappearance of leukæmic cells from the cranial and spinal cord dura. On the other hand, there was no definite evidence of a decrease of leukæmic cell infiltration in the arachnoid space. The transplantability of the spleen was reduced below that of the untreated controls on day 4 or day 7. Following therapy, the transplants of spleen grew with the 1 : 10 dilution of inoculum, but there were 'no takes' in three animals inoculated with the 1 : 100 dilution. In contrast, as compared with the corresponding untreated controls, there was only a slight reduction in the ability of the brain to 'take' on transplant. That there was some decrease in the total number of the leukæmic cells in the arachnoid space is suggested by the observation that the median survival-time for the brain transplants was slightly increased as compared with the corresponding controls.

The depletion of leukæmia cells in the spleen, following three days of therapy, was reflected in the relatively low value of dihydrofolate reductase for the spleen (56.1).

Despite therapy with 'Cytoxan', there was a substantial increase in the value for dihydrofolate reductase of the brain (129). This value was lower, however, than that observed for the untreated leukæmic controls for the seventh day, suggesting again that there was some retardation of leukæmic growth on therapy.

On the eleventh day (after a total of seven injections, the last injection was given on day 10), the pathological findings were similar to those seen on treatment to the seventh day (group 6). The somatic tissues appeared to be devoid of leukæmic cells, but the arachnoid space was densely infiltrated. In addition, the infiltration in the intracerebral perivascular spaces was more extensive than previously observed. The reduction in the number of leukæmic cells in the spleen was maintained so that only two out of three transplants took successfully at a 1 : 10 dilution. The median survival-time was 22.5 days. There was a slight increase in the number of takes on transplant of brain tissue, one out of three animals succumbing at a 1 : 10,000 dilution. The decrease in the number of cells in the spleen was reflected by a further decrease in the dihydrofolate reductase activity (20.5). In contrast, the dihydrofolate reductase of the brain on the eleventh day was 280.

On the fourteenth day (therapy days 4–13) there was still no evidence of leukæmic cells in the spleen, liver, bone marrow or other extracranial tissues (group 7). There was a dense infiltration of leukæmic cells in the arachnoid space and deep infiltration in the fissures between the lobes of the brain. There was progressive increase in the number of cells infiltrating the intracerebral perivascular spaces and along the intracerebral needle track. Only one tumour take occurred on reinoculation of the spleen, at a 1 : 10 dilution. The dihydrofolate reductase of the spleen remained at a very low level (18.5). Brain tissue continued to give 100 per cent takes down to a 1 : 1,000 dilution, and the dihydrofolate reductase remained at a high value (298).

The present investigation illustrates a close correlation of biochemical, biological and pathological observations on the influence of 'Cytoxan' on the growth of a sub-line of leukæmia *L*1210 with high dihydrofolate reductase. The 'Cytoxan' was effective in increasing the survival-time of the animals. The survival-time was approximately doubled over that of untreated controls. There was good correlation of the value for dihydrofolate reductase, the retransplantability of the tissues, and the pathological findings. Regression of the systemic disease was virtually complete after treatment with 'Cytoxan', as shown by study of histological sections and by the low level of dihydrofolate reductase activity in the spleen and the reduced transplantability of spleen suspension. The leukæmic cell infiltrate in the arachnoid space appeared to progress despite successful systemic therapy. This was associated with a progressive increase in dihydrofolate reductase in the brain, and the success of re-transplant with brain tissue inoculum.

Enzymatic markers of this type may be useful in investigations of fate and distribution of drugs, and studies of the blood-brain barrier. They may be employed in investigation of tumour invasiveness, and the efficacy of therapy and the origin of resistance[7,15]. High dihydrofolate reductase in antifolic resistant leukæmia can serve as a biochemical marker which, in conjunction with biological and pathological studies, may provide a means for detailed investigation of chemotherapeutic agents.

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REACTION OF CYTOCHROME *a* WITH CHEMICALLY MODIFIED CYTOCHROME *c* AND BASIC PROTEINS

By SHIGEKI TAKEMORI, KEISHIRO WADA, ICHIRO SEKUZU and KAZUO OKUNUKI
Department of Biology, University of Osaka

IN previous communications[1], we have suggested not only that cytochrome *c* acts as an electron donor but also that it has some coenzymatic function in the cytochrome oxidase reaction The questions are what part of the protein moiety of cytochrome *c* plays the essential part in the reaction with cytochrome *a* and how These questions may be partly solved by studying the reaction between cytochrome *a* and cytochrome *c* chemically modified by substitution on the functional groups As cytochrome *c* is a strongly basic protein with an isoelectric point at about *p*H 10 5 (ref 2) and as lysine constitutes about 20 per cent of the total amino-acid residues[3], the blocking of the amino-groups was first undertaken

The activity of a chemically modified cytochrome *c* such as acetyl or succinyl-derivative in the cytochrome oxidase reaction was measured with a highly purified preparation of cytochrome *a* from bovine heart muscle

As shown in Table 1, the cytochrome *c* with more than 32 per cent of the amino groups acetylated or more than 22 per cent succinylated showed no activity in the cytochrome oxidase reaction when the Warburg manometric method was used The reducibility of the cytochrome *a* by the chemically modified cytochrome *c*'s was estimated spectrophotometrically by the appearance of the absorption at 605 mμ of cytochrome *a* after the addition of hydroquinone as an electron donor to the reaction mixture As shown in Fig 1, the reducibility of the cytochrome *a* by the acetylated cytochrome *c* was decreased slowly in accordance with the progress of the acetylation However, with the succinylated cytochrome *c*'s was observed the rapid decrease of reducibility

Table 1 EFFECT OF CHEMICALLY MODIFIED CYTOCHROME *c*'S ON THE CYTOCHROME OXIDASE ACTIVITY

Sample	NH_2-groups reacted (per cent)	Relative activity (per cent)
Native cytochrome *c*	0	100
	32	0
Acetylated cytochrome *c*	41	0
	71	0
	22	0
Succinylated cytochrome *c*	31	0
	65	0
	49	110
Guanidinated cytochrome *c*	70	116
	90	123

The activity was measured manometrically by using hydroquinone as substrate

When larger quantities of the chemically modified cytochrome *c*'s were added to the reaction mixture containing cytochrome *a* and cytochrome *c*, essentially no inhibition was observed The oxidation of the chemically modified reduced cytochrome *c*'s by cytochrome *a* was further investigated Although the reduced cytochrome *c* was rapidly oxidized by cytochrome *a*, chemically modified reduced cytochrome *c*'s were scarcely oxidized On the addition of a small amount of cytochrome *c* to the reaction mixture, however, the latter was also oxidized rapidly We have attempted to determine the chemical structure of the active site of cytochrome *c* in the cytochrome oxidase reaction, with 2,4,6-trinitrobenzenesulphonate (TNBS) which is known to bind specifically and stoichiometrically with the site related to the enzymatic activity

According to Satake *et al.*[4], TNBS binds only with the amino-groups of proteins at neutral *p*H The specific binding of TNBS with 4 moles of the ε-amino-groups of the lysine residues per mole of cytochrome *c* caused the loss of the cytochrome oxidase activity, although the reducibility on cytochrome *a* was scarcely changed In addition, guanidinated cytochrome *c*'s, which are more basic than naturally occurring cytochrome *c*, even when as much as 90 per cent of the amino-groups were guanidinated,

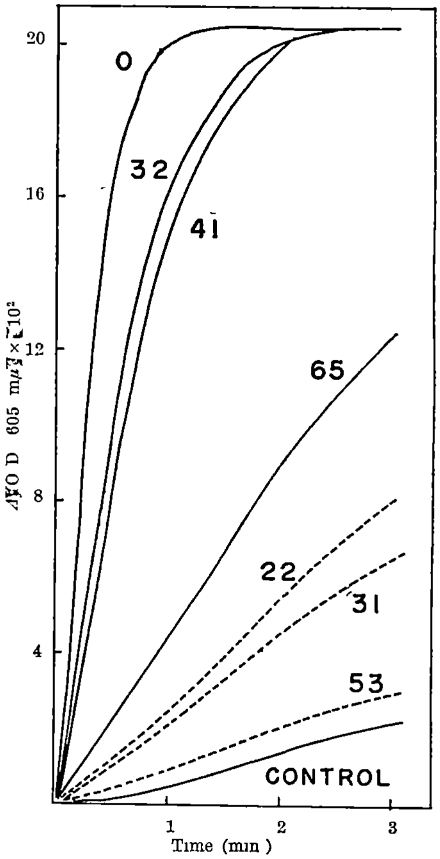

Fig 1 Effect of chemically modified cytochrome *c*'s on the reduction of cytochrome *a*
The assay system (3 ml final volume) contained 9 0 × 10^{-2} *M* phosphate buffer at *p*H 7 4, 1 per cent 'Emasol' 1130, 1 0 × 10^{-3} *M* potassium cyanide, 3 1 × 10^{-5} *M* cytochrome *a*, 1 0 × 10^{-3} *M* hydroquinone, and 1 1 × 10^{-5} *M* cytochrome *c* or a chemically modified cytochrome *c* The percentages shown in the figure represent the extent of modification of the free amino-groups The control shows only the reduction of cytochrome *a* by hydroquinone
——, acetylated cytochrome *c*, – – –, succinylated cytochrome *c*

were as active as the latter in the cytochrome oxidase activity and in the reducibility on cytochrome *a* This is in accord with the data reported by Minakami *et al* [5], who have obtained similar results in the succinic oxidase system of rat liver homogenate

From these results, it may be suggested that some free ε-amino-groups of the cytochrome *c* molecule, which can be easily blocked by TNBS, participate in the cytochrome oxidase reaction with cytochrome *a*, whereas other ε-amino-groups of the molecule, which can be less readily blocked, function in the reduction of cytochrome *a* What is essential for the maintenance of the activity is the positive charges on the lysine side-chains, and it does not matter that these positive charges result from the free ε-amino-groups or from the guanidino groups

Since cytochrome *a* is an acidic protein[6], and cytochrome *c* is a basic protein, the effect of basic proteins on the cytochrome oxidase activity was tested

As shown in Table 2, protamines such as salmine and culpeine strongly inhibit the cytochrome oxidase activity at low concentrations The inhibition was found to be non-competitive with cytochrome *c* However, lysozyme had a less inhibitory effect than did the protamines The synthetic polypeptides which were used here were valuable for demonstrating the interactions between macromolecular basic polyions and cytochrome *a* Poly-L-lysine (polymerization degree, $n = 260$) showed the same inhibitory effect as the protamines However, copoly-1 1-(L-glutamic acid, L-lysine) ($n = 70$) had little inhibitory effect on the cytochrome oxidase activity at low concentration-levels This is not unexpected, since the net charge of the copolymer is zero Furthermore, Nagumo[7] recently reported that the complexes between acidic proteins, such as α-casein and catalase, and basic proteins, such as lysozyme and protamines, were proved by both ultracentrifugal and spectroscopic analyses These results suggest that the inhibitory effect of the basic proteins on the cytochrome oxidase activity depends on the formation of the complex between the acidic protein, cytochrome *a* and any one of the basic proteins

Table 2 EFFECT OF BASIC PROTEINS AND POLYPEPTIDES ON THE CYTOCHROME OXIDASE ACTIVITY

Sample	Concentration (*M*)	Inhibition (per cent)
Salmine	3×10^{-5}	83
	5×10^{-6}	55
Culpeine	3×10^{-5}	80
	5×10^{-6}	54
Lysozyme	3×10^{-5}	20
Poly-L-lysine	3×10^{-5}	70
	5×10^{-6}	52
Co-poly-1 1-(L-glutamic acid, L-lysine)	3×10^{-5}	17

We thank Prof. J Noguchi, University of Kanazawa, and Dr K Iwai, University of Tokyo, for providing samples of compounds used in this work, which was supported in part by research grant *RG*–5871 from the Public Health Service of the U S National Institutes of Health

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IMMUNOGENIC SUB-CELLULAR PARTICLES OBTAINED FROM SPLEENS OF ANTIGEN-INJECTED MICE

By ROBERT E. FRANZL

The Rockefeller Institute, New York

RECENT work[1,2] has lent new impetus to the concept that the phagocytic activity of the reticulo-endothelial system plays a significant part in antibody synthesis, and that the interaction of antigen and a particular type of phagocytic cell produces the specific factor (the antigenic stimulus) capable of initiating the synthesis of an antibody In the hope of learning more about the nature of the antigenic stimulus, a preliminary investigation was made of the immunogenic properties of an ingested antigen prepared from two highly phagocytic tissues of the mouse prior to the appearance of antibody For this purpose the cell homogenates of spleens and livers from approximately 40 donor mice (Rockefeller Swiss, Nelson-Collins strain), previously injected intravenously with 2×10^8 sheep red blood cells (446 µg nitrogen), were fractionated by differential centrifugation[3] at various time-intervals before the appearance of serum antibody Each of the resulting cell fractions—nuclei, mitochondria, lysosomes, microsomes and supernatants—was then injected intraperitoneally in nine recipient mice, sensitized five weeks previously by an intravenous injection of 2×10^6 sheep red blood cells The ability of the sub-cellular fractions to stimulate a secondary hæmolysin response in the recipient animals was utilized as an assay for immunogenic activity The quantitative hæmolysin titres of pooled sera obtained from three recipients on the fourth, sixth and eighth day after the injection of cell fractions were determined by the method of Taliaferro and Taliaferro[4]

As illustrated in Fig 1, spleen lysosome fractions consistently elicited high hæmolysin titres in sensitized recipient mice The enhanced immunogenic activity of these fractions was also manifested by their ability to induce an antibody response as early as on the fourth day after their administration The other spleen fractions either possessed lower activity (hæmolysin appearing on the sixth and eighth day), or were totally inactive The latter was also true for fractions isolated from spleens of immunized donors at the time of appearance of serum antibody (4 days after immunization) It must be stressed

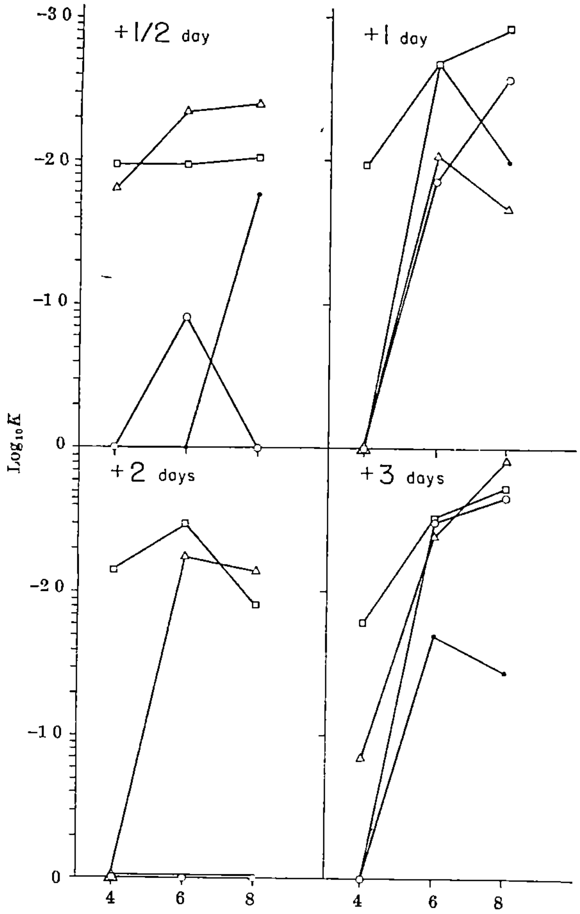

Fig 1 Secondary hæmolysin responses induced by spleen cell fractions from antigen-injected donors Spleen cell fractions, nuclei (*N*, ●), mitochondria (*M*, ○), lysosomes (*L*, □), and microsomes (*P*, Δ) were prepared from donor mice + 1/2, + 1, + 2, and + 3 days after the intravenous injection of 2 × 10^8 sheep red blood cells The fractions were then administered intraperitoneally to sensitized recipient mice which, five weeks previously, had been injected intravenously with 2 × 10^6 sheep red blood cells The dose-ranges (mg N per mouse) were *N* (0 41–2 79), *M* (0 06–0 46), *L* (0 32–0 49), *P* (0 43–0 61) The antibody titres ($\log_{10}K$) of the undiluted sera of recipient mice are expressed as the log of the number of 50 per cent anti-sheep red blood cell hæmolysin units per ml

that, throughout the entire experimental period. all liver cell fractions from antigen-treated donors were completely ineffective in stimulating the secondary hæmolysin response, even though the doses administered contained the same amounts of phagocytized sheep red blood cells present in the active spleen fractions Control experiments with liver and spleen fractions prepared from untreated donor mice were also immunogenically inactive Thus we may conclude that, under the conditions of our experiment, a particulate antigen, sheep red blood cell, after phagocytosis by liver macrophages, loses its ability to stimulate the anamnestic hæmolysin response in mice, whereas its ingestion by spleen macrophages appears to have no deleterious effect on its antibody-stimulating capacity

In the absence of any contrary evidence, the association of immunogenic activity with sub-cellular particles of spleen can be interpreted, in accordance with Straus's findings[5], as probably being due to the presence of contaminating phagocytic vacuoles containing an activated ingested sheep red blood cell antigen

A comparison of the secondary hæmolysin responses elicited by the injection of spleen cell fractions (Fig 1), and the responses evoked by the injection of known amounts of sheep red blood cells into identically sensitized mice, permits the evaluation of the immunogenicity of each administered cell fraction in terms of an equivalent number of sheep red blood cells (Table 1, col 3) These values are only approximate, primarily because cell fractions stimulate an anamnestic response different in character from that obtained with sheep red blood cells alone Nevertheless, the results obtained clearly show that the spleen lysosome fractions had the highest specific and total immunogenicity (Table 1) It is also of interest that the sum of the total immunogenicities of spleen fractions was much higher on the first and third day after the injection of antigen than on the second This effect was found to be reproducible, yet its significance at the present can only be an object of speculation

Table 1 IMMUNOGENICITY OF SPLEEN CELL FRACTIONS AT VARIOUS PERIODS AFTER ANTIGEN INJECTION

(1) Days after injection	(2) Cell fraction	(3) Immunogenicity (× 10^{-6})	(4) Specific immunogen (× 10^{-3})	(5) Total immunogen (× 10^{-6})
+1/2	*N*	0	0	0
	M	0 01	0 18	0 12
	L	1 5	3 4	16 8
	P	1 0	1 8	11 4
	S	0	0	0
+1	*N*	0 1	0 02	1 4
	M	0 1	0 25	1 1
	L	10 0	30 0	110 0
	P	0 02	0 03	0 22
	S	0	0	0
+2	*N*	0	0	0
	M	0	0	0
	L	2 0	4 1	22 0
	P	0 02	0 05	0 22
	S	0	0	0
+ 3	*N*	0 02	0 05	0 28
	M	0 1	0 26	1 1
	L	10 0	34 0	110 0
	P	1 0	1 7	11 0
	S	0	0	0

(1), (2) For explanation of symbols see legend to Fig 1

(3) The immunogenicity of the injected dose of the spleen cell fractions (Fig 1) is expressed as the number of sheep red blood cells necessary to evoke a comparable secondary hæmolysin response in sensitized recipient mice

(4) Specific immunogenicity is the immunogenicity (column 3) per µg N of the cell fraction

(5) Total immunogenicity is the product of the specific immunogenicity (column 4) and the N content (in µg) of the total cell fraction

From the estimated total immunogenicity of donor spleens (Table 1), and the known distribution of injected foreign erythrocytes in the mouse[6], it can be estimated that only 15 per cent of the antigen phagocytized by the spleen was active in stimulating the secondary hæmolysin response This signifies that no more than 1 5 per cent of the originally administered particulate antigen was immunogenic. These results are in agreement with White's[7] hypothesis that only some of the macrophages of the reticulo-endothelial system of the spleen participate in antibody synthesis

I thank Dr J Primosigh for the nitrogen analysis This work was supported in part by U S Public Health Service grant *E*–3297(*C*1)

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PROTON TRANSFER IN NUCLEIC ACID

KYOGOKU *et al* [1] have reported that mixed crystals have been obtained with the nucleoside pairs, adenosine (A)–uridine (U) and guanosine (G)–cytidine (C), and that the infra-red spectra of the mixed crystals show new bands not present in the individual components, indicating a proton transfer from U to A and from G to C (see formulæ (1) and (2) in ref 1)

These experiments were then taken as evidence that certain absorption bands in film spectra of DNA and RNA indicated base pairing by proton transfer[2] The question raised by Kyogoku *et al* [1] is an important one, since it would put the hydrogen-bonding schemes and interchain attractive forces of nucleic acids on quite different bases from those which are usually considered to exist

If the arrangement of bases were that shown in formulæ (1) and (2)[1], the electrostatic attraction between the bases would be expected to account for an important part of the molecular stabilization The hydrogen-bonding schemes presented by Watson and Crick[3] and modified by Pauling and Corey[4] are widely accepted to-day, as is the view of these authors that the hydrogen bonds hold together the polynucleotide chains An increasing understanding of the importance of hydrophobic bonding in biological macromolecules makes it appear likely that this is also an important stabilizing factor (for a discussion of this phenomenon with special reference to the proteins, see, for example, W Kauzmann[5], for a discussion of its importance in DNA see Herskovits *et al* [6])

All the spectra reported here were measured with a Beckman *IR* 7 spectrophotometer, those in Fig 1 as Nujol mulls and those in Fig 2 as deuterium oxide solutions (if the mull and deuterium oxide spectra are compared, it should be borne in mind that the former contain NH_2 deformations which the latter lack, that mull spectra may also contain additional bands attributable to the solid state, and that the frequencies and intensities of bands usually vary with the medium in which they are measured Adding further to the difficulty of interpreting solid-state spectra are the possibilities that different polymorphs of the same compound may have different spectra and that extraneous bands may result from water that is adsorbed or incorporated into the crystal) The samples of pairs of nucleotides were prepared in two ways (*a*) equimolar amounts of nucleosides were thoroughly mixed by brief vibration in an electrically driven agitator (Fig 1, *C* and *G*), (*b*) equimolar amounts of nucleosides were dissolved in water and slowly evaporated to dryness in a desiccator (Fig 1, *D* and *H*) The deuterium oxide solutions of the nucleosides and polynucleotides were prepared and measured in fixed cells of 25μ path-length, solvent compensated, as described in earlier papers[7] The results are given in Figs 1 and 2 and Table 1 (The report[1] that UC and AG also formed mixed crystals with new absorption bands, indicating proton transfer prompted analogous experiments with these pairs The only bands observed in these experiments were those present in the component nucleosides)

The nucleosides were obtained from Pabst and from the California Corporation

Table 1 INFRA-RED SPECTRA IN DEUTERIUM OXIDE SOLUTION

Material	Frequency (cm^{-1})
Cytidine	1,650 , 1,618
Cytidine, *p*H 2	1,712 , 1,657 , 1,591
Inosine	1,673
Inosine, *p*H 12	1,597 1,563
Poly I	1,677
Poly C	1,653 , 1,617
Poly (I+C)	1,697 , 1,648 , 1,630 (sh)

The essential points made by Kyogoku *et al* [1] regarding the solid-state spectra of the nucleosides may be summarized (*a*) neither A nor U has a band at 1,700 cm^{-1}, whereas the mixed crystal AU does have a band at this frequency, similarly, GC has a band at 1,720 cm^{-1} which the separate nucleosides lack, (*b*) the acid forms of A and C have higher frequency bands (compared with the neutral molecules) at 1,693 cm^{-1} and 1,725 cm^{-1}, respectively, (*c*) these higher frequency bands in the mixed crystals are an indication of protonation of the bases by proton transfer

Regarding these points, the following observations are made (*a*) It may be seen in Fig 1 that uridine has a band at 1,698 cm^{-1} and guanosine has a band at 1,732 cm^{-1}, and that the nucleoside pairs, whether obtained by mechanical mixing or by evaporation from aqueous solution, do not differ in any major way from a summation of the appropriate members of the pairs measured separately (though the resolution of some bands of the evaporated mixtures is poorer, no new bands appear) (*b*) The protonated forms of cytidine and adenosine do have new bands at higher frequencies[7-9] (*c*) There is no need to assume proton transfer to explain bands

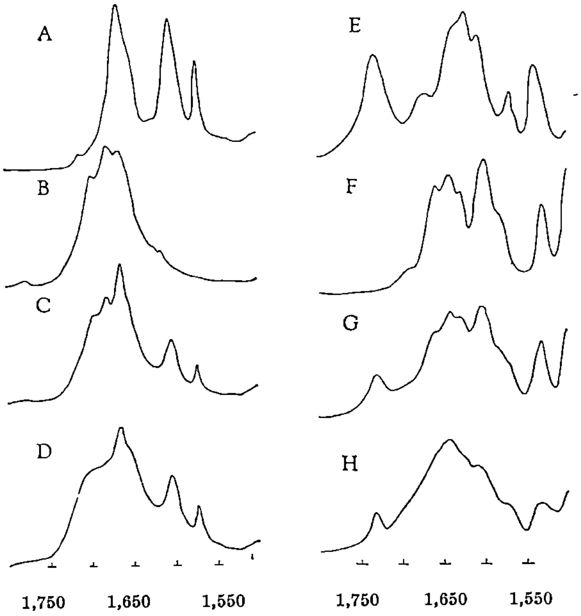

Fig 1 Infra-red spectra of Nujol mulls of the following nucleosides and nucleoside mixtures *A*, adenosine , *B*, uridine , *C*, adenosine + uridine, mechanical mixture , *D*, adenosine + uridine, evaporated from solution , *E*, guanosine , *F*, cytidine , *G*, guanosine + cytidine, mechanical mixture , *H*, guanosine + cytidine, evaporated from solution

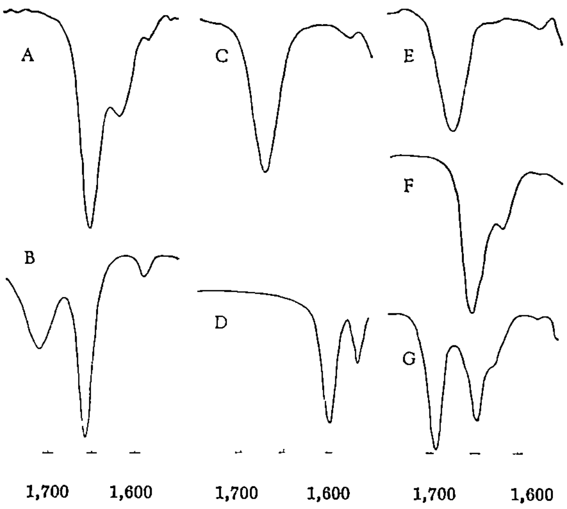

Fig 2 Infra-red spectra of deuterium oxide solutions *A*, cytidine, *B*, cytidine, acid solution, *C*, inosine, *D*, inosine, basic solution, *E*, polyinosinic acid, *F*, polycytidylic acid *G*, interaction product of poly I and poly C (poly (I + C))*

which are present in the individual nucleosides (It is possible that the conditions used here for obtaining the mixed nucleosides from solution do not duplicate those of the paper in question[1], since no experimental details were given This possibility is immaterial, however, in view of point (*c*)

While these observations do not appear to be consistent with the cited[1] basis for expecting proton transfer in nucleic acids, they do not prove that it does not in fact occur in these macromolecules Evidence on this point can, however, be provided by a study of DNA-like[10,11] polynucleotide helices, the example of the interaction product of poly I and poly C (poly (I + C)) being chosen in the present case because of the clear resolution of the bands[7,12] If proton transfer from poly I to poly C occurred, then the hypoxanthine units would exist as anions and have a spectrum similar to that of a basic solution of inosine (Fig 2*D*), and the cytosine units would exist as cations having spectra similar to that of acid cytidine (Fig 2*B*) Proton transfer in this helix would thus require strong bands near 1,710 cm $^{-1}$ and 1,597 cm $^{-1}$, with a band of moderate intensity near 1,563 cm $^{-1}$ (Fig 2, Table 1)[12] Since none of these bands is present, it follows that this kind of proton transfer does not occur, and that the infra-red spectral changes occurring on polynucleotide helix formation must arise from other causes. (Though there would be some overlapping of pertinent bands in the helical interaction product of poly A and poly U (poly (A + U)), the same conclusion can be reached in this case (for spectra see ref 7) Thus, the high-frequency band of uridine would be absent in the anionic[8] form, whereas it is actually observed at 1,695 cm $^{-1}$ in poly (A + U)[7] The 1,666 cm $^{-1}$ band of protonated adenosine would probably be obscured by the 1,672 cm $^{-1}$ uridine band, the neutral adenosine band would be absent, whereas a band reasonably assigned to this source is observed at 1,637 cm $^{-1}$ (ref 7) The spectra[13] of the helical interaction products[14] of poly A and poly I (poly (A + I) and poly (A + 2I)), also rule out the possibility of proton transfer Thus poly (A + I) has a strong inosine band at 1,682 cm $^{-1}$ and a strong poly A band at 1,628 cm.$^{-1}$ If the proton had been transferred from the inosine to the adenosine rings there would be bands near 1,666 cm $^{-1}$ and 1,600 cm $^{-1}$, whereas no absorption is observed near either of these frequencies The same conclusion may be reached from poly (A + 2I) which has a strong band at 1,681 cm $^{-1}$ and a weak one at 1,627 cm $^{-1}$ and no absorption near 1,666 cm $^{-1}$ or 1,600 cm $^{-1}$)

H Todd Miles

National Institute of Arthritis and
Metabolic Diseases,
National Institutes of Health,
Bethesda, Maryland

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[9] Miles, H T, *Biochim Biophys Acta*, **27**, 46 (1958) In acid deuterium oxide solution adenosine has bands at 1,665 (*S*) and 1,616 cm $^{-1}$ (*W*) A gel of poly A in acid deuterium oxide (*p*H 2 5) has a strong band at 1,666 cm $^{-1}$ Details of these spectra will be published at a later date

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In our previous article[1], we presented a view that a band at 1,710 cm $^{-1}$ of nucleic acids is to be assigned to the adenine–uracil (or thymine) and guanine–cytosine specific base pairings in their secondary structures, in which a proton transfer of the type given in formula (1)[1] and (2)[1] occurs

By later work, a number of experimental facts have been found which show that the 1,710 cm $^{-1}$ band is certainly characteristic of the secondary structure of nucleic acids[2,3]. Thus, drying, heating (for 10 min at 100° C), treating with deoxyribonuclease (for 10 min at 37° C) or treating with formamide causes a marked decrease in intensity of the 1,710 cm $^{-1}$ band of DNA On the other hand, as to what scheme in the secondary structure is responsible for the appearance of the 1,710 cm $^{-1}$ band, our view is still open to question In the preceding communication, H Todd Miles has dealt with this point

In order to obtain information on the specific base pairings, we have further studied the mixed products of adenosine and uridine, and found a few different modifications of the 50–50 per cent products A crystalline product is obtainable from equimolar aqueous mixture of adenosine and uridine by a slow evaporation in a desiccator (The infra-red spectra shown in Fig 1*a* and *b* are apparently different from those given by Todd Miles These differences might come from different experimental conditions both in the procedures of preparing samples and of measuring spectra We used a Perkin–Elmer 112*G* single-beam, double-pass spectrophotometer with a 75 lines/mm grating and a potassium bromide fore-prism In the spectral region in question, atmospheric water

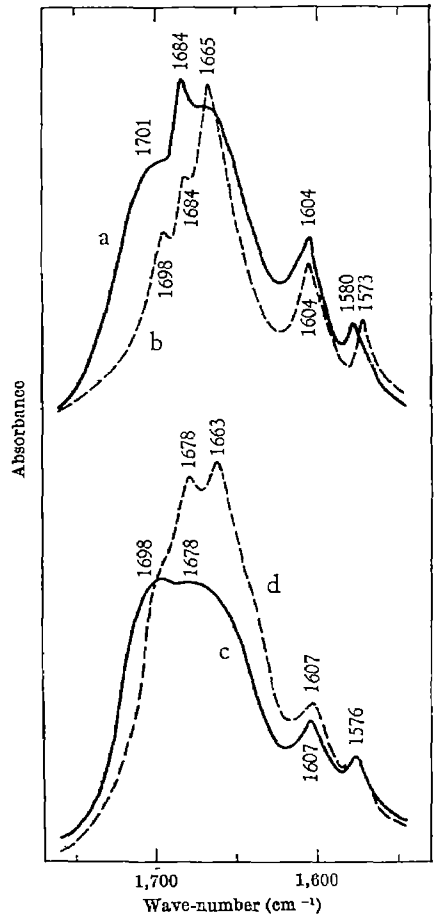

Fig 1. Infra-red spectra of Nujol mulls· *a*, A product obtained from an equimolar aqueous mixture by slow evaporation in a desiccator, *b*, an equimolar mechanical mixture of crystalline adenosine and crystalline uridine powder, obtained from a commercial source (Nutritional Biochemicals Corp), *c*, a product obtained from an equimolar aqueous mixture by quick lyophilization, *d*, an equimolar mechanical mixture of adenosine and uridine powder each of which was obtained by quick lyophilization separately from each aqueous solution

vapour (at 40 per cent relative humidity) seriously interferes with the observation, and therefore extremely dry air (water pressure 5×10^{-3} mm mercury) was flushed into the spectrometer) This gives an infra-red absorption spectrum (Fig 1*a*) and an X-ray powder diagram different from those of the 50–50 per cent mechanical mixture of adenosine and uridine crystals obtained from a commercial source (Nutritional Biochemicals Corp , Cleveland 28, Ohio) (see Fig 1*b*) (The X-ray examination was kindly made by Dr Y Iitaka) An amorphous product is obtained from the similar aqueous mixture by quick lyophilization What we mentioned in the previous article[1] was this amorphous product The words "complex crystal" we used were not correct This gives an infra-red absorption spectrum (Fig 1*c*) different from the crystalline product (Fig 1*a*) and also from mechanical mixtures (Fig 1*b* and *d*) As may be seen in Fig 1, both these products from the aqueous mixture give absorptions in the 1,710 cm^{-1} region. Especially in the case of the amorphous product (Fig 1*c*) this band is much stronger than those of adenosine–uridine mechanical mixtures Thus, a support of our view does still exist here

On the other hand, a few facts have been found which are apparently less favourable for our view None of them, however, is found to contradict seriously with it

In deuterium oxide solution, 1 1-complex of polyadenylic acid and polyuridylic acid shows a 6μ-region spectrum[4] which is not the same as the spectral sum of the protonated adenosine and deprotonated uridine[5] (Fig 2) This fact shows that in this complex the adenine residue is not exactly the same form as that it takes in acidic solution of adenosine, and that the uracil residue is not exactly the same as that it takes in alkaline solution of uridine However, in our model[1] the base residues are involved in a strong hydrogen bond even after the proton transfer takes place Therefore, the electronic states of the residues should not necessarily be the exact duplicates of those of protonated and deprotonated nucleosides freely moving around by themselves in solutions A similar statement may be valid for the 1 1-complex of polyinosinic acid and polycytidylic acid, mentioned by Todd Miles in the preceding communication

Solid-state guanosine was found to take two modifications[5], namely, form (I) and form (II) Form (I) does not give any definite band above 1,700 cm^{-1}, while form (II) gives a strong band at 1,736 cm^{-1} (The modification previously named form (I) was recently found not to be a crystal but an amorphous form The infra-red spectrum of the complex product of guanosine and cytidine observed by Todd Miles is practically the same as that which we observed[1] Both show a band near 1,730 cm^{-1} The apparent difference is that he observed this

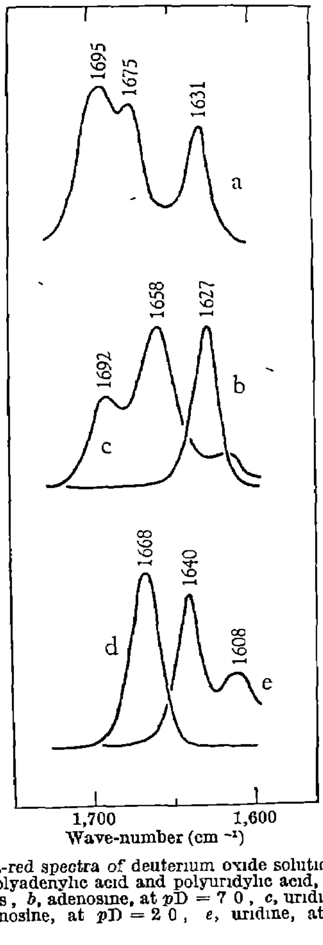

Fig 2 Infra-red spectra of deuterium oxide solutions *a*, 1 1 complex of polyadenylic acid and polyuridylic acid, observed by H Todd Miles, *b*, adenosine, at *p*D = 7 0, *c*, uridine, at *p*D = 7 0, *d*, adenosine, at *p*D = 2 0, *e*, uridine, at *p*D = 11 0

band also in guanosine and we did not This difference comes from the fact that there are two modifications of guanosine By comparison with the spectra given in our later paper[5], what Todd Miles observed is identified as that of form (II), and what we first observed[1] was that of form (I)) This means that guanine residue by itself can give 1,736 cm^{-1} in certain circumstances Polyadenylic acid film obtained from an aqueous solution of *p*H 4 5 shows a strong band at 1,710 cm^{-1} (refs 6 and 7). This band seems to be characteristic of the secondary structure of this polymer, and it disappears on drying the film On deuteration, the 1,710 cm^{-1} band disappears In the deuterated film, no band is observed in the 1,600–1,750 cm^{-1} region the intensity of which is lowered on drying the film Then, the 1,710 cm^{-1} band, in this case, should be assigned to the scissoring vibration of the strongly hydrogen-bonded NH_2 group or strongly bound water molecule These facts suggest that, in the assignment of the 1,710 cm^{-1} band in nucleic acid (for example, ribonucleic acid), not only the specific base pairings but also other factors (such as non-specific base–base interactions) should be taken into consideration

In conclusion, our models, (1) and (2), still remain as one of the possible explanations of the 1,710 cm^{-1} band, although the critical evidence has not yet been established Conclusive assignment of this 1,710 cm^{-1} band will be made on the basis of a quantitative work of the normal modes of vibrations of the base residues, which is now in progress

Y KYOGOKU
T SHIMANOUCHI
Faculty of Science,
M TSUBOI
Faculty of Pharmaceutical Sciences,
University of Tokyo,
Bunkyo-ku, Tokyo
I WATANABE[*]
Institute for Virus Research,
University of Kyoto,
Sakyo-ku, Kyoto

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[*] This part of the investigation has been aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research

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SOME ELECTRICAL PROPERTIES OF THE MEMBRANE OF A CELL NUCLEUS

By PROF WERNER R LOEWENSTEIN and DR YOSHINOBU KANNO
Physiology Department, Columbia University, College of Physicians and Surgeons, New York

THE past fifteen years, since Graham and Gerard[1] and Ling and Gerard[2] introduced the microelectrode, have seen a considerable increase in our knowledge of the electrical properties of the cell membrane Membranes of a great variety of cells have been studied The studies, however, have been limited to the plasma membrane Membranes of cellular organelles such as the nucleus, although they are the sites of the passage of materials of particular biological importance, have not yet been examined The reasons for this neglect are not difficult to find Cells are small and nuclei are even smaller The size of most nuclei places them beyond the reach of the microelectrode Besides, electrophysiologists traditionally have devoted little attention to membranes other than 'excitable' ones where large electrical changes occur during activity There are, however, a few animal cells with nuclei large enough for electrophysiological work An example is the salivary gland cell of the fruit fly (*Drosophila*) larva In a large gland cell, the nucleus measures 30–40μ in diameter The nucleus can be viewed *in vivo* without staining aids under an ordinary microscope Its surface[3], like that of other nuclei[4–11], appears as a double-layered membrane under the electron microscope and presents mechanical properties distinctly different from those of the cytoplasm and nucleus interior on micromanipulation[4,12,13] This article deals with some electrical properties of this membrane It will be shown that the nuclear membrane has a high electrical resistance and capacitance, and that there is a potential across it which is of about the same magnitude and sign as that across the cell membrane

Nuclei of salivary gland cells of *Drosophila flavorepleta* larvæ (third instar) were used in the experiments These nuclei do not divide during the third instar stage The glands were isolated and mounted in a bath of Schen's solution The cells of freshly obtained gland cells are quite transparent The nucleus, its outline and some of its internal details are clearly seen under a phase contrast or dark field microscope Electrical measurements were made with microelectrodes (3 *M* potassium chloride) of tip diameters below 0 5μ and tip potentials below 2 mV (ref 14) Such electrodes could usually be inserted into the cell and nucleus a few times without apparent change in membrane potential Two electrodes were inserted into each nucleus or cell with the aid of a pair of micromanipulators (Fig 1) One electrode served to pass square pulses of current of measured intensity across the membrane, the other to record the resulting passive potential, and the 'resting' potential Potentials were fed into the d -c amplifier of an oscilloscope through a cathode follower with a variable negative capacitance The passive potential had an exponential time course The membrane resistance was calculated from the 'steady state' potential, and the capacitance from the time constants of the rising or falling phase of the potential The nucleus is nearly spherical, its membrane area could thus be measured with a high degree of accuracy

Fig 2 illustrates an experiment in which the cell and its nucleus were successively impaled with a potential measuring electrode As the electrode is advanced from the cell exterior towards the nucleus one finds (1) an abrupt change in potential as the

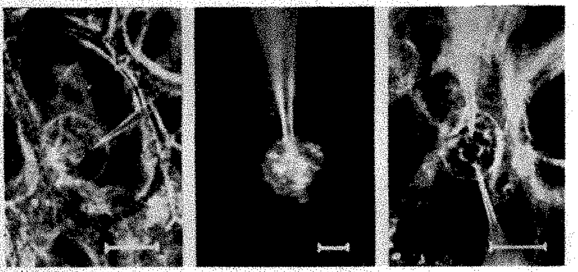

Fig. 1. Dark-field photomicrograph of unstained, fresh salivary gland cells. Views of nuclei impaled *in situ* with one (left) or two microelectrodes (right); or in semi-isolation after destroying mechanically the cell membrane (centre, the portion of cytoplasm that adheres to and surrounds the nucleus is not visible in this photomicrograph). (Calibration 25μ)

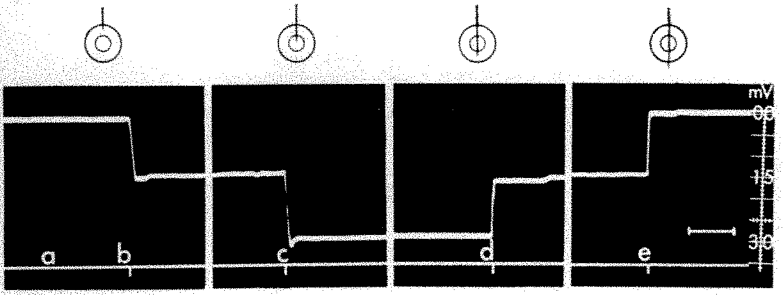

Fig. 2. Potentials across cell and nucleus membranes. A micro-electrode is advanced progressively in the direction of cell exterior-nucleus. Upper beam records the potential (downward deflexion negative) as the electrode tip is *a*, outside the cell; *b*, penetrates the cell membrane; *c*, enters the nucleus; *d*, leaves the nucleus and enters again into the cytoplasm; *e*, emerges into the cell exterior. Reference electrode is in the solution bathing the cell exterior. Time calibration: 0·05 sec., film interrupted for about 1 sec. in between photographs

electrode penetrates the cell membrane; (2) no further change in potential as the electrode moves through the cytoplasm; (3) another abrupt change in potential when it enters the nucleus. The latter change occurs clearly at the surface of the nucleus. It is associated with the penetration of the electrode tip into the nucleus and is preceded by a visible dimpling of the nucleus surface. No change in potential is seen, as the electrode tip moves through the interior of the nucleus.

The mean potential across the cell membrane of 25 cases was 12 mV. with a standard error of ± 0·7 mV. (cytoplasm negative). This is about 10 mV. lower than the cell membrane potential of mammalian salivary gland cells (type I)[15]. The mean potential between the nucleus interior (negative) and the cytoplasm (hereafter referred to as nucleus membrane potential) was 13 ± 1·1 mV. (18 cases): and the total potential between nucleus interior and cell exterior was 25 mV.

The surface of the nucleus has a high electrical resistance. When the current delivering electrode is inside the nucleus, and the potential recording electrode is advanced from the cytoplasm into the nucleus, one measures a sharp increase in resistance on penetration into the nucleus. The increase in resistance coincides with the appearance of the nucleus membrane potential. It is clearly associated with the nucleus surface: as the electrode moves through the nucleus interior, changes in resistance are several orders of magnitude lower than at the surface. The resistance that appears to be associated with the nucleus boundary will here be referred to as nucleus membrane resistance. Table 1 gives resistance values of seven representative nuclei. Typically the nucleus membrane shows no rectification; its resistance is constant over a wide range of inward or outward currents (Fig. 3). Moreover, it gives no sign of excitation over the entire range of current that can be used without destroying the membrane. This applies to the nucleus both semi-isolated and *in situ*. The mean nucleus membrane resistance was 1·5 ± 0·4 Ω cm.^{2} and the capacitance 412 ± 62 μF./cm.^{2} (8 cases). Resistance and capacitance were measured in each case under two different conditions: first, in the nucleus *in situ*, surrounded by the cytoplasm and the 'intact' cell membrane (the resistance and capacitance of the cell membrane were previously measured, and the nucleus and cell membranes were treated as leaky capacitors in series); and then, in the semi-isolated nucleus, immediately after rupturing the cell membrane, and thereby eliminating completely the cell membrane resistance and capacitance, the nucleus being still completely surrounded by a mass of cytoplasm. There was, in most cases, fair agreement between the values obtained under the two conditions.

The capacitance of the nucleus membrane is high compared with that of the cell membrane. This result will be discussed in a future paper. The resistance, on the other hand, is smaller than that of the cell membrane (Table 1). It is also smaller than that of the cell membrane of a number of other cells[16-21]. It is, however, large enough to indicate that the nuclear membrane must be a formidable ion barrier. In this connexion it is interesting to consider some recent electron microscope studies which picture the nuclear membrane with large pore-like structures, 400–1000 Å. in diameter[3-10]. From the present results it appears that these 'pores' cannot provide a direct contact between the nucleo- and cyto-plasm. On the basis of a pore diameter of

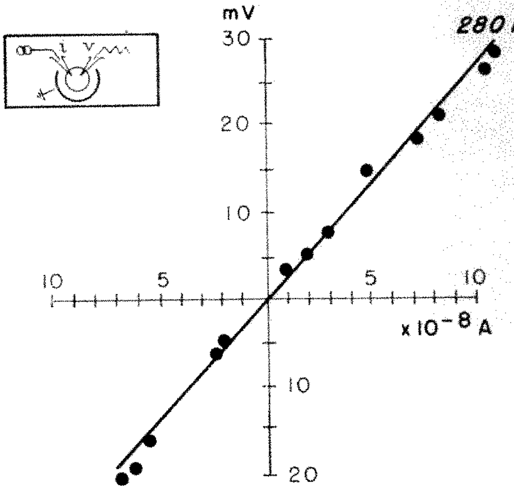

Fig. 3. Current-voltage relation in a nucleus membrane. Square current pulses of 24 msec. duration are passed through the membrane of a semi-isolated nucleus with one microelectrode (*i*) and the corresponding electrotonic potentials are recorded with a second microelectrode (*v*). Abscissæ, total nucleus membrane current, inward current left (membrane area = 1·6 × 10^{-5} cm.^{2}); ordinates, steady-state electrotonic potential, hyperpolarization downwards

Table 1. CELL AND NUCLEUS MEMBRANE CONSTANTS

	Cell membrane			Nucleus membrane		
Case no.	Potential (mV.*)	Resistance (Ωcm.^{2})	Capacitance (μF./cm.^{2})†	Potential (mV.*)	Resistance (Ωcm.^{2})	Capacitance (μF./cm.^{2})†
A–32	9·0	221·7	1·69	12·0	1·95	115·2
A–34	12·5	424·5	2·78	9·5	2·09	439·1
A–35	11·0	1,183·1	2·13	21·0	5·25	523·1
B–17	9·0	299·7	3·34	11·0	1·08	416·0
B–18	6·0	173·4	2·53	9·5	0·93	294·5
B–19	15·0	730·0	9·93	10·5	0·86	300·9
B–20	8·0	846·0	3·99	9·0	0·51	441·8

* Values include up to 2 mV. tip potentials

† Capacitance values as calculated from time constants of the apparent reactance term of membrane impedance.

500 Å., a spacing of 1500 Å. in between pore centres, a total membrane thickness of 200 Å.[3] and a resistivity of 50 Ωcm. for nucleoplasm and cytoplasm, it is calculated that the nucleus surface exposed to the cytoplasm would be 15–23 per cent and that such a perforated membrane would have a resistance of 7×10^{-4} to 4×10^{-4} Ωcm.^{2}, a value four orders of magnitude lower than the observed one. It would seem, therefore, that the 'pores', which have often been associated with the passage of large molecules through the nucleus membrane, are either not freely communicating fenestrations, or that the material within them is of very high resistivity.

We thank Prof. G. Hoecker, University of Chile, for pointing out the salivary gland of the fruit fly as a convenient material for our investigations, and Prof. T. Dobzhansky, Columbia University, for kindly providing us with the specimens which started out our fly colony. We also thank the National Cystic Fibrosis Research Foundation, the National Science Foundation, and the National Institutes of Health for financial support.

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ADAPTATIONS OF THE MAMMALIAN SKIN TO THE AQUATIC MODE OF LIFE

By DR. W. SOKOLOV
Department of Vertebrate Zoology, University of Moscow

THE aquatic mode of life is natural to various mammals. The degree of their adaptation to an aquatic medium varies. It is possible to establish a number of transitory forms from almost exclusively terrestrial to purely aquatic ones. The main function fulfilled by the cutaneous covering of aquatic mammals, when they are in water, consists in providing the thermo-insulation of the organism, which is explained by a high degree of heat conduction of water exceeding many times that of air. Among its other functions are: ensuring heat delivery; protection of the organism against mechanical injuries; facilitation of gliding of the body in water when swimming and so on.

A thick-haired animal, when in water, still retains an air layer in its pelage, which serves as a heat insulator. However, in mammals leading an exclusively aquatic mode of life such an air layer cannot remain, for in case of the slightest mechanical action on their fur it would be disturbed, and to restore it the animal would be compelled to return to dry land. Being unable to fulfil its chief function, that is, heat protection, the pelage of such mammals becomes reduced. Instead, a subcutaneous layer of fat tissue is developed of such a thickness which is never found among land animals leading an active mode of life. In hibernating species, an equally thick fat tissue develops before hibernation commences. Yet, this fat content in hibernating animals changes sharply according to season, its function consisting rather in the accumulation of food reserves than in the preservation of heat in the organism. On the other hand, in purely aquatic forms the fat tissue undergoes but slight alterations during the course of the year. Investigations carried out by Scholander *et al.*[1] have shown that when the animal is in water its subcutaneous fatty layer proves to be a much better thermo-insulator than the air layer in its fur. Thus, the impossibility on the part of strictly aquatic animals to keep an air layer in their pelage and, on the other hand, higher thermo-insulating properties of subcutaneous fat than those of that layer, have determined this trend of the evolutionary process. A definite economy is brought about in those animals owing to their large dimensions corresponding to a relatively small body surface.

Amphibian mammals of moderate size, which spend a considerable time on dry land, do not follow

Table 1 HAIR PECULIARITIES OF SEMI-AQUATIC AND SOME OF LAND MAMMALS

Species	Correlation of axes of the axial hair cross-section	No of kinks in		Breadth of the medullary layer of an axial hair in per cent to its whole breadth
		axial hairs	wool hairs	
Ornithorhynchus anatinus	1 6	absent	4	16
Tachyglossus aculeatus	1 3	1 5–2	absent	absent
Chironectes minimus	1 3	2–3	5–6	57
Neomys fodiens	1 2	3	2–3	50
Sorex araneus	1 2	1–1 5	1–2	58
Galemys pyrenaicus	1 3 6	3–4	4–5	absent
Desmana moschata	1 4	3–4	7–8	,,
Talpa europaeus	1 1 5	3	3	60
Lutra lutra	1 2 3	absent	5–6	58
Mustela lutreola	1 2	,,	1–2	66
Martes martes	1 1 7	,,	3	85
Marmota baibacina	1 2 5	,,	3–5	92
Castor fiber	1 2 3	,,	7	46
Microtus arvalis	1 3	,,	3–5	92
Ondatra zibethica	1 2 6	,,	4–5	57
Arvicola terrestris	1 2	,,	2–3	75
Myopotamus coypus	1 2 5	,,	5–7	76

the path of fur reduction On the contrary, their pelage is highly developed Scholander *et al* [1] indicate that the subcutaneous cellular tissue is a thermo-insulator of poor efficiency in the air medium Thus, a layer of blubber in the seal is little more efficient in its insulating properties than the skin of the lemming notwithstanding its much greater thickness Therefore, with a reduced pelage, the animal would need a very thick layer of subcutaneous fat to ensure reliable heat protection in the air, this would impair its mobility on dry land It is natural that all mammals forced by their nature to move swiftly on dry land, for example, all beasts of prey, cannot possess a thick layer of subcutaneous fat On the other hand, the thickness of the subcutaneous cellular tissue is in contradiction with the animal's dimensions The smaller the latter, the relatively thicker should be the fat tissue (a relative magnitude of heat delivery is found to increase in animals of small size). Mammals rather slight in build such as the duck-bill or musk-shrew which on principle could develop a subcutaneous fat tissue instead of a pelage fail to do so because that tissue would be necessarily so comparatively thick, much thicker than, for example, in the seal, that it could not be formed in a small animal

According to the character of their cutaneous covering aquatic animals which I have examined may be divided into two groups (1) semi-aquatic forms with a dense pelage (*Ornithorhynchus anatinus, Chironectes minimus, Neomys fodiens, Galemys pyrenaicus, Desmana moschata, Lutra lutra, Mustela lutreola, Castor fiber, Ondatra zibethica, Arvicola terrestris, Myopotamus coypus*), (2) animals more adapted to life in water or even purely aquatic ones with a well-developed subcutaneous fat tissue The second group may be divided into two sub-groups (*a*) animals with a pelage at different stages of reduction (*Callorhinus ursinus, Eumetopias jubatus, Odobaenus rosmarus, Phoca vitulina*), (*b*) those which are devoid of hairs (*Eubalaena glacialis, Balaenoptera physalus, B borealis, B acutorostrata, Physeter catodon, Berardius bairdii, Delphinus delphis, Lagaenorhynchus obliquidens, Orcinus orca*)

The hairs of group (1) may be distinctly subdivided into the categories directing, axial and wool hairs Even in Insectivora (in *N fodiens* and particularly in *G pyrenaicus* and *D moschata*) these three types of hairs sharply differ from each other, while in terrestrial species of this order, for example, in members of the genus *Sorex*, wool and axial hairs are alike The latter are broader in semi-aquatic mammals (Table 1) Wool hairs of aquatic species have a large number of kinks (Table 1)

These wool hairs are relatively less thick Thus, in the small *Sorex araneus* and in the much larger *D moschata* the thickness of wool hairs is the same, that is, 0 015 mm The difference in length between wool and axial hairs of semi-aquatic animals is greater than in terrestrial forms For example, in *S araneus* and *S minutus* these hairs are nearly equal, but in *D moschata* axial hairs are 2–5 5 mm longer than wool hairs In most axial hairs the thick part, the so-called granna, rises over the wool hairs, and at its base the hair forms a kink In the structure of hairs of semi-aquatic species a slighter development of the medullary layer is characteristic (Table 1), which makes hairs more solid and flexible in swimming, due to that fact their specific gravity approximates 1 In *D moschata* the medulla of the wool hairs is half as thick as that of *N fodiens* and a third as thick as that of *Sorex araneus* Hairs of semi-aquatic animals form well-marked tufts and groups They are present even in *G pyrenaicus* and *D. moschata*, although in all terrestrial Insectivora and in *N fodiens* (less adapted to aquatic life) the hairs are located singly The tufts produce a denser fur Hairs of semi-aquatic species surpass in thickness those of most terrestrial animals Thus, the number of hairs per 1 cm^2 of skin reaches in *S araneus* 15,000 on the back and 14,000 on the abdomen, in *N fodiens* 16,000 and 15,000, in *D moschata* 30,000 and 37,000, in *O zibethica* 11,000 and 12,000[2], in *L lutra* 35,000 and 51,000[3] and in *M coypus* 6,000 and 14,000[4] It is characteristic that the abdomen of semi-aquatic animals (contrary to land animals) is protected from cooling by a denser and higher fur The density of the pelage in individuals of semi-aquatic species dwelling in the north and south differs to a much lesser degree than it does in terrestrial forms This peculiarity may be explained by more stable temperature conditions of the water medium in various latitudes Also, in connexion with the fact that the temperature of water, as compared with air, changes less in the different seasons, a slighter seasonal variability of the pelage is noticed

All peculiarities of the hair cover of semi-aquatic animals (dense fur, high and broad axial hairs, slight development of the medullary layer) allow the retention of an air layer while swimming In this case the dilated upper parts (grannas) of axial hairs, rising over the down, bend parallel to the body surface and cover the down Owing to the surface tension of water ends of these hairs, forming a T-shaped covering, keep the air in the underfur Kinks in wool hairs increase their resilience and they probably do not allow for the pressure of water in diving to reduce the thickness of the air layer in the fur

In amphibian mammals the thickness of skin, epidermis and dermis is as a rule greater on the abdomen than on the back, which is the reverse of terrestrial species No differences are present in the structure of the epidermis A stronger development of the papillary layer characterizes the dermis in the skin of semi-aquatic animals Bundles of collagen fibres mostly form a lax type of plexus The subcutaneous fat tissue is either poorly developed or even quite absent Sebaceous and sweat glands are usually large The giving off of excess heat when the organism becomes overheated is accomplished in water mostly by parts of the body with no pelage In the majority of semi-aquatic forms the tail serves as such a site for giving off excess heat It is usually of large dimensions with few hairs or without them, but with a well-developed blood system In air, surplus of heat is given off by evaporation of sweat in most species or, evidently, at the expense of breathing if the sweat glands are absent, as, for example, in *M coypus*

Animals with a pelage in the state of reduction (sub-group *a* of the second group-order Pinnipedia) are as a rule more adapted to aquatic life than those of the first group Their hairs may be divided into axial and wool, but the former have no characteristic grannas, while the latter are of the same form as the axial hairs They lose their function of creating an air layer, when the animal is in water Probably their main task consists in protecting the body surface against mechanical injuries and in promoting movements on the ice, as, for example, in seals The animal covered with such a fur can easily glide over the snow or ice, and at the same time elastic hairs prevent its rolling back The hairs in animals of the second group sharply decrease in length, but increase in breadth, the number of hairs being reduced, but the hairs still grow in tufts The M m arrectorus pilorum is absent New-born cubs of seals with little-developed subcutaneous fat tissue have a dense pelage with well-marked wool hairs The cubs do not swim at that time With the beginning of an aquatic mode of life accumulation of fat begins under their skin and the juvenile pelage is replaced by adult fur

In animals of that sub-group the epidermis becomes considerably thicker In most cases all the five layers may be found therein, the corneous layer being particularly well developed In the walrus the epidermis reaches its greatest thickness and has an alveolar structure on its inner surface In this mammal is the greatest reduction of hair cover among Pinnipedia and here only its epidermis protects the organism against mechanical action The dermis is much thickened, the reticular layer forming the greatest part Bundles of collagen fibres form intricate plexuses In the dermis there is a specific net of smooth muscle bundles unconnected with hairs Probably these muscles can change the configuration of the skin contour in swimming and make the folds, which probably have an anti-turbulent function The elastic fibre plexuses which are absent in land mammals assist the displaced tissues to return to their usual places I cannot suggest another function for these morphological structures The thermo-insulating function passes to the subcutaneous fat tissue of great thickness Sweat and sebaceous glands are well developed

The densest and highest pelage is found in the fur-seal It is evidently connected with the relatively active mode of life led by the fur-seals on land during summer

Giving off of excess heat in Pinnipedia, in the case of their becoming overheated in water, occurs among sparsely haired species directly from the body surface But in densely haired forms during short immersions, when there remains an air layer in the hairs, it takes place mostly from the surface of flippers In the air medium heat is delivered by sweat evaporation

In mammals leading an exclusively aquatic mode of life (sub-group *b* of the second group-order Cetacea) hairs either completely disappear as, for example, in most Odontoceti, or remain on the snout to serve as tactile vibrissæ (in Mystacoceti) The thickness of the epidermis sharply increases, because now it has to fulfil its main protective function against mechanical actions It has an alveolar structure on its inner surface and its external layers remain uncornified It is necessary to add that the latter property is not a specific feature of the epidermis in animals of the sub-group *b*, but it is characteristic of epithelial coverings of some organs in terrestrial mammals Thus, an alveolar structure is natural to the thick epidermis of some hairless parts of the skin, for example, to the soles of human feet The tongue epithelium is likewise alveolar without being always cornified However, in Cetacea the alveoli are distributed in a strictly definite order, and their form is specific for diverse species Perhaps they assist to make small folds (see, for example, F S Essapian, 1955, Pl 8, 9) which may be have an antiturbulent function Large skin folds (F S Essapian, 1955, Pl 3, 5) (also antiturbulent ?) probably are formed by the subcutaneous muscles The dermis of Cetacea is comparatively thin, without being subdivided into layers The bundles of its collagen fibres show usually a complex interweaving The subcutaneous fat tissue, fulfilling the important function of thermo-insulation, occupies the largest part of the skin, being formed by sparsely disposed bundles of collagenic fibres and by an enormous accumulation of fat cells There are no cutaneous glands in Cetacea The giving off of surplus heat is carried out by the whole body surface of the animal and to a relatively high degree by the fins

A change in the chemical composition of the subcutaneous fat in animals belonging to the second group is of interest In most terrestrial species and in semi-aquatic forms of the first group the fat is characterized by a considerable content of saturated fatty acids (fats with a low iodine number) The subcutaneous blubber of Pinnipedia and Cetacea contains a vast number of non-saturated fatty acids (fats with a high iodine number), which provide a low temperature of the fat setting (Table 2) The latter circumstance is of great importance for Pinnipidea and Cetacea, particularly for those inhabiting arctic and antarctic waters because the outer layer of their skin has usually a low temperature only just surpassing that of the surrounding water

Table 2 THE IODINE NO AND THE TEMPERATURE OF THE SUBCUTANEOUS FAT SETTING IN SOME ANIMALS (refs 5, 6)

Species	Iodine No	Temperature of the fat setting (°C)
Phoca sibirica	130–133	+ 3
Balaena mysticetus	69–144	+ 7 to + 9 5
Balaenoptera musculus	99–136	+ 5 to + 8
Balaenoptera physalus	107–155	+ 3 to + 5
Mustela putorius	65	+ 31
Mortes zibellina	57	+ 28 to + 30
Citellus pygmaeus	118	0 to + 2

In hibernating species the subcutaneous fat has also a high iodine number Their temperature during the hibernation is near to the environment Such a chemical composition of subcutaneous fat of Pinnipedia, Cetacea and hibernating animals protects the animals against the setting of external layer of their body

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MESSENGER RIBONUCLEIC ACID IN PEA SEEDLINGS

By U E LOENING

Department of Botany, University of Edinburgh

AN unstable ribonucleic acid (RNA) carrying information from deoxyribonucleic acid (DNA) to ribosomes for protein synthesis was postulated by Jacob and Monod on the basis of enzyme induction and other experiments with bacteria[1] The presence of such a messenger RNA could be shown in a number of cases[2–4] It is characterized by a high rate of turnover and by a base composition similar to that of the DNA, uridylic acid taking the place of thymidylic The messenger will also hybridize with its homologous heat-denatured DNA, and naturally occurring hybrids have been found[5,6] The experimental demonstration of the presence of messenger RNA is greatly facilitated under conditions of continued protein synthesis but no net RNA synthesis In this way the turnover of the messenger and its base composition can be studied by radioisotope incorporation without interference due to incorporation into 'bulk' RNA These conditions have been obtained in phage-infected bacteria[2,3] and in 'step-down' cultures[4]

Under normal conditions of growth, however, the messenger RNA must be separated from other RNA components for identification This is partly achieved in bacterial homogenates, from which messenger RNA can be sedimented bound to the ribosomes at a high magnesium ion concentration, and released by lowering the magnesium concentration[3] This technique has been applied in experiments with pea seedlings described here An RNA with a high rate of turnover was detected by its sedimentation with ribosomes at a high magnesium ion concentration, using the centrifugation technique described previously[7] It could be partially separated from the ribosomes by electrophoresis at a low magnesium concentration The structural RNA of the ribosomes themselves remained unlabelled for some time after incubation of the seedlings in radioactive phosphate The base composition of the rapidly labelled RNA could therefore be determined without interference from other labelled RNA components, and was found to be closely similar to the DNA of the tissue The experimental procedure was as follows

Batches of 60 two-day-old pea seedlings were given a 'pulse' incubation by immersing the first centimetre of the 3–4 cm long roots in a medium at 25° containing 1–2 mc carrier-free phosphate labelled with phosphorus-32, 0 1 m*M* magnesium chloride and 0 1 m*M* potassium nitrate at *p*H 6 8 in a total volume of 25 ml This solution was used only once, since it was found that repeated use resulted in a greatly reduced uptake of phosphorus-32 into RNA The addition of the small concentrations of salts was an attempt to prevent this *Tris* buffer also reduced the uptake of phosphate For the 'chase' incubation, the seedlings were transferred to a large volume of 2×10^{-5} *M* phosphorus-31 phosphate at *p*H 6 8, also at 25° After cooling to 0°, 6–7 mm root tips were cut and homogenized in 3 ml medium containing 0·5 *M* sucrose, 35 m*M* *tris* buffer at *p*H 7 4, and 5 m*M* magnesium chloride The larger cell components were removed by centrifuging at up to 30,000*g* and the microsomes sedimented at 140,000*g* for 90 min Vesicular material was removed from the free ribosomes by centrifuging the tubes inverted at about 300*g*, the ribosomes then remain as firm pellets at the tips of the tubes This technique has been described previously[7]

The ribosomes were examined by electrophoresis on cellulose acetate membrane[8,9] using 50 m*M* *tris* buffer at *p*H 7 4 containing 0 1 m*M* magnesium chloride 120 V was applied across 10-cm strips of the membrane at 0° The ribosomes moved about 4 cm in 3 hr The membranes were dried at 0°, washed in 0 5 *N* perchloric acid, rinsed in *tris* buffer and re-dried The strips were then scanned for radioactivity at 1 5-mm intervals, using an automatic scanning device fitted to the Ekco *N664A* scintillation head The position and quantity of ribosomes was determined by scanning the strips at 260 mμ after clearing in 'Ondina oil 17' (Scottish Oils and Shellmex, Ltd) The absorption curve of the nucleoprotein in the oil-cleared membrane is similar to that in buffer

Scans were obtained after various periods of pulse and chase incubations Fig 1 shows the result after a 10 min pulse and 2 min chase The peak on the origin is due to traces of vesicular material, and its relatively high radioactivity reflects the high rate of turnover of the phospholipid The absorption peak shows the position of the ribosomes at about 3 5 cm , but the main radioactive peak has travelled considerably slower and covers a region of relatively little ultra-violet absorption This peak could be entirely removed by incubating the strips moistened with ribonuclease and acid washing This indicates that the activity was due to labelled RNA The phospholipid on the origin remained almost unaffected by ribonuclease treatment This 'pulse-labelled' RNA gradually disappeared during the chase incubation, and it was only after a long chase that the ribosomes became labelled This is shown in Fig 2, where after a 10-min pulse and 50-min chase, the absorption and radioactive peaks are coincident From scans similar to those shown in Figs 1 and 2 the amount of phosphorus-32 incorporated into RNA after various times of incubation was determined This is expressed as total counts per min irrespective of the position of the radioactivity on the electrophoresis strips (but neglecting the phospholipid on the origin) and calculated per unit ultra-violet absorption of the ribosomes and per unit radioactivity of the incubation medium The time course of this labelling during a 10-min pulse and subsequent chase is plotted in Fig 3 The first rise up to 12 min incubation is due to radioactivity migrating slower than the ribosomes as in Fig 1 This is largely removed during the chase incubation The second rise after 50 min. is due to labelling of the ribosomes as shown in Fig 2 At the

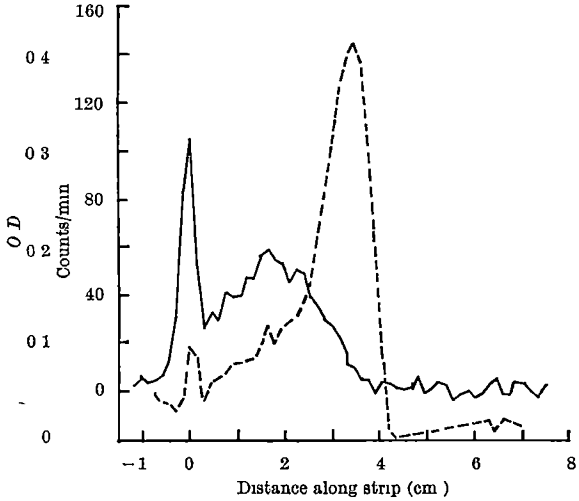

Fig 1 Pulse-labelled RNA associated with ribosomes Seedlings were incubated in ^{32}P (6 34 × 10^{7} counts/min /ml) for 10 min and in ^{31}P (2×10^{-5} *M*) for 2 min Electrophoresis of the isolated ribosomes at 12 V /cm for 2½ hr in 50 m*M tris* buffer at *p*H 7 4, containing 0 1 m*M* MgCl$_2$ Optical density scan (at 260 mμ) of the oil-cleared strip shown by the dotted line and radioactivity scan (counts/min) by the continuous line Readings for both scans were made at 1 5-mm. intervals

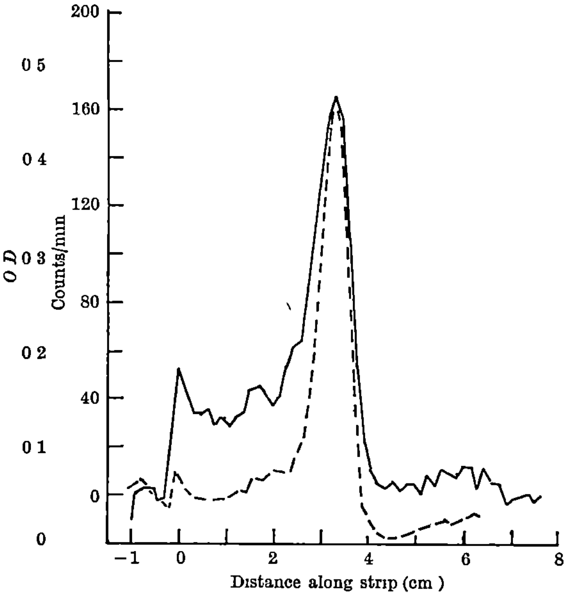

Fig 2 Ribosomal RNA labelled after a long chase. Seedlings were incubated in ^{32}P (1 88×10^{7} counts/min /ml) for 10 min and in ^{31}P for 50 min Electrophoresis as in Fig 1 Dotted line, optical density, continuous line, radioactivity

minimum labelling at 40 min, the pulse RNA and ribosomes each account for about half the radioactivity, as is shown by the double peak in Fig 4 It appears, therefore, that there is a ribonucleic acid fraction sedimented with the ribosomes which is turning over rapidly, so resembling messenger RNA Its detection is unequivocal because of the delay before the ribosomes become labelled It is interesting to note that the amount of radio-phosphorus in the supernatant from which the microsomes had been sedimented rises rapidly during the pulse but does not fall during a 30-min chase Since the phosphate concentration in the chase incubation medium is less than 1 per cent of the intra-cellular phosphate[10], it seems likely that phosphate taken up by the roots is available for metabolism for a short time only and thereafter is taken up into vacuoles or other cell structures Evidence for this has been obtained by electrophoresis of the vesicular fractions, when 3–5 times as much inorganic phosphate is released from the vesicles in citrate buffer as in *tris* buffer containing 0 1 m*M* magnesium ion Ts'o and Sato[10] also found that much of the intracellular phosphate was bound and not available for metabolism If this were not so it would be impossible to chase out the pulse-labelled RNA The effect of repeated incubations in the same solution, and of *tris* buffer, seems

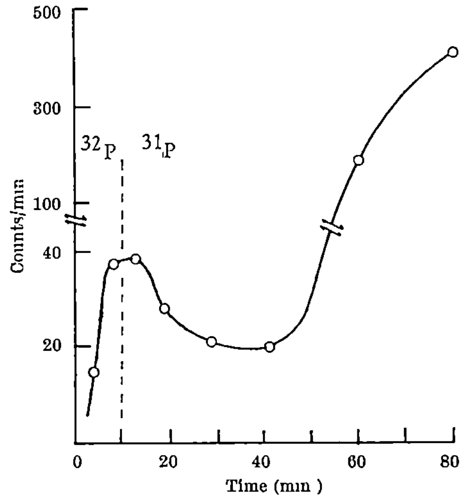

Fig 3 Time course of labelling of ribosomes and associated RNA Seedlings were incubated for 10 min in ^{32}P and then in ^{31}P, as indicated Values of radioactivity of RNA per unit ribosomal ultra-violet absorption were obtained from scans similar to those in Figs 1, 2, and 4, neglecting the peaks due to phospholipid on the origins

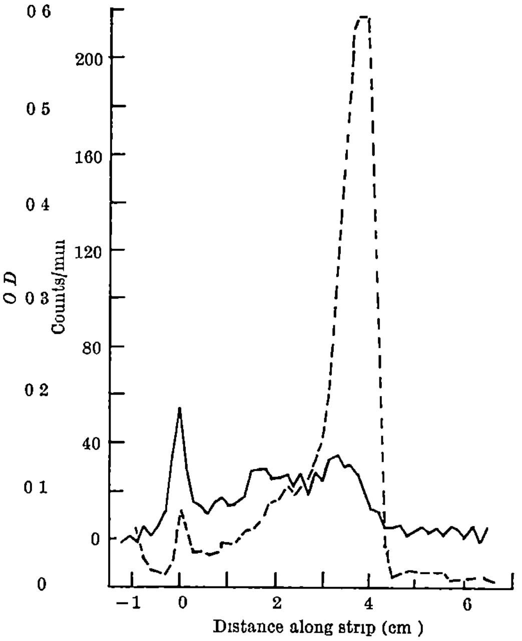

Fig 4 RNA labelled after a chase of intermediate length Details as in Fig 1, except the incubation in ^{31}P, which was for 31 min Dotted line, optical density, continuous line, radioactivity

to be to reduce the amount of metabolically available phosphate rather than its total uptake

Electrophoresis of the vesicular microsome fractions releases some ribosomes, but little or none of the pulse-labelled RNA was found There was, however, some leakage of acid-insoluble labelled substances from the highly radioactive phospholipid peak on the origin and this may have obscured the results The insoluble material on the origin does contain some labelled RNA, but this has not yet been obtained sufficiently pure to measure its specific activity or its base composition It is not known whether any of this RNA includes the ribosome precursors, or in which cell fractions these precursors occur during the first 40 min incubation The membrane-associated ribosomes do not become labelled sooner than free ribosomes

In order to determine the base composition of the labelled RNA by the radioactivity of the nucleotides after alkaline hydrolysis, it was necessary to free the RNA from traces of other radioactive materials, and considerable difficulty was experienced in doing this Precipitation of the ribosomes with 0 3 *M* magnesium chloride (R Bock, personal communication) resulted in some purification, but the cytidylic and uridylic acids were frequently contaminated The method recently described by Cox and Arnstein[11], in which ribosomal RNA is precipitated free of protein by guanidine chloride solutions, was satisfactory Usually $\frac{1}{2}$ vol of ethanol, in addition to the guanidine chloride, was required to effect the precipitation The small precipitate sometimes obtained without ethanol contained a higher proportion of the pulse-labelled RNA. Usually, however, total ribosomal RNA was prepared. After hydrolysis with 0 3 *N* potassium hydroxide at 37° and acidification, the nucleotides were separated by electrophoresis on cellulose acetate membrane in a citrate–phosphate buffer at *p*H 3 15 The nucleotide bands and adjacent blanks were cut from the paper and their phosphorus-32 contents determined by scintillation counting with the papers in contact with a plastic phosphor Ultra-violet absorption was determined after elution in 0 1 *N* hydrochloric acid Published molar extinction coefficients were applied[12] Ribosomal RNA obtained after a short pulse and a long chase incubation was uniformly labelled, since the specific activities of the four nucleotides were closely similar After a 15-min pulse only, however, the guanylic and cytidylic acids had a much lower specific activity than adenylic and uridylic The composition of pulse and chase labelled RNA is shown in Table 1, and compared with published figures for total DNA and RNA[13] There is no doubt that the composition of the pulse-labelled RNA corresponds closely to that of DNA

Table 1

	Adenylic	Uridylic (Thymidylic)	Cytidylic	Guanylic
Pulse RNA	10	8 4	6 8	6 6
DNA (Heyes[13])	10	8 9	6 2	6 5
Chase RNA	10	8 1	7 3	10 3
RNA (Heyes[13])	10	9 3	8 9	11 7

RNA was prepared from ribosomes and the radioactivity of the mixed 2′ and 3′ nucleotides determined as described in the text Pulse RNA was prepared from a 15 min incubation of the seedlings in phosphorus-32, and chase RNA after a further 80–90 min in phosphorus-31 Average of 4 and 3 determinations respectively

These results provide good evidence that informational or messenger RNA, synthesized on the DNA model and rapidly destroyed, occurs in pea seedlings Spiegelman pointed out, however, that a more complete proof of the identity of informational RNA involves an RNA–DNA hybridization experiment[3] Unfortunately this cannot be obtained at present in the higher organisms, since even DNA alone cannot be re-natured by slow cooling It may be possible, however, to isolate a naturally occurring hybrid, and Bonner and his co-workers already have evidence that this exists[14]

If this messenger RNA is similar to that in bacteria, it is also likely that its production is controlled by the inducer and represser mechanisms described by Jacob and Monod This system could then provide a mechanism for the control of cell development With this in mind, the relationship between the pulse RNA and the state of development of the pea seedling root tip cells is now being investigated

This work was supported by the Nuffield Foundation

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STATUS OF THE EAST AFRICAN SALT-WATER-BREEDING VARIANT OF *ANOPHELES GAMBIAE* GILES

By H E PATERSON

Poliomyelitis Research Foundation, South African Institute for Medical Research, Johannesburg

SEVERAL reports[1–3] have been published of the breeding of *Anopheles gambiae* Giles s l (Diptera, Culicidae) in East African estuarine waters at salinities greater than the normal tolerance limits of the species[4] Records apparently referring to this salt-water-breeding variant range from Zululand[5] to Somalia[6] On Mauritius a similar or identical form occurs[3,7] It has been demonstrated[2,3,5] that this east coast form is not identical with *Anopheles melas* Theobald, which occupies a similar niche on the west coast There are apparently no clear-cut morphological characters which separate the form unambiguously from the typical fresh-water-breeding *A gambiae*[3,5]

The object of this article is to present the results of some preliminary crossing experiments between members of a colony of salt-water-breeding *A. gambiae* from Tanga, Tanganyika (*T*), which was established by Dr. F. Kuhlow, and members of two colonies of typical, fresh-water-breeding *A. gambiae* from Kisumu, Kenya (*K*) and Muheza, Tanganyika (*M*). The following crosses and their reciprocals were set up: *K*♂♂ × *T*♀♀, *M*♂♂ × *T*♀♀. Ultimately, the method used for feeding the larvæ was that described by Shute[8].

F_1 generations were obtained for all the crosses. Table 1 presents the rearing success in each case.

Table 1. Rearing Success in Crosses

Cross (F_1)	No. of eggs laid	Percentage hatch	Percentage first stage larvæ reaching maturity
TK	4,276	30·7	8·7
KT	2,486	40·7	14·7
TM	1,056	21·9	14·8
MT	541	42·9	22·8

TK = Tanga ♂♂ × Kisumu ♀♀, *KT* = Kisumu ♂♂ × Tanga ♀♀, *TM* = Tanga ♂♂ × Muheza ♀♀, *MT* = Muheza ♂♂ × Tanga ♀♀.

Evidence of genetic discordance between the two forms is as follows: (1) Although the feeding regimen used yields healthy adults when applied to the pure parental forms, the adults obtained from the crosses were, in most cases, feeble, many being incapable of flight, despite the fact that the pupæ were large and active. (2) The sex-ratios of the F_1 generations deviated from the expected 1 : 1 as shown in Table 2. It will be noted that although the cross *M*♂♂ × *T*♀♀ yielded a ratio which is not significantly different from the expected, the deviation is in the same direction as that obtained for the corresponding cross, *K*♂♂ × *T*♀♀. (3) F_1 males from crosses which produced a deficiency of males (*T*♂♂ × *M*♀♀ + *T*♂♂ × *K*♀♀) were found to have highly degenerate testes, which were scarcely thicker than the vasa deferentia, and were quite clearly incapable of producing sperms. The F_1 males from the reciprocal crosses had testes of normal size, and spermatogenesis was noted to occur in them. No evidence is available on whether the F_1 females and the F_1 males with apparently normal testes are fertile or not. Because of the weakness of most of the F_1 imagines, only 8 females from the cross *T*♂♂ × *K*♀♀ were available for a backcross to *K*♂♂. Three of these females died within the first nine days and a single female which appeared gravid was then killed and dissected. It was found to have been fertilized and that its ovaries held eggs which were at late Stage 4 or early Stage 5 (ref. 9). The remaining females, although apparently gravid, produced no eggs in the next seven days, when the attempt ended with their accidental death while *en route* to Johannesburg. The ovaries of newly hatched hybrids appeared small but otherwise seemed normal.

Table 2. Sex-ratios in Hybrid F_1 Generations

Cross (F_1)	Males	Females	$\chi^2_{(1)}$	P
TK	26	82	29·04	$< 0·001$
KT	103	14	67·70	$< 0·001$
TM	8	23	7·29	$< 0·010$
MT	31	22	1·53	$0·25 > P > 0·20$*

* Not significant.

Although little time was devoted to looking for morphological differences between the two forms of *A. gambiae*, the eggs were examined for characters. Table 3 presents data on the length of the eggs of the three colonies as well as of P_1 females of the crosses *K*♂♂ × *T*♀♀ and *T*♂♂ × *K*♀♀. The mean egg-lengths of the *K*, *M* and *T* samples are all significantly different from each other ($P < 0·01$), and the means of the F_1 egg samples of the crosses *K*♂♂ × *T*♀♀ and *T*♂♂ × *K*♀♀ are significantly different from the means of the *K* and *T* samples ($P < 0·05$). This is somewhat surprising as egg characters are generally believed to be characters of the mother. It is possible that the results are due to sampling errors, despite efforts to minimize these.

Table 3. Egg-lengths

	n	x (mm.)	S	$S\bar{x}$	C (per cent)	Range
K	100	0·481	0·0196	0·00196	4·1	0·429–0·525
T	100	0·575	0·0172	0·00172	3·0	0·525–0·627
M	100	0·490	0·0252	0·00252	5·1	0·397–0·531
*KT**	100	0·545	0·0234	0·00234	4·3	0·435–0·589
*TK**	50	0·488	0·0179	0·00253	3·7	0·454–0·531

* Eggs of the P_1♀♀.

Of interest, too, is the fact that means of the Muheza and Kisumu colonies should differ significantly. It may be the case that the eggs of the Kisumu colony are smaller than those of most wild populations as a result of some selective influence acting during the years since it was colonized.

Preliminary work indicates that the F_1 hybrid offspring of crosses between the Tanga and the Kisumu and Muheza colonies were intermediate in their tolerance to high salinities.

It is apparent from the crossing experiments that genetic differences exist between the salt-water-breeding variant and typical *A. gambiae* and that at least some of their F_1 hybrids are sterile, and that the others have often impaired viability when compared with the parental forms. These points, taken together with the facts that the two forms are often sympatric in Nature and that these sympatric associations are stable (as at Dar-es-Salaam[1,2]), suggest very strongly that we are dealing with two distinct biological species[10,11]. The alternative possibilities that we are dealing with two sub-species or two polymorphs of a single species, seem unlikely for these reasons: (*a*) If they were sub-species it would be necessary to demonstrate some extrinsic barrier which prevents or strongly limits gene flow between the two populations. Such a barrier does not seem to exist[2]. (*b*) If they were simply 'polymorphs' maintained, perhaps, by disruptive selection[12], one would not find the, at least partial, sterility among the F_1 offspring, nor their relative inviability.

With regard to the naming of the species, only one point needs to be made here; the name *litoralis* given by Halcrow to the salt-water-breeding variant of *A. gambiae* found on Mauritius is not available as it is preoccupied by *A. litoralis* King (1932).

The work described here was performed during the tenure of a World Health Organization short-term consultantship at the East African Institute of Malaria and Vector-borne Diseases, Amani, Tanganyika. I thank the director, Dr. G. Pringle, and his staff for assistance.

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STIMULATIVE EFFECTS OF ALUMINIUM ON PLANT GROWTH

By C HACKETT

Imperial College of Science and Technology, Field Station, Sunninghill, Ascot

DURING the course of an investigation of the nutrition of a calcifuge, *Deschampsia flexuosa* (L) Trin , experiments were carried out with different levels of aluminium in water-culture solutions The results strongly suggest that aluminium can be of benefit to plant growth The treatments were applied to seeds (Stage 1), to 4–10 day old seedlings (Stage 2), and to established plants 10–12 weeks old (Stage 3)

The basal nutrient had the general composition given in Table 1, but there were some modifications between experiments The *p*H was maintained at 3 5–3 6, which prevented precipitation of aluminium and phosphate The concentration of sulphur present in the solutions is given in Table 2, amounts added as sulphuric acid in *p*H adjustments are included

Table 1 COMPOSITION OF THE BASAL NUTRIENT SOLUTION

Source	Ca	Fe	K	p p m. Mg	Mn	N	P	S
$CaSO_4$ $2H_2O$	10							7 8
KNO_3			11			3 6		
$MgSO_4$ $7H_2O$				3				4 8
$MnSO_4$ $4H_2O$					1			0 6
KH_2PO_4			2				2	
Fe citrate or Fe-EDTA		0 5						
$Al_2(SO_4)_3$ $16H_2O$								

Trace additions (p p m) Cu 0 01 ($CuSO_4$ $5H_2O$) B 0 14 (HBO_3), Zn 0 04 ($ZnSO_4$ $7H_2O$), and Mo 0 005 ($(NH_4)_6Mo_7O_{21}$ $4H_2O$)

Table 2 CONCENTRATION OF SULPHUR AT DIFFERENT ALUMINIUM-LEVELS

p p m Al added	0	2	5	10	25
p p m S present	20	24	29	38	64

The methods, treatments and results can be summarized as follows

Stage 1 Experimental layout 0, 5 and 25 p p m aluminium × 9 replicate trays × 4 spp /tray

The species used for comparison with *Deschampsia* were *Alopecurus pratensis*, *Festuca pratensis* and *Lolium perenne* The seeds were supported on thin, rigid glass-fibre matting over holes in waxed cork sheet, which was then floated on the surface of 1,500 ml of solution in painted baking trays so that the seeds were in direct contact with the free solution The glass-fibre matting had been soaked for three days in portions of the treatment solutions High relative humidity was maintained by covering the trays with polythene sheet and panes of glass The experiment was carried out under laboratory conditions, with temperatures fluctuating between 13 and 23° C *Deschampsia* was weighed after 11 days and the other species after 7 days, the nutrient was replaced on the fifth day Differences in rates of germination did not contribute to the results showing stimulation by aluminium

The stimulative effect of aluminium appeared in the roots It was highly significant for the overall treatment means (Table 3), but there was some difference in response between the species (Table 4)

Stage 2 Experimental layout 0, 2 and 10 p p m aluminium × 0 1, 1 and 8 p p m phosphorus × 5 spp /jar × 2 replicate jars × 3 replicate groups of seedlings per jar *Agrostis tenuis* was added to the species already named

Table 3 EFFECT OF ALUMINIUM ON IMMEDIATE POST-GERMINATION GROWTH

	Root		Shoot	
	Trans-formed means ($\sqrt{100x}$)	Original data mg/plant fresh wt	Trans-formed means ($\sqrt{x}$)	Original data mg/plant fresh wt
p p m Al added				
0	2 18	(0 09)	1 78	(3 4)
5	3 82	(0 17)	1 73	(3 3)
25	1 75	(0 06)	1 53	(2 5)
S D at 0 05 =	0 80			
at 0 01 =	1 08		at 0 01 = 0 11	

Table 4 EFFECT OF ALUMINIUM ON IMMEDIATE POST-GERMINATION ROOT GROWTH

Means from $\sqrt{100}\ x$ transformation
(In brackets—original data—mg/plant—fresh wt)

p p m Al added	*Alopecurus*	*Deschampsia*	*Festuca*	*Lolium*
0	1 3 (0 5)	2 5 (0 7)	1 2 (0 6)	3 8 (1 5)
5	3 3 (1 3)	3 3 (1 1)	3 1 (1 2)	5 5 (3 1)
25	0 8 (0 3)	3 6 (1 4)	0 0 (0 0)	2 6 (0 9)
		S D at 0 01 = 2 0		

The seedlings were supported in groups of 2 or 3 in holes in waxed cork This framework was floated on nutrient solution held in wrapped 350-ml jars The seedlings were grown for 3 weeks under greenhouse conditions, with three changes of solution per week

Again the stimulation appeared only in root growth The mean fresh weight per seedling group is given in Table 5, with shoot weights for comparison There were no aluminium × phosphorus or Spp × aluminium interactions

Table 5 EFFECT OF ALUMINIUM ON THE GROWTH OF 4–10 DAY OLD SEEDLINGS

	Root	(Means per seedling group)	Shoot	
p p m Al added	Transformed means (log x)	Original data mg fresh wt	Transformed means (log x)	Original data mg fresh wt
0	0 93	(11 1)	1 41	(32 3)
2	1 16	(18 9)	1 26	(26 2)
10	0 98	(12 0)	1 17	(18 9)
S D at 0 01 =	0 16		= 0 19	

Stage 3 Experimental layout 0, 2, 5, 10 and 25 p p m aluminium × 3 replicate jars × 3 groups of plants per jar, using *Deschampsia* only

The plants were held in waxed corks in waxed wooden squares which rested on the neck of 2,700-ml wrapped jars The plants were grown for 6 weeks under greenhouse conditions, with the solutions changed every week

Similar trends to those at Stages 1 and 2 were found, but the stimulative effects were not significant The shoots were affected more than the roots

Table 6 EFFECT OF ALUMINIUM ON THE GROWTH OF ESTABLISHED PLANTS OF *Deschampsia*

p p m Al added	Root (mg dry wt /plant group)	Shoot
0	48	177
2	50	208
5	52	181
10	33	148
25	39	168

(Since these experiments, similar effects have been obtained with young plants of oats and rye, barley did not respond)

In a brief review of the elements considered essential in plant nutrition, Hewitt[1] tentatively described aluminium as a potential micronutrient He referred to the number of reports between 1920 and 1945 of beneficial effects of aluminium on plant growth but noted that the reliability of the conclusions had afterwards been questioned by Hutchinson[2] in a detailed review of aluminium and plant growth Since these reviews, work with aluminium has been concentrated on those levels causing toxicity, and it seems possible that some important effects of lower concentrations have been overlooked, effects which could be of physiological and ecological importance

This work was carried out under the general supervision of Dr A J Rutter and with the financial support of the Department of Scientific and Industrial Research

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SOIL DEVELOPMENT AND THE AVAILABILITY OF PHOSPHORUS AND POTASSIUM TO *ASPERGILLUS NIGER*

By Dr B. N ROY

School of Agriculture, Kwame Nkrumah University of Science and Technology, Ghana

THE extent to which the active or kinetic release of minerals to micro-organisms from the various stages of weathering of the soil parent material is not well understood It appears that there is practically no published literature in this respect In the investigation reported here the release of phosphorus and potassium from fresh rock, weathered rock, detritus, lithosol and soil of a basic metamorphic rock (hornblende schist) was studied using the *Aspergillus niger* method[1,2] This method provides a sensitive measure of the amount of phosphorus and potassium available to the organism and should throw a light on the release of these two minerals as the decomposition of the rock advances

The samples representing the sequence of weathering from fresh rock to the soil were collected from the same site, at Creag Liath, Ballater, Scotland, ensuring that all had been subjected to the same soil-forming factors The size fraction (< 2 0 mm) of fresh rock, weathered rock and detritus was prepared by hand grinding in a mortar until all particles passed through a 2-mm sieve This fraction contains a complete size-range of mineral matter similar to soil, namely, coarse sand, fine sand, silt and clay Lithosol and soil were passed through a 2-mm sieve

Table 1 TOTAL PHOSPHORUS AND POTASSIUM CONTENT OF ROCK IN DIFFERENT STAGES OF WEATHERING WITH THE CALCULATED PROPORTIONAL LOSS OF CONSTITUENTS

Constituents	I Fresh rock (per cent)	II Weathered rock (per cent)	III Detritus (per cent)	IV Percentage of each constituent lost*	
				Weathered rock	Detritus
P_1O_5	0 50	0 20	0 10	83 80	93 00
K_1O	2 30	1 60	1 20	64 30	82 70

* Fe_2O_3 used as a constant factor for calculation Calculations for the proportional loss of constituents were carried out according to Merrill[3]

Table 2 PAD WEIGHTS AND THE PHOSPHORUS AND POTASSIUM CONTENT OF THE MYCELIUM OF *A niger* GROWN ON ROCK IN DIFFERENT STAGES OF WEATHERING AND ON SOIL

Sample	Total weights of mycelium from 4 cultures (mgm)		Amount of P_2O_5 and K_2O in 4 pads by analysis* (mgm)		P_2O_5 and K_2O absorbed by *A niger* from 100 gm of sample (mgm)	
	P_4O_5	K_2O	P_2O_5	K_2O	P_2O_5	K_2O
Fresh rock	1,231	814	1 16	0 924	5 80	9 24
Weathered rock	1,067	1,140	1 04	1 202	5 20	12 02
Detritus	1,344	1,148	1 24	1 562	6 20	15 62
Lithosol	1,958	1,841	2 06	2 035	10 30	20 35
Soil	2,278	2,466	2 29	2 634	11 45	26 34

* Total phosphorus and potassium in the mycelial pads were determined according to Mehlich *et al* [6]

Phosphorus and potassium both exhibit a correlation between the amounts of these minerals released and the stage of weathering It will be seen that in spite of a great loss of phosphorus and potassium in the course of weathering, as revealed by rock analysis (Table 1), the release of these two minerals to *A niger* is found to increase with the advancement of decomposition The pad weight in relation to phosphorus (Table 2) diminishes slightly from fresh rock to weathered rock, but again the weight increases significantly on passing from detritus on to the soil The significant release of phosphorus from detritus to soil is of interest in view of the findings of Polynov[4] and Nikiforoff[5], who have concluded that apart from physical and chemical weathering which render minerals available, the effect of pioneer organisms—especially lichens and mosses—in organic weathering cannot be overlooked The presence of these organisms in detritus, lithosol and soil is frequent The increase in pad weight, in relation to potassium (Table 2), is significant throughout The total phosphorus and potassium in the mycelial pads (Table 2) grown on samples in general, parallels the weights of all the pads The release of these two minerals increases in a manner corresponding with the increase of pad weights These determinations, however, tend to emphasize that the amounts of phosphorus released to micro-organisms increases sharply in the later stages of weathering, but for potassium there is a definite gradual trend to release more as the decomposition of the rock advances

This work was carried out at the Department of Soil Science, University of Aberdeen I thank Dr W T H Williamson for his advice

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METABOLISM OF PHENOLIC COMPOUNDS BY YEASTS

By G. HARRIS and R W RICKETTS

Brewing Industry Research Foundation, Nutfield, Surrey

THE ability of several species of bacteria to metabolize phenolic compounds supplied as sole carbon source is well known, and it has been recognized that certain fungi, including species of *Aspergillus*, *Penicillium* and *Neurospora*, likewise bring about some of the reactions involved[1] Strains of the yeast-like fungi, such as *Oospora*, also metabolize phenolic substances[2], including those derived from lignin[3], but less is known of the fate of these materials in yeasts themselves That *Saccharomyces cerevisiae* could be trained to tolerate poisons such as phenol or 'sublimate' and that such training resulted in morphological and physiological changes in the cells was established by Imshenetsky and Perova[4] Moreover, Zimmerman[5] has described the destruction of phenol by four yeasts comprising one strain each of *Saccharomyces cerevisiae*, *Candida tropicalis*, *Rhodotorula glutinis*, and an unidentified 'white yeast' In a survey of the properties of moulds and yeasts from soils and allied habitats, di Menna[6] observed that his strain of *Candida tropicalis* failed to attack the lignin derivatives, including ferulic acid, p-hydroxybenzoic acid and vanillin, but others of these yeasts did so This article describes the preliminary findings of a survey of the ability of a wider range of yeasts from the British National Collection of Yeast Cultures to grow on various phenolic substances (cf Tables 1 and 2)

The initial investigations were made using catechol as, first, this compound is a common intermediate in the bacterial dissimilation of phenols[1] and, secondly, of a number of phenols tested in preliminary trials it supported the most rapid growth of yeasts The medium consisted of a mixture of catechol solution (200 ml , 0 1 per cent) and Wickerham's 'carbon assimilation' medium[7] (20 ml) at ten times its normal concentration Portions (10 ml) were inoculated heavily with the selected yeasts, which had been grown up on malt extract–yeast extract–glucose–peptone medium (MYGP), and the cultures were shaken aerobically at 25° C The amounts of growth, estimated after 3, 7 and 14 days, are given in Table 1 Clearly, ability to grow on catechol under these conditions is limited to relatively few genera, mainly *Candida*, *Debaryomyces*, *Pichia*, *Saccharomyces* and *Torulopsis* and usually to only a few species However, of the seven strains of *Debaryomyces* tested, three actively metabolized catechol in contrast to the strains examined by di Menna[6], and all the strains of *Torulopsis* used grew on the same substance With the latter genus, however, growth was only weak, except in the case of *Torulopsis famata*, which grew as well on catechol as on MYGP The most active yeasts were strains No 405 and 470 of *Candida tropicalis* and strain No 459 of *Debaryomyces subglosus*, which grew strongly within three days Even using these yeasts, however, growth was inhibited when the concentration of catechol was increased above 0 1 per cent and inhibition of the growth of *Debaryomyces subglosus* (459) was complete when this concentration was 0 5 per cent

The substitution of other phenolic compounds (Table 2) for catechol permitted growth in certain instances Generally speaking, the faculty of growing on catechol was paralleled by ability to metabolize

Table 1 GROWTH OF SELECTED YEASTS ON CATECHOL AS SOLE CARBON SOURCE

Yeast			Growth†		
Genus	Species	Numbers*	3 Days	7 Days	14 Days
Brettanomyces	*anomalus*	449	−	−	−
Candida	*humicola*	461	−	−	−
	krusei	42, 43, 45	−	−	−
	lipolytica	376	−	−	−
	mycoderma	116, 178, 327	−	−	−
	parapsilosis	458	−	−	−
	pseudotropicalis	188	−	−	−
	pulcherrima	166, 372	−	−	−
	rugosa	130, 189	−	−	−
	tropicalis	405	+	+	+ +(+ +)
		470	+	+ +	+ +(+ +)
	utilis	168, 359	−	−	−
Cryptococcus	*albidus*	445	−	−	−
	laurentii	136	−	−	−
	terreus	393	−	−	−
Debaryomyces	*hansenii*	9	+ +	+ +	+ +(+ +)
		10, 102, 103	−	−	−
	kloeckeri	8	+	+ +	+ +(+)
	marama	382	−	−	−
	subglosus	459	+ +	+ +	+ +(+)
Endomycopsis	*capsularis*	127	−	−	−
Hansenula	*anomala*	18	−	−	−
	schneggii	375	−	−	−
	suavolens	57	−	−	−
	subpelliculosa	16	−	−	−
Kloeckera	*apiculata*	36	−	−	−
Nadsonia	*elongata*	379	−	−	−
	fulvescens	46	−	−	−
Oospora		403	−	−	−
	lactis	47	−	−	−
Pichia	*fermentans*	105	−	−	−
	membranaefaciens	19	−	[+]	[+]
		169, 334	−	−	−
Rhodotorula	*glutinis*	154G, 377	−	−	−
	minuta	62	−	−	−
	mucilaginosa	158, 159, 195	−	−	−
Saccharomyces	*bisporus*	171	−	−	−
	carlsbergensis	112	−	−	−
	cerevisae	198, 210	−	−	−
		240	−	+	+
	cerevisiae var *ellipsoideus*	96	−	−	−
	delbruckei	147	−	−	−
	fragilis	179	−	−	−
	fructum	410	−	−	−
	mellis	173	−	−	[+](+)
	pastori	175	−	−	[+](+ +)
	pastorianus	392	−	−	−(+ +)
	rouxii	170	−	−	[+](+)
		381	−	−	−
	willianus	99	−	−	[+](+)
Schizosaccharomyces	*pombé*	132	−	−	−
Torulopsis	*datilla*	144	−	−	[+](+)
	etchellsii	407	−	−	[+](+)
	famata	1	[+]	+	+ (+)
	glabrata	350	−	−	[+](+)
	holmii	137	−	−	[+](+)
	magnoliae	38	−	−	[+](+)
	sphaerica	150	−	−	[+](+)
	versatilis	409	−	−	[+]([+])

* Numbers in British National Collection of Yeast Cultures

† − no growth [+] slight growth + good growth + + heavy growth Growth in MYGP is shown in parenthesis for comparison

Table 2 GROWTH OF SELECTED YEASTS ON VARIOUS PHENOLIC COMPOUNDS AS SOLE CARBON SOURCE

Yeast			Phenolic Compound						
Genus	Species	Number*	Catechol	Phenol	Resorcinol	*p*-Hydroxy-benzoic acid	Proto-catechuic	*p*-Cresol	Phloro-glucinol
Candida	*tropicalis*	4	+ + +	+ +	+ +	–	–	–	–
		5	+ + +	+ +	+ +	–	–	–	–
		405	+ + +	+ +	+ +	–	–	–	–
		470	+ + +	+ +	+ +	–	–	–	–
Debaromyces	*hansenii*	9	+ + +	+ +	+ +	–	+	–	–
	subglosus	459	+ + +	+ +	+ +	+ +	+ +	–	–
Pichia	*membranaefaciens*	19	+	+	+	–	–	–	–
Saccharomyces	*cerevisiae*	240	+	+	+	–	–	–	–
	pastori	175	+ + +	–	–	–	–	–	–

* Number in British National Collection of Yeast Cultures – no growth + slow growth + + good growth in 3–7 days + + + rapid growth

both phenol and resorcinol In the case of *Debaryomyces subglosus* (459) it was accompanied by the capacity to utilize *p*-hydroxybenzoic acid and protocatechuic acid However, *Saccharomyces pastori* (175) although able to metabolize catechol slowly, failed to grow on either phenol or resorcinol

The routes by which phenolic compounds are metabolized by bacteria are rapidly becoming clear As indicated here, phenol, for example, is degraded via catechol and then either *o*-quinone, *cis-cis*-muconic acid and β-oxoadipic acid or 2-hydroxymuconic acid and its transformation products, the simple acids finally formed entering the terminal respiratory systems In the present investigation the formation of a quinone from phenol in the presence of *Candida tropicalis* spp was observed but addition of aniline to the media during dissimilation of the other substrates by the yeasts (Table 2) failed to yield quinone anil except from protocatechuic acid The anil was isolated as described by Evans[8] as follows a washed culture of *Debaryomyces subglosus* (459) from MYGP (20 ml) was inoculated into Wickerham's medium[5] (2 l) containing aniline and potassium protocatechuate each at 0 1 per cent concentration and the whole shaken aerobically at 25° C After seven days the mixture of yeast and thick red precipitate was removed, extracted by means of boiling ether and the ethereal extract dried and evaporated Dianilino-*o*-benzoquinone (100 mgm), m p 190°, separated on crystallization of the product from light petroleum (b p 100°–120°) and failed to depress the m p (191°) of authentic material[9]

It is of interest that the oxidation of protocatechuic acid by the bacterium, *Pseudomonas fluorescens*, for example, in presence of aniline fails to yield an anil because cleavage of the aromatic ring of the acid occurs without decarboxylation[10–13], a change occurring also in *Neurospora* spp [14] Possibly, therefore, the production of quinone from protocatechuic acid described here depends on prior decarboxylation of an unstable acidic intermediate The metabolism of protocatechuic acid but not *p*-hydroxybenzoic acid by *Debaryomyces hansenii*[9], in contrast to *Debaryomyces subglosus*, which utilizes both acids, may reflect the failure by the former yeast to adapt to the mono-hydroxy compound under the conditions used, as in bacteria this acid is converted into protocatechuic acid[8,15] Both these findings, together with the inability to detect dianilino-*o*-quinone during the metabolism of the above phenols other than protocatechuic acid, suggest that adaptation experiments as introduced into this field by Stanier[16] may help to decide which oxidative pathways for phenols exist in yeasts

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MODIFICATIONS OF GREEN ALGAE TO BE USED AS FOOD

By D STRIETELMEIER* and ROBERT B KOCH†

Chemistry and Microbiology Branch, Food Division, Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U S Army, Chicago 9

THE most undesirable characteristics of the green alga *Chlorella* as a food are the deep green colour, a bitter taste, and a tough cell wall which inhibits the efficient absorption of nutrients from the cell by non-ruminant animals[1] Green algae have been found to be acceptable, however, if added to food in small concentrations Thus, when less than 10 per cent is incorporated in Japanese and Western style soups, bread, noodles, cakes and ice cream, no significant effect on acceptability is observed As much as 20 per cent has been added to Japanese green tea with equally good results[2]

This article represents one phase in an attempt to develop a high protein product for use as a food extender from a strain of *Chlorella* (Sorokin) with a high-temperature optimum for growth In this work it was postulated that an extraction procedure could be developed which would modify the colour and remove the bitter principle of *Chlorella* Thus, high concentrations of extracted algae could be added to low-protein foods without a significant reduction in the acceptability of the food

Evidence in the literature suggests that methanol extraction would minimize the colour and bitter principle problems[3,4] The literature does not indicate, however, the colour of the extracted algae in precise, mathematical terms The 1931 C I E colour values have been used in the present work to represent the colour of the algae When these values are plotted on the C I E -Munsell system conversion tables, the Munsell notation indicates the colour of the algae as it appears to the human eye

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* Present address Industrial Division, Morton Salt Company, Chicago

† Present address Research Center, Minneapolis-Honeywell Regulator Co , Hopkins, Minnesota

Extraction by methanol has an additional advantage in that recent work using the albino rat has shown that the extraction increased nutrient absorption from 54 5 to 81 7 per cent[4] The extraction also increased the digestibility of protein from 46 2 to 65 5 per cent

Conditions of growth influence the composition of algae[5] Variations in chlorophyll content and cell wall composition could result in corresponding variations in the time required for extraction and the completeness of extraction For this reason, an effort was made to maintain constant conditions for growth and collection

To maintain careful control of the culturing process, the algae were grown in batch culture in Burk's[6] nutrient medium, except for the substitution of urea as a nitrogen source The algae were grown in a 100-l tank and illuminated with fluorescent light The suspension, 0 1–0 8 gm of algae/l, was circulated by pump to create sufficient turbulence for maintaining a uniform suspension and to effect maximum solubilization of carbon dioxide The air supply was enriched with carbon dioxide to a 5 per cent level using a dry-ice converter Collection was accomplished by centrifugation at 25,000 r p m, with a Sharpless-Super centrifuge, resulting in a paste of 30 per cent dry solids Usually, 200–230 gm of algae paste was gathered every two days The supernatant was re-cycled, and fresh nutrients were added to replace that depleted by the algae The quantity of fresh nutrients required depended on the amount of algae gathered

After a wash to remove residual nutrients, a quantity of paste equivalent to 39–40 gm dry solids was blended in a Waring blender in 200 ml of methanol for 30 sec The heat evolved by blending in excess of 1 min appeared to fix pigment and produce a brownish discoloration that could not be extracted by methanol The methanol suspension of algae was poured into a single-strength cellulose extraction thimble of the dimensions 60 mm × 180 mm The thimble was transferred to a Soxhlet, and the latter was fitted to a 3-l boiling flask containing 1 l of methanol The methanol was distilled at the rate of 2 5 l/hr The Soxhlet cycle-time averaged 3 min with the withdrawal of 125 ml of extract at the end of the cycle

The problem of solvent channelling was solved by continuous stirring of the methanol suspension during the extraction period A glass stirring rod with attached propeller was extended through the Soxhlet condenser into the extraction thimble The stirring was accomplished at 200–400 r p m, with the result that the algae were kept in suspension except for a layer of solids on the sides of the thimble It was observed that rate of stirring influenced the rate of extraction When stirring at 100 r p m, 5 hr were required for complete extraction, while only 2 5 hr were required at 200 r p m

Following extraction, the suspension was filtered The methanol-extracted algae had a noticeable green colour In an attempt to remove the last traces of green colour, the extracted algae were suspended in a 3 per cent solution of hydrogen peroxide At room temperature, a more complete decolorization was effected in 18 hr The bleaching time was reduced to 5 hr when a 5 per cent solution of hydrogen peroxide was used The white paste, obtained after centrifugation and washing with distilled water, was freeze-dehydrated to remove residual solvents

Light reflectance measurements were made with a 'Color Eye' tristimulus colorimeter using a white vitrolite standard calibrated against magnesium oxide (Table 1) The tristimulus Y values showed that the bleached methanol extracted was lighter in colour than the methanol extracted algae However when the x and y values of the bleached algae were plotted on the C I E -Munsell system conversion tables, the Munsell notation indicated a slight greenish hue

Table 1 EFFECT OF METHANOL EXTRACTION AND HYDROGEN PEROXIDE ON THE COLOUR AND PROTEIN CONTENT OF A TYPICAL SAMPLE OF *Chlorella* C–32

Treatment of algae	Dry weight (gm)	Crude protein ($N \times 6$ 25) dry basis (per cent)	Colour—1931 C I E values of freeze-dehydrated product		
			Y	x	y
(1) Raw	39	48 0	13 61	0 3478	0 1725
(2) Methanol extracted	27	68 2	63 32	0 3159	0 3569
(3) Methanol extracted, 3 per cent H_2O_2	25	69 0	67 20	0 2973	0 3363

Methanol extraction removed about 12 gm of solids or 31 per cent of the total dry weight of the raw algae (Table 1) The methanol-extracted algae was found to have 0 3 per cent ether-soluble fat Approximately 2 gm of dry solids were recovered in the supernatant from the hydrogen peroxide bleach, accounting for the difference in weight between the methanol extracted and bleached algae (Table 1) The bitter principle present in this strain of algae was found to have been completely removed by the methanol

Table 1 shows an increase in the crude protein content from 48 per cent in the raw to 69 per cent in the bleached methanol-extracted algae Comparable decolorization was obtained by 'solarization' (illumination at very high light intensities), by which the algae are forced into a state of rapid respiration accompanied by the cessation of photosynthesis However, the crude protein content was reduced to only 6 per cent Therefore, if it is important to retain high protein content, methanol extraction is preferred to the 'solarization' method of decolorization

Various methods of cell rupture in green algae were also investigated These included sonic oscillation at 10 kc/s, extended treatment in a Waring blender and a Virtis 45 homogenizer, and grinding in a ball mill These methods were all effective, but required treatment for extended periods of time However, it was found that the methanol extraction process was as effective as these supplemental treatments The 2 5 hr treatment, already described, resulted in as many as ten fractures of the cell membrane per cell (observed under 970 magnification) This may account for the earlier report[4] that methanol extraction increases the absorption of cell constituents to an acceptable level

Methanol extraction effectively removed the bitter taste of *Chlorella*, and also fractured the cell membrane, making the constituents more available for assimilation Methanol extraction with supplemental treatment with hydrogen peroxide decolorized *Chlorella*, resulting in a colour described by

Munsell notation as near white with a slight greenish hue. The methanol-extracted algae were found to be 65 per cent crude protein.

The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

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THE BIOLOGICAL CLOCK AT THE SOUTH POLE

By Prof. K. C. HAMNER, J. C. FINN, jun.,* G. S. SIROHI, T. HOSHIZAKI†
and B. H. CARPENTER

Department of Botany, University of California, Los Angeles

FOR many years biologists have known that plants and animals possess a mechanism—the biological clock—which permits accurate measurement of time. The twenty-fifth Symposium of Quantitative Biology at Cold Spring Harbor[1] was devoted entirely to the subject of biological clocks. Since, in many cases, periodic oscillations in metabolic or physical activities have been shown to persist even when an organism is placed under conditions of constant temperature and constant light or darkness, they have been regarded as manifestations of endogenous rhythms.

Diurnal endogenous rhythms have been designated 'circadian rhythms' because their periods are not exactly 24 hr. in duration. The use of biological clocks in compensating for the Sun's apparent motion has been well demonstrated[1] in experiments which show that bees, birds and many other animals navigate to a particular goal by utilizing the Sun for orientation. It has also been shown that certain birds navigate apparently by 'sighting' on the stars and using their internal clocks much as a ship's captain uses a chronometer[2,3]. Photoperiodic responses of plants and animals appear to be dependent on biological clocks[1]. Organisms which are situated a sufficient distance from the equator are thus afforded an accurate estimate of the calendar day. Hence, biological clocks serve as a key factor in survival of many species.

One of the basic questions raised by students of biological clocks has been whether organisms growing under supposedly constant conditions actually may be receiving information from their environment. F. A. Brown, jun.[4], has pointed out that there are diurnal fluctuations in the air pressure and slight periodic fluctuations in gravity associated with the rotation of the Earth in relation to the Sun and Moon. Brown claims correlation between certain rhythms which he has been measuring and cosmic-ray intensity. However, no one has definitely shown that the clock mechanism present in many organisms, perhaps in all, is guided by any particular factor of the external environment. Since Brown has been the chief proponent of the hypothesis that external factors, over which the experimenter has no control even under so-called constant conditions, are influencing the clock, it seems worth while to quote briefly from two of his recent reviews.

He states: "The characteristics of the rhythmicity in living things of the kind that have just been described led many investigators of the phenomenon many years ago to the conclusion that living things possess within themselves clock mechanisms that would permit them when isolated from all environmental changes which are conceded to be able to influence them, to measure off accurately periods closely corresponding to the length of the solar day and other natural periods. In other words, there was postulated to be operating in living things a completely independent complex of rhythms which parallel in their natural periods the complex known for the external physical environment. The thesis supported in this article, namely, that during the timing of cycle-length in the rhythm in animals and plants in so-called 'constant conditions' the organisms are still continuously receiving from the external environment information about the natural geophysical cycles"[5]. Brown further states, "However, short of the demonstration that biological cycles of the natural geophysical periods can still persist in organisms travelling in space well outside of the Earth's rhythmic field of influence, there is no logical basis on which this hypothesis can be rejected"[6].

It is obviously difficult at present to examine rhythms in organisms travelling in space away from the influence of the Earth. However, there are two places on the Earth, the geographical poles, where all diurnal variables can be essentially eliminated. Organisms could be placed on turntables which, if revolved counter to the Earth's rotation once each 24 hr., would completely eliminate diurnal rotation. In such circumstances it would be inconceivable that organisms could receive any information of a diurnal nature from the external environment, except that which might be accidentally introduced by the experimenters and that which might be associated with the daily 'rotation' of the magnetic pole about the geographical pole. These latter variations are very slight indeed and will be discussed later.

A project which would enable us to conduct appropriate experiments on the functioning of the biological clock at the South Pole was submitted to the National Science Foundation for inclusion in the 1960–61 U.S. Antarctic Research Program. The project was accepted and funds became available in July 1960. Departure was scheduled for Antarctica in November 1960. Special temperature-controlled shipping containers were constructed for transport of organisms from Los Angeles to the South Pole. Only a very

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* Present address: North American Aviation, Inc., Space and Information Systems Division, Downey, California.

† Present address: Space Biology Laboratory, Department of Anatomy, University of California Los Angeles Medical Center.

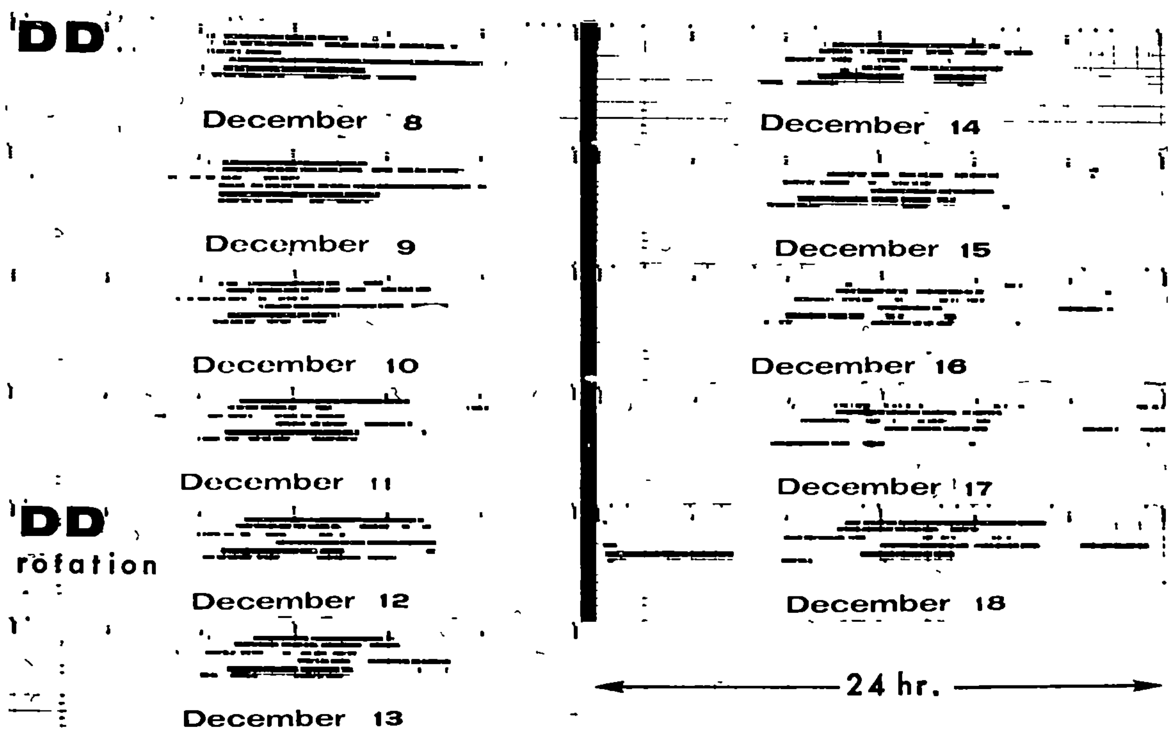

Fig 1 Effect of rotation in constant darkness on the running activity of six different hamsters Prior to December 8, the animals received 12 hr of light during 8 a m –8 p m New Zealand time During the 4 days of the experiment the animals received constant darkness and no rotation The turntables beneath four of the animals were activated on December 12 The activity of the animals is indicated by the vertical pen markings on the chart, which fuse to give a solid band during intensive activity The daily records presented are from noon to noon For example, the December 8 record includes the time-span from noon of December 7 to noon of December 8 All the other calendar days are similarly shown The individual treatments, which were initiated on December 12, are shown in sequence from top to bottom of each daily record Periods of rotation of the animals with respect to the Sun were 24 0, 28 8, 20 0, 24 0, 20 0 and 28 8 hr

limited period of time was available following funding for the design and fabrication of equipment, and the testing of various organisms An arbitrary selection was made of five different manifestations of the biological clock, all of which represent circadian rhythms running activity of the hamster, rhythmic activity of the cockroach, growth rhythms of the fungus *Neurospora*, eclosion of *Drosophila* and leaf movements of bean plants

Hamsters (*Mesocricetus auratus*, Syrian golden hamster) Eight cages with exercise wheels (Geo H Wahmann Manufacturing Co , Baltimore, Md) were each fitted with a plywood box, reinforced with aluminium framework The front of the box consisted of a sliding door which, when closed, made the interior of the box light-tight The plywood box served as a shipping crate and, at the Pole, as an experimental dark room for each cage A box, together with its cage, was mounted on the appropriate turntable for experimentation Each exercise wheel was connected by a reducing gear to a microswitch that sent impulses to a recorder Ten revolutions of the exercise wheel produced a single impulse Each cage was fitted with an automatic watering device and each box was provided with lightproof ventilation Food consisted of apples and a commercial preparation in the form of dried pellets Each of eight aluminium turntables was driven by a synchronous motor through a set of gears which permitted rotation in either direction at speeds equivalent to one revolution in 12 hr , one, two, four, six or eight days

Bean plants (*Phaseolus vulgaris* L , var Scotia) Plastic cups with holes for bottom drainage were used as containers for the vermiculite medium Seeds were planted about 1 in below the surface Cool white fluorescent lamps spaced 2 in. apart and 24 in from the plants provided the only illumination

A record of leaf movements while plants were on the turntables in complete darkness was made in infra-red radiation with a time-lapse camera actuated by a intervalometer Since it was necessary to hold all the plants in the same focal plane, eight turntables were mounted in each of three rows, one row above the other Each turntable was large enough to hold one potted plant Each turntable in a row was attached to one gear train of eight gears so that adjacent turntables rotated in opposite directions Each of the three gear trains was driven by a common shaft The common shaft was driven by a motor through an attached gearbox which permitted all the turntables to be rotated once every 24 hr , and every one, two, four, six or eight days The entire mechanism was housed in an aluminium frame Any of the turntables could be disconnected from the rest of the set to provide stationary controls

Neurospora (*N crassa* 21863), Dr W H Brandt, Oregon State University) Cultures were grown on a thin layer of 'complete' medium in glass tubes about 0 5 in in diameter and about 16 in long, having turned-up ends fitted with cotton stoppers A short layover at McMurdo N A F , the main air base and supply centre for Antarctica, enabled us to provide fresh culture tubes in the laboratory of Dr Wohlschlag In preliminary tests, cultures were exposed to light for about 12 hr after inoculation and then placed in complete darkness Subsequent growth exhibited distinct zonation, one band each 24 hr Zonations were produced by differences in the

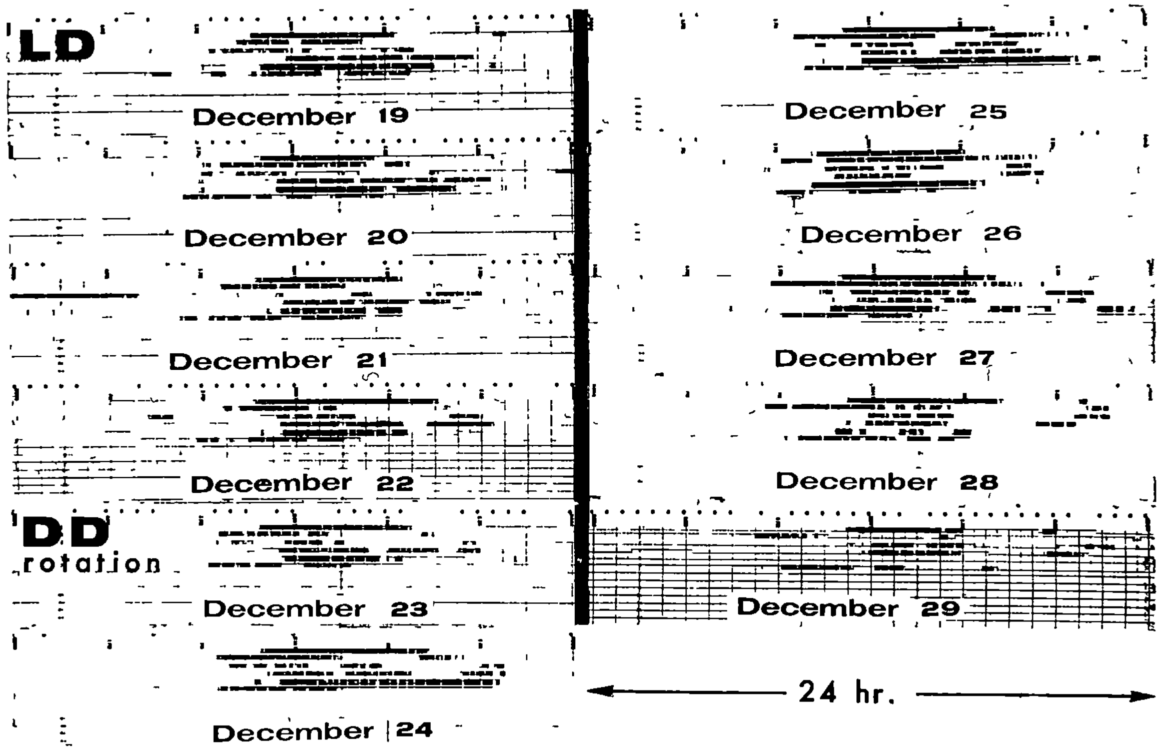

Fig 2 Running activity of six different hamsters The treatments were started on December 23 Prior to that date the animals received light during 8 a m -8 p m The presentation is similar to Fig 1 except the treatment of the animals, which is as follows The six animals, in the order of their activity from top to bottom of the chart, received the following treatments controls 1, 4 and 5 received no rotation, 2, 3 and 6 were rotated once each 24 hr counter to the Earth's rotation

character of growth and colour of the mycelium Hamster turntables were also used for *Neurospora*

Drosophila (*D pseudo-obscura*, Chiricahua-8, Prof Carl C Epling, Department of Botany, University of California, Los Angeles) Cultures were maintained in cotton-stoppered, half-pint milk bottles on a standard media Fresh media were prepared at McMurdo N A F Hamster turntables were also used for this series of experiments

Cockroach (*Periplaneta americana*, Dr I B Tarshis, Department of Entomology, University of California, Los Angeles) Activity was recorded by the same camera system used for recording leaf movement, during quiescent periods the cockroaches were stationary on film and blurred during active periods Cockroaches were maintained in 1-gallon large-mouth glass jars containing hamster food, water and a wire screen for exercise Again, hamster turntables were used

Experiments were conducted in an unused garage about 800 meters from the geographical South Pole Circulation of the air within the building was provided by electric fans Since the floor of the building remained quite cold, all the experimental animals were maintained on tables about 3 ft above the floor Except for one sudden drop in temperature to about 60° F, which was caused by a failure in the heating system, the temperature remained between 68° and 72° F throughout the experimental period

Hamsters The activity of the hamster was adjusted to New Zealand time during a short stay there and during the period of transportation to the Pole by exposing the animals each day to light from 8 a m until 8 p m, New Zealand time One day after arrival at the Pole the animals were placed in experimental cages and recorders were connected Cages were placed in the light-tight boxes and the boxes were mounted one each on a turntable The turntables were not activated in the first four days, during which period the light treatments were continued. The animals began their running activity about 8 p m, were active during the night and inactive during the day On December 8 a large quantity of water and food was placed in each cage, the light-tight boxes were closed The turntables under four of the cages were activated on December 11, while the other two remained inoperative, serving as controls Two cages moved clockwise at the rate of one revolution in six days, the other two moved at the same speed counterclockwise Thus, with respect to the Sun, controls rotated once every 24 hr, two cages rotated once every 20 hr and two cages once every 28 8 hr

A record was made of the activity of the animals for the next eight days Results are shown in Fig 1 During the first five or six days in complete darkness, the animals' activity persisted during that period corresponding to the New Zealand night, whether turntables were rotated or not Toward the end of the experiment, activity of some of the animals became erratic, possibly due to exhaustion of food and water supplies

A second experiment with hamsters was begun immediately on conclusion of the first Some of the animals were replaced, then all were exposed to light from 8 a m until 8 p m, New Zealand time, for four days prior to rotation of the turntables On the fifth day the cages were closed and three of the turntables were activated so that they rotated counter to the Earth's rotation once each 24 hr The other three were inactive and served as controls Results of this second experiment (Fig 2) show that there was no apparent effect of rotation on the rhythmic activity of these animals. As in the previous experiment, the animals' activity became irregular during the last two or three days, although this time food and water were not lacking. The control animals exhibited more erratic activity than the test animals.

Drosophila. Stock cultures were exposed to light each day between 8 a.m. and 8 p.m., New Zealand time, both in New Zealand and also at McMurdo Station. Each experimental bottle was supplied with several mature males and mature females on December 14. The cultures were kept in 12 hr. light from 8 a.m. until 8 p.m., New Zealand time, for 16 days during the period of egg-laying. Cultures were then placed in continuous darkness on December 30. Each of four groups, six bottles per group, were placed in a light-tight box. One group served as a control and remained in a fixed position. Each of the other groups was mounted on a turntable. One turntable was adjusted so as to rotate counter to the Earth's rotation once every 24 hr. Another turntable rotated counter to the Earth's rotation once every six days. A third turntable rotated in the direction of the Earth's rotation once every six days. With respect to the Sun, therefore, the cultures rotated as follows: the control rotated once every 24 hr.; the second group did not rotate; the third experienced one rotation every 28·8 hr.; and the fourth underwent one rotation every 20 hr. Flies began to emerge during the first day of continuous darkness. The flies in individual cultures were removed every 2 hr. in total darkness by shaking the flies down into a receiver flask; flies were then exposed to ether and counted. In every case, cultures were returned precisely to their former position and orientation on the turntable. The results are presented in Fig. 3. Rotation of *Drosophila* on the turntables had no effect on the very well-defined rhythmicity of eclosion.

Neurospora. (Prof. Geo. Meyer, University of Texas, was kind enough to inoculate our experimental culture tubes and also to confirm our observations at the conclusion of the experiments.) After the experimental tubes were inoculated they were exposed to continuous light for 48 hr., during which time the mycelia grew approximately 1 in. in the tubes. A mark was made on each tube indicating the advancing edge of the mycelia. The cultures were divided into four groups of five tubes each. Tubes were wrapped carefully in photographic dark cloth so that the cultures were in complete darkness. One group on a stationary turntable served as the control throughout the experimental period. A second turntable revolved counter to the Earth's rotation once in six days; a third turntable turned counter to the Earth's rotation once in 24 hr.; and a fourth rotated in the direction of the Earth's rotation once every six days.

On inspection of the tubes after seven days, seven distinct zones of growth were noted. There was no difference between the zones of any of the cultures. A photograph of the cultures is shown in Fig. 4. Zonation is not as evident in the photographs as it was to the naked eye because of the film's reduced sensitivity to differences in colour. Rotation of *Neurospora* on turntables did not cause a change in the circadian rhythm of zonation.

Bean plants. Cultures were watered with nutrient solutions prepared from the proprietary mixture 'Spoon-it'. The entire growing area, including the fluorescent lights, was enclosed in a plastic covering in order to increase the normally low relative humidity to approximately 60 per cent at 70° F. The seeds germinated rapidly and plants grew with reasonable vigour. After the plants were about 8 in. high and one tri-foliate leaf was evident, they were placed in a dark room constructed of photographic dark cloth. Relative humidity in the dark room was maintained between 50 and 70 per cent at 70° F. Some of the plants served as stationary controls, while others were rotated either counter to the Earth's rotation or in the direction of the Earth's rotation at various rates. Plants were photographed every 20 sec. in infra-red light for several days until film revealed a reduction in plant vigour. The film clearly shows that the rhythmic leaf movement in all twelve experiments was unaffected despite rotation on the turntables.

An interesting secondary result appeared in these photographs. In the literature there have been

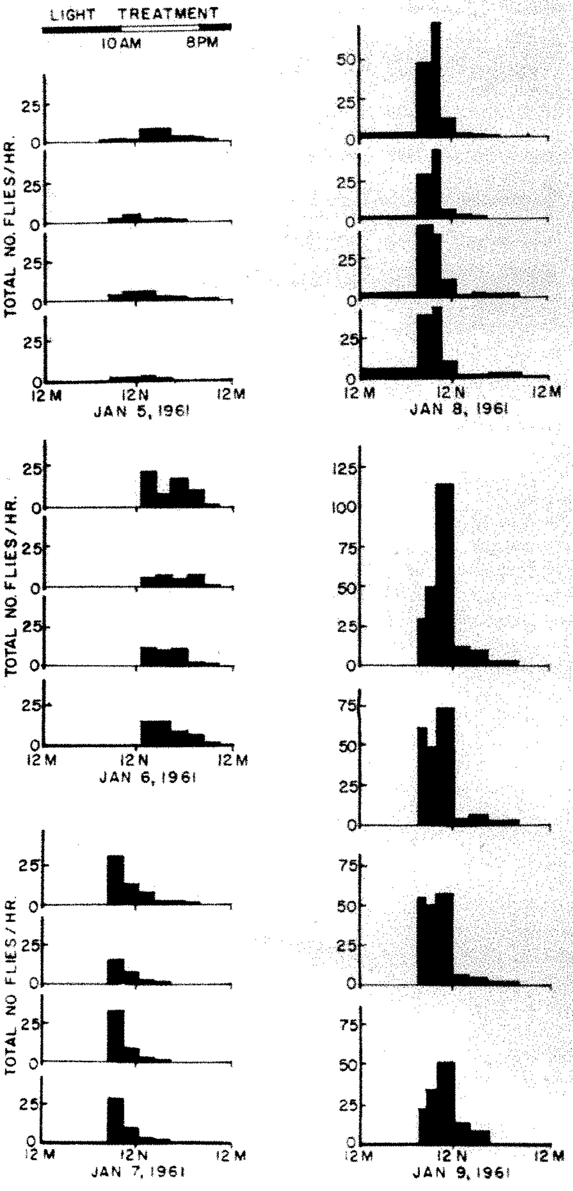

Fig. 3. Effect of rotation on the eclosion rhythms of *Drosophila pseudo-obscura* in constant darkness. The daily record presented is from midnight to midnight. Total number of flies per six cultures of each treatment (ordinate) is shown against time (abscissa) *M*, midnight; *N*, noon

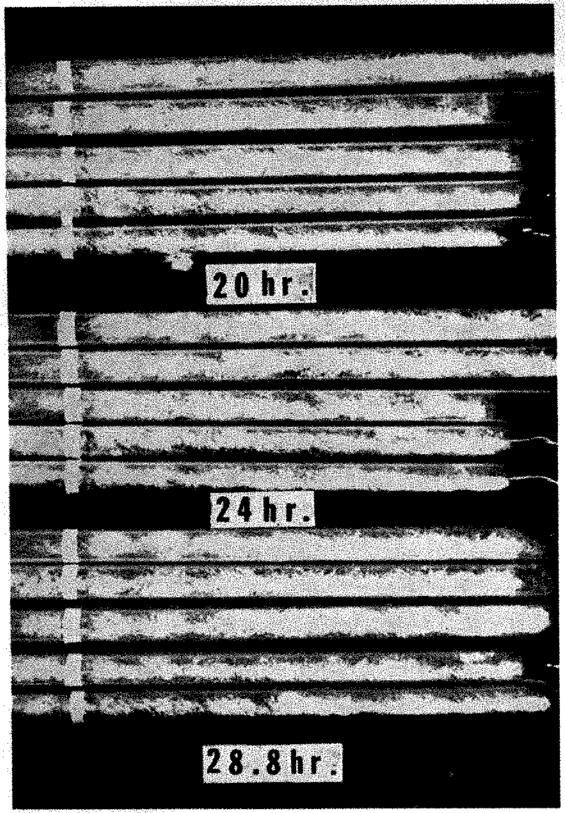

Fig. 4. Effect of rotation on the zonation pattern in the growth of *Neurospora* in constant darkness. The white band on the left-hand side is the marker to denote the extent of growth when the tubes were transferred to total darkness. The tubes were rotated for seven days with respect to the Sun as follows. Top to bottom: (1) Five tubes, once each 20 hr.; (2) five tubes, once each 24 hr.; (3) five tubes, once each 28·8 hr.

claims that the twining of plants in the southern hemisphere is counter to that in the northern hemisphere. All the plants in this experiment exhibited very vigorous counter-clockwise twining, regardless of the direction of turntable rotation or rates of rotation. In one experiment the plants were rotated counter to the Earth's rotation once each 12 hr., which means that with respect to the Sun they were rotated in the opposite direction to the Earth once every 24 hr. In spite of this, twining continued in the same direction and at the same rate as in the control plants.

Cockroaches. Neither the controls nor the cultures on the turntables gave any evidence of rhythmicity in activity. On return to Los Angeles the same strain of cockroaches were found to exhibit no evidence of rhythmic activity. Therefore, the negative results obtained at the Pole station were due entirely to the unfortunate selection of this cockroach strain and hence had nothing to do with the polar location of the experiments.

Prior to the experimental programme at Antarctica, one of three results was anticipated; (1) all external expressions of rhythmicity would cease because the experiments were carried out at the Pole; (2) the rhythms would be altered to new frequencies on the turntables; (3) the diurnal rhythms of none of the organisms would be influenced by either location at the Pole or by rotation on turntables at the Pole. Our data conclusively support the last possibility.

From these results it can be stated that external environmental variables of a diurnal nature associated with the rotation of the Earth have no detectable influence on the basic mechanism, the *Zeitgeber*, of the biological clock. During the period of experimentation there was essentially no diurnal variation in human activity within the building since the continuous light of the summer period permits uninterrupted surface activity at the Pole. It is true that there are slight variations of a diurnal nature in the magnetic field at the South Pole. These are associated with the 'rotation' of the magnetic pole around the geographic pole. On return to Los Angeles several experiments involving *Drosophila*, *Neurospora* and bean plants were conducted to determine the effect of magnetic fields on rhythms. A permanent magnet was placed to one side of a large turntable. As the turntable rotated at speeds comparable with those used at the South Pole, organisms mounted near the periphery were exposed alternately to high (25 gauss) and low (0·68 gauss) intensity magnetic fields. At no time were the circadian rhythms disturbed by changes in the magnetic flux.

It might be argued that with organisms such as the hamster and *Drosophila* the pre-conditioning of the organisms in New Zealand and at the McMurdo Station might have produced a carry-over effect during the experiment at the Pole. However, it is difficult to see how such an argument would hold for movements of plant leaves and for zonation in *Neurospora*. The bean plants were grown from seed at the Pole station, and at no time were the seedlings exposed to diurnal fluctuations in temperature or light. While the *Neurospora* were exposed to diurnal variations during transportation to the Pole, only a small amount of inocculum was used in preparing the experimental culture. Thus, it would seem likely that in the period of growth in the experiment any effect of the previous treatment would have disappeared.

We conclude, therefore, that although it is possible that some external periodic stimulus may regulate the biological clock, it does not arise from any factor associated with the Earth's rotation. We feel that these results give a negative answer to one particular hypothesis concerning regulation of the biological clock by geophysical stimuli. We appreciate the fact that these experiments have not added any information concerning the real nature of the clock other than to make highly improbable this one particular hypothesis concerning regulation of the clock's mechanism.

We thank the U.S. Military Air Transport Service for carrying more than one ton of equipment with dispatch, and without damage, from San Francisco to New Zealand. Likewise, we thank the U.S. Antarctica Research Project in getting our equipment from New Zealand to the South Pole without mishap. The co-operation of U.S. Navy and U.S. Air Force personnel is greatly appreciated. We especially thank Messrs. Edward E. Goodale, George R. Toney, Drs. George H. Meyer and Donald E. Wohlschlag and Lieut. Philip K. Schwartz, U.S.N., without whose support the execution of this work would not have been possible.

This work was supported in part by a grant from the U.S. National Science Foundation and by the U.S. Antarctic Research Project.

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LETTERS TO THE EDITOR

GEOPHYSICS

Unusual Fluctuations of 5577A OI Airglow Emission Intensity on October 28–29, 1961

In a recent article, Kidd, Silverman, Whitney and Aarons[1] reported an unusual inversion of the frequency dependence of radio star scintillations during the magnetic storm of October 28, 1961. It is the purpose of this communication to report on the unusually large fluctuations in the 5577*A* *OI* airglow line intensity during the same period.

The October 28, 1961, event was unusual in that there was no obvious connexion with solar activity[2]. During October several class-2 flares and no class-3 flares were reported. The latest class-2 flare, however, was on October 16. Class-1 flares were reported on October 20 and 27. Some Type III solar radio bursts were reported for October 27 and 28. Magnetic storms were reported on October 26 (1940 *Z*) and October 28 (0812 *Z*) and during this latter storm K_p was as high as 8 and 9. A Forbush decrease is evident on the Deep River neutron monitor cosmic ray counter on October 28.

The airglow data were obtained at Sacramento Peak, New Mexico (geographic lat. 32°43′ N., geomagnetic lat. 41.6° N.), using a birefringent photometer as part of a routine airglow observing programme. Measurements of the green line are normally taken every 10 min. Fig. 1 shows the green line zenith intensity during the night of October 28–29, 1961 (all times are 105° W.M.T.), as well as, for comparison, the more typical night of October 21–22, 1961. It will be noted that the intensity fluctuations for October 28–29 are very marked, and are, in fact, unusual in that no such marked variations have previously been noted. The intensity variations were sufficiently rapid that even during the 36 sec. that the photometer is in the zenith position a variation by as much as a factor of two was observed. In order to be able to make some quantitative comparison of the intensity fluctuations during this night with other nights a fluctuation index equal to $\Delta I/\bar{I}$, where ΔI is the difference between the maximum and minimum values for a given time period and $\bar{I}$ is the mean intensity for this period, was defined, analogous to the scintillation index used in radio star scintillation work. This fluctuation index was calculated for all nights between September and December 1961 for which observations were available, a total of 75 nights. The time-period 2100–2300 hr. was chosen to reduce the effects of the normal diurnal variation on the index and still allow for sufficient experimental points to make the index significant. It was found that the fluctuation index for October 28–29 was at least 1.5 times as high as that for any other night during this four-month period, and greater than most nights by a factor of three to seven, thus emphasizing the unusual nature of the fluctuations for this night.

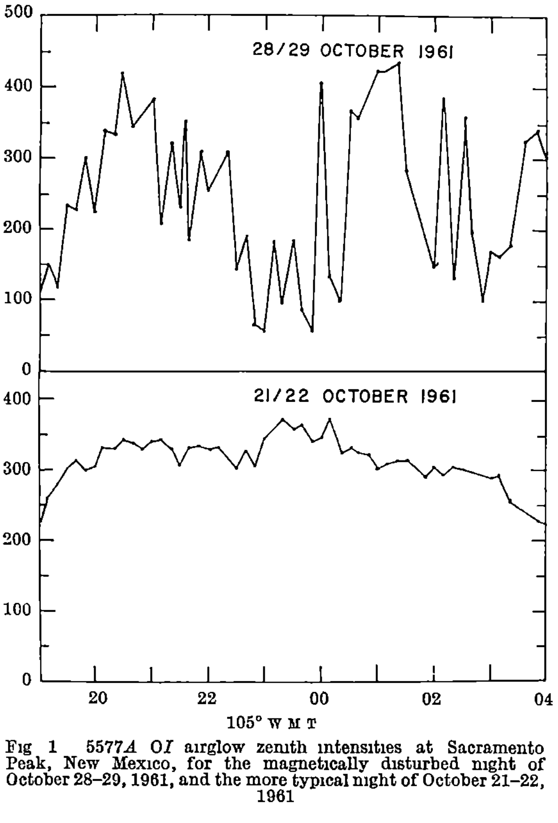

Fig. 1. 5577*A* *OI* airglow zenith intensities at Sacramento Peak, New Mexico, for the magnetically disturbed night of October 28–29, 1961, and the more typical night of October 21–22, 1961

Kidd *et al.*[1] state that localized multiple ionospheric small-scale irregularities of high electron density are responsible for the scintillations. They feel that the irregularities probably start at the *E*-layer and extend to greater heights aligned along the Earth's magnetic field. The airglow data are consistent with a picture of highly localized density variations. Emission of the green line takes place at the 90–100 km. level. The mechanism for production of the normal airglow emission does not involve electrons, but is quite sensitive to the concentration of oxygen atoms, if the Chapman mechanism is valid. Hence, a high degree of inhomogeneity of the atmosphere, as indicated by the radio star scintillation results, would lead to considerable patchiness of emission with resulting marked fluctuations in the zenith intensities, as is observed. The airglow data tend to support a greater than normal inhomogeneity at the lower heights since the 6300*A* *OI* emission, which occurs at a much greater height (of the order of 250–400 km.), showed fluctuations on October 28–29 of a sort which have been observed on a number of magnetically disturbed nights which did not show correspondingly high green line fluctuations. Parenthetically, it may be pointed out that normally for a magnetic storm of the magnitude observed on October 28 the red line shows a definite enhancement to the north[3], corresponding to auroral activity, while on this night no such enhancement was observed.

Examination of the airglow fluctuation index for other nights for which the radio star scintillation inversion effect has been noted (Aarons, J., private communication) shows that the fluctuation index is higher on these nights than on neighbouring nights, indicating that a real correlation exists between the two phenomena It must be pointed out, however, that the airglow fluctuation index, as here defined, may be expected to be sensitive to a number of factors These would include instrumental factors, observing conditions, the normal diurnal variation, patchiness of the airglow, and vertical transport Additional work is now in progress to define the effects of these factors on the fluctuation index and to examine other possible indices which may be more suitable for comparison with other upper atmospheric parameters

In summary, then, we may say that very unusual fluctuations of the 5577*A* *OI* airglow emission intensity were noted on October 28–29, 1961 The phenomenon is consistent with the picture of small-scale irregularities at *E*-region heights obtained from studies of radio star scintillations during the same day A fluctuation index has been defined which enables a semiquantitative comparison of the fluctuations for different days to be made Examination of the fluctuation index for other days on which the radio star scintillation inversion effect was noted shows that a correlation probably exists between the occurrence of the inversion effect and a high airglow fluctuation index

I thank Dr J Aarons for directing my attention to the radio star scintillation investigations and for many helpful discussions Acknowledgments are also due to personnel of Geo-Science, Inc, for the operation of the Sacramento Peak photometer and the scaling of the charts

S M SILVERMAN

Geophysics Research Directorate,
Air Force Cambridge Research Laboratories,
Bedford, Mass

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PHYSICS

Relationship between Viscous Flow and Diffusion

A MOST peculiar paradox in the general area of viscous flow and diffusion is the fact that while the absence of slip between the surface of very large diffusing particles and the surrounding medium is usually taken for granted, at the same time in the case of diffusing particles that are identical as those of the medium, that is, in self-diffusion, it is the presence of slip between the 'diffusing particles' and the 'medium' which is a triviality It amounts to nothing more than the generally accepted fact that in a fluid the molecules are in translatory motion and, hence, move past each other However, in those cases of diffusion where neither of these two extreme situations is encountered the question as to the presence or absence of 'slip' does not always appear to be a trivial one

For the case of highly dilute solutions of very large particles the theory is generally regarded as well founded As, among others, H C Longuet-Higgins[1] pointed out, the Stokes–Einstein relation

$$D\eta = \frac{kT}{6\pi a} \tag{1}$$

is "one of the most soundly based relationships in the whole theory of transport phenomena" As is well known, in the derivation of equation (1) the medium was assumed to adhere to the surface of the diffusing particles and hence the only intrinsic friction coefficient in this equation is that between the solvent molecules

Expressions of essentially the same form as equation (1) have been suggested[2–5] also for the case where the diffusing particles are of commensurable size as the molecules of the medium The fact that none of these relationships proved satisfactory is due, to a large degree, to the failure of these equations to allow for the presence of slip and, hence, friction between the diffusing particles and the molecules of the medium For example, if one expects an expression of the form of equation (1) to hold for a dilute solution of ethanol in benzene, where D is now replaced with the 'activity corrected' mutual diffusion coefficient, $\mathscr{D}_{12}$, and η is the viscosity of pure benzene, this is tantamount to proposing either that the intrinsic friction coefficient between the ethanol and benzene molecules is the same as that between benzene molecules or that there is no slip between the ethanol and the benzene molecules Neither of these propositions seems at all plausible The analogy between the physical situation for which the Stokes–Einstein formula was derived and the case of, say, a non-dilute solution of ethanol in benzene becomes even more remote In such solutions it has been customary to use the viscosity of the solution with either the 'activity corrected' mutual diffusion coefficient[2,3] or the self-diffusion coefficient[4], D_{ii} (i = 1,2), of either constituent One should not be surprised that such relationships did not, in general, behave in a similar fashion as the Stokes–Einstein relation

From the very important treatment by Lamm[6] of the relationship between the diffusion coefficients and certain intrinsic friction coefficients introduced in his theory there follows that, at least for binary mixtures of non-electrolytes consisting of molecules of similar sizes, the following relations hold

$$\mathscr{D}_{12} = \frac{RT}{\varphi_{12} + \varphi_{21}} \tag{2}$$

$$D_{11} = \frac{RT}{\Sigma\varphi_{11} + \varphi_{12}} \tag{3a}$$

and

$$D_{22} = \frac{RT}{\Sigma\varphi_{22} + \varphi_{21}} \tag{3b}$$

Here φ_{12} and φ_{21} is the friction coefficient between molecules of type 1 and 2 per one mole of 1 or 2, respectively, $\Sigma\varphi_{11}$ is the friction coefficient between molecules of type 1 and 1 per two moles of 1, and $\Sigma\varphi_{22}$ is the friction coefficient between molecules of type 2 and 2 per two moles of 2

In the case of no slip between molecules 1 and 2, equations (2) and (3) must evidently be given a modified interpretation However, one would expect the above interpretation to be the correct one in a large number of binary mixtures For these one can calculate the molar friction coefficient η^* in the solution

$$\eta^* = N_1(\varphi_{12} + 1/2\,\Sigma\varphi_{11}) + 1/2\,N_2\,\Sigma\varphi_{22} = \frac{RT}{2}\left(\frac{N_1}{D_{11}} + \frac{N_2}{D_{22}}\right) \tag{4}$$

where equations (2) and (3) have been used

On the other hand, the viscosity of a fluid is the friction coefficient per unit area in the plane of shear multiplied by δ, an average distance between the centres of molecules such that touch each other (or collide with each other), measured in the direction perpendicular to the plane of shear Thus the following relationship has been obtained between η and η^*

$$\frac{\eta}{\eta^*} = \frac{\delta^2}{V_m} \qquad (5)$$

where V_m is the molar volume of the solution Equation (5) via equation (4) is the correct relationship between the viscosity and the diffusion coefficients of mixtures where there is 'slip' between molecules of type 1 and 2 as well as between 1 and 1, or 2 and 2 Equations (4) and (5) apply equally to liquids and gases, to mixtures and pure fluids Their extension to multi-component mixtures appears immediate If applied to pure gases, use of the elementary mean-free-path formulæ for η and D_{11} gives, as expected, the well-known value of 2/3 λ for δ (λ is mean-free-path) However, δ is equally meaningful in the case of liquids as well as gases

Using equations (4) and (5), δ has been calculated for pure liquids and binary solutions where the necessary experimental data are available These include the systems ethyl iodide–butyl iodide, nitromethane–carbontetrachloride, nitromethane–benzene, benzene–bromobenzene, methanol–benzene, ethanol–benzene, and sucrose–water In all the cases investigated the value of δ/r_{av} was found to be between 1 and 1 3 (r_{av} is average molecular radius) at all compositions of the solutions For the truly spherical molecules of argon and mercury this value was 1 24 and 1 27, respectively This result can be shown to be consistent with Bernal's[7] theory of the structure of monatomic liquids where the centres of all neighbouring atoms were assumed to be equidistant The average separation (δ) of planes passing through adjacent atomic centres in such a liquid can indeed be expected to be in the neighbourhood of 1 3 r

The foregoing results indicate identical mechanism of diffusion and viscous flow in the solutions investigated (No such conclusion can be reached for the sucrose–water system which, owing to the great size difference between sucrose and water molecules, probably does not qualify in this treatment) A further important consequence is the fact that, contrary to the widespread view held by many authorities in the field, the 'kinetic units' in such associating solutions as the methanol–benzene and the ethanol–benzene systems are the single molecules The 'anomalous' concentration dependence of $\mathscr{D}_{12}$ in these solutions could be interpreted by realizing that the friction coefficients in equations (2) and (3) are proportional to the areas of contact between the various kinds of molecules φ_{12} and φ_{21} are proportional to the fraction of the surface area of a molecule 1 and 2 that, on the average, is in contact with molecules 2 or 1 respectively $\Sigma\varphi_{11}$ and $\Sigma\varphi_{22}$, on the other hand, are proportional to the fraction of the surface area of a molecule 1 and 2 that, on the average, is in contact with other molecules 1 or 2, respectively Now, in associating solutions these fractions are very different from the random case, resulting in different concentration dependence of the friction coefficients and, hence, the diffusion coefficients

A further consequence of the present results is the fact that there does not seem to exist an exact relationship between the viscosity and the mutual diffusion coefficient in mixtures consisting of molecules of comparable sizes This fact is evident from equations (4) and (5), which do not involve $\mathscr{D}_2$ For regular or ideal solutions one has in a good approximation[4]

$$\mathscr{D}_{12} = N_2 D_{11} + N_1 D_{22} \qquad (6)$$

and this explains the well-known fact that for such solutions $\eta \mathscr{D}_{12}$ varies but little with composition (cf equation (4)) But this obviously constitutes a special case, an exception

F A L DULLIEN

School of Chemical Engineering,
Oklahoma State University,
Stillwater Oklahoma

* Present address Jersey Production Research Co , Tulsa, Oklahoma

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Internal Friction of Glass under High Strain

IT is now known that, if proper care is taken over surface treatment, specimens of certain types of glass can be made which will withstand a strain of at least 5 per cent without failure It is of interest to investigate whether any of the physical properties of glass change when it is subjected to these high values of strain

In this communication, some experiments are described which show that the internal friction of glass increases by a considerable factor when subject to a high torsional bias strain Samples of 3 mm rod of *X*8 glass (a soda-lime glass) were drawn down in the centre so as to form a portion of approximately constant diameter of about 400µ The samples were treated by etching in hydrofluoric acid so that the thin portions would withstand a surface strain of 3–4 per cent Pairs of these samples, chosen to have similar minimum diameters, were then joined together end to end and an inertia disk was attached to the central thick portion of the resulting specimen A torsional bias stress was applied to the specimens by twisting one end relative to the other and then clamping both ends The logarithmic decrement of torsional oscillations of the central inertia disk was measured as a function of the torsional bias strain The observations were made at room temperature, and the frequency of the oscillations was of the order of 1 c /s

In the case of both chilled specimens and specimens which had been heat-treated for 12 hr at 530° C , the logarithmic decrement increased with bias strain An example typical of the results is shown in Fig 1, where the logarithmic decrement is plotted against the maximum value of strain on the specimen, namely, that at the surface of the section of minimum diameter The logarithmic decrement was corrected for air damping by performing an auxiliary experiment with a fused silica fibre and the same inertia disk Thus the corrected value refers only to that part of the damping which is due to internal friction in the *X*8 glass Since the glass is not under homogeneous strain the internal friction as a function of strain cannot be deduced from the results directly.

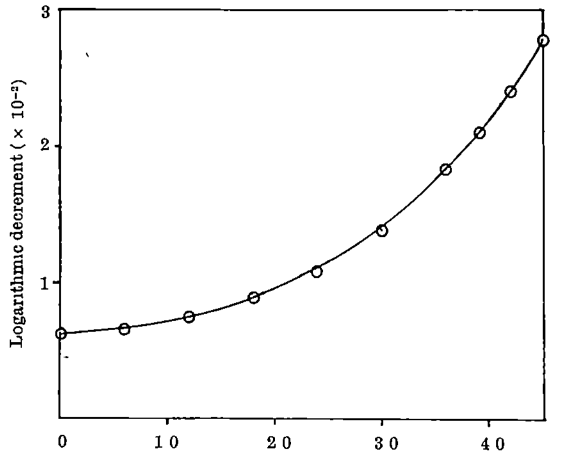

Fig. 1. Logarithmic decrement of torsional oscillations of heat-treated *X8* glass as a function of torsional bias stress

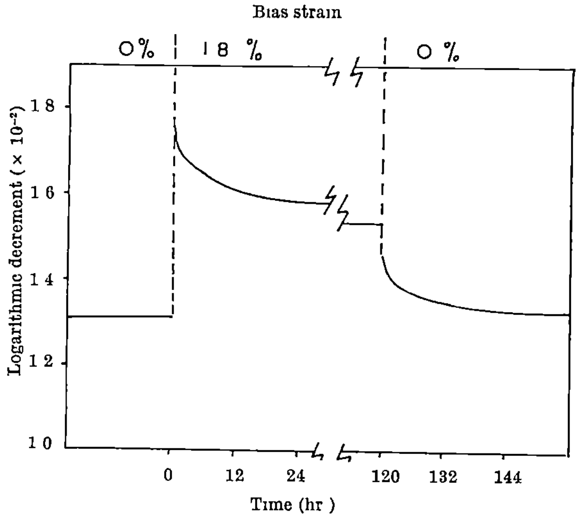

Fig. 2. Variation of logarithmic decrement with time after application and after removal of a torsional bias stress

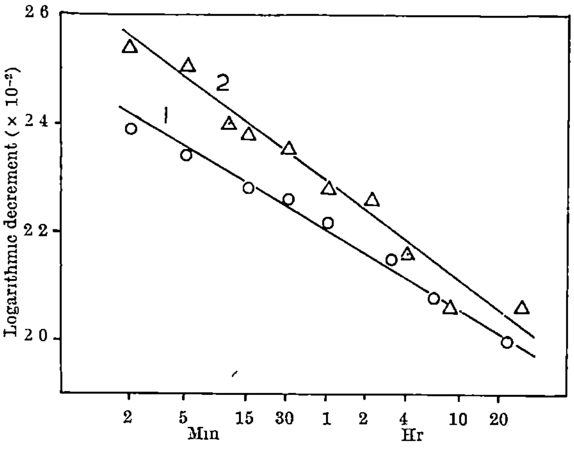

Fig. 3. Variation of logarithmic decrement with time under torsional bias stress. Curve 1, strain 2.3 per cent, clockwise twist, immediately after a period when the specimen was unstrained; curve 2, strain 2.3 per cent, clockwise twist, after 24 hr immediately preceding at 2.3 per cent strain, anticlockwise twist

However, it seems likely that it increases in a similar fashion and by an even larger factor.

The value of logarithmic decrement was also found to depend on previous mechanical history. Some examples of this phenomenon are shown in Figs. 2 and 3. In Fig. 2, it is seen that the value of logarithmic decrement decreases with time after the application of a bias stress. Moreover, if the specimen is later released to zero bias stress, the logarithmic decrement takes up a value which is higher than the initial zero-stress value, and then decreases gradually with time. Fig. 3 shows that the logarithmic decrement under torsional bias stress is higher immediately after a period when the specimen was twisted in the opposite direction than immediately after a period when the specimen was unstrained.

'Pyrex' glass also shows an increase in internal friction with torsional bias strain and shows similar relaxation of the values with time after straining.

Investigations into these phenomena are continuing with a view to determining the actual variation of internal friction with strain and the dependence of this variation on temperature.

D. M. SCHLAPP

Department of Physics,
University of Keele, Staffs.

CRYSTALLOGRAPHY

Preparation of Single Crystals of Cadmium Oxide

ELECTRICAL measurements on cadmium oxide have hitherto been made on thin layers[1], prepared by oxidizing an evaporated cadmium-metal layer or on ceramic samples[2-4]. So far as I know, single crystals have never been made. The preparation of such crystals is described in this communication.

If cadmium oxalate is heated it decomposes in the same way as the more-or-less noble metal oxalates, that is to say, following the reaction

$$CdC_2O_4 \rightarrow Cd + 2CO_2$$

When heating the oxalate in air the cadmium is oxidized immediately after it is formed. When there is no oxygen present, however, cadmium metal is formed.

On this reaction a method was based of growing single crystals of cadmium oxide. The oxalate was placed in a closed alumina vessel, in which a small hole was made. The vessel was placed in an electric furnace, which was heated at about 650° C. During the reaction the temperature was kept constant within 0.1° C. A stream of air was passed over the boat. As soon as the boat is placed in the hot furnace the oxalate starts to decompose, and the carbon dioxide which is liberated drives out the air. Only when the reaction comes to an end can air diffuse through the small hole and react with the cadmium vapour present. It is found that needles of cadmium oxide grow from the inside of the hole. By regulating the temperature needles of maximum 1 mm × 1 mm × 12 mm were obtained, which are very suitable for electrical measurements.

Cadmium oxide has the rock-salt structure. X-ray diagrams revealed that the needle axis is the [111]-direction. For the lattice parameter at 20° C a value of $a = 4.6949$ Å was found.

The stoichiometric composition of the crystals can be influenced by heating them at different temperatures in air or oxygen, or by heating in a closed quartz tube in which a definite cadmium pressure is maintained.

Note added in proof. In a recent paper, Haul and Just[5] mention the preparation of cadmium oxide crystals by gas-phase oxidation of cadmium metal.

S. van Houten

Philips Research Laboratories,
N.V. Philips' Gloeilampenfabrieken,
Eindhoven.

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Selective Epitaxial Deposition of Silicon

Epitaxial layers of silicon have been prepared by the hydrogen reduction of silicon tetrachloride at raised temperatures[1,2]. The rate of deposition of silicon is dependent on the total gas flow-rate, the molecular percentage of silicon tetrachloride in the hydrogen and on the silicon substrate temperature.

In this work the silicon substrates, after being chemically polished in a mixture of nitric, acetic and hydrofluoric acids, were placed on an electrically heated graphite electrode supported in a horizontal quartz tube. The substrates were heated to 1,200° C., and a 0·02 molecular ratio of silicon tetrachloride to hydrogen was used in all experiments. The gas composition was obtained by bubbling the hydrogen through silicon tetrachloride, maintained at 10° C., and further dilution with hydrogen provided the required molecular ratio. Conductivity type and required resistivity value of the epitaxial silicon were controlled by adding suitable quantities of phosphorus trichloride and boron tribromide to the silicon tetrachloride.

Oxide layers of various thicknesses were produced on the silicon substrate surface by heating the samples in an atmosphere of steam at different temperatures[3]. Windows were produced in the oxide layers by masking with photo-resist and etching away the unmasked areas[4]. By this means an oxide mask is produced directly on the substrate surface.

Prior to deposition the oxide-masked substrates were thermally etched in hydrogen for 10 min. at 1,200° C. This procedure removes the thin oxide layer present on the unmasked silicon surface[1], presumably by reducing it to silicon monoxide, which is volatile at 1,200° C.

The silicon substrate temperature is maintained at 1,200° C. and the silicon tetrachloride–hydrogen mole fraction at 0·02, therefore the sole factor affecting the rate of deposition of silicon is the gas flow-rate. Using a gas flow-rate of 4 l./min. a polycrystalline silicon deposit was formed on the oxide layer and a single crystal deposit where the oxide layer had been removed (Fig. 1). The polycrystalline deposit was not satisfactorily removed by ultrasonic etching in hydrofluoric acid, which should attack the underlying oxide layer. Oxide masks up to 30,000 Å. thick were used in these experiments.

On decreasing the gas flow-rate it was found that the amount of polycrystalline silicon on the oxide layer also decreased. At flow-rates below 1 l./min. virtually no polycrystalline deposit is formed on the oxide mask, while epitaxial silicon continues to grow on the unmasked areas.

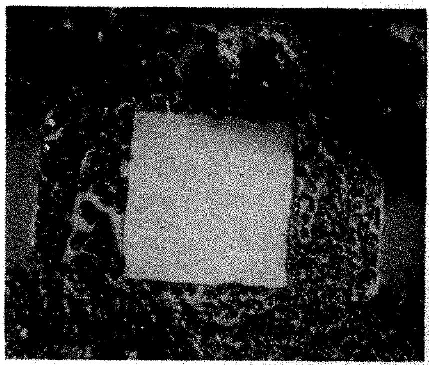

Fig. 1. Single crystal silicon deposit on 0·050 in. × 0·050 in. unmasked area. Polycrystalline deposit on surrounding oxide mask

Examples of these selective depositions are shown in Figs. 2 and 3. The substrate surfaces are perpendicular to the [111] crystal axis. Although the epitaxial deposits are only approximately 5μ thick they are bounded by small facets shown clearly in Fig. 2. These facets are probably characteristic of growth in the [111] direction.

Selective deposition of epitaxial silicon layers has been obtained at low gas flow-rates using oxide masking techniques. Under these conditions the rate of nucleation of polycrystalline silicon has been minimized on the oxide layer, and it appears that the rate of nucleation is greater on the silicon than on the silicon dioxide surface. This may be due, in part, to the fact that the rate of nucleation of silicon on the oxide layer has to compete with the rate of reduction of the oxide surface in a hydrogen atmosphere to form the volatile silicon monoxide. It is very probable that at 1,200° C. any silicon nucleating on the silicon dioxide surface will react with it to form silicon monoxide, according to:

$$Si + SiO_2 = 2\ SiO$$

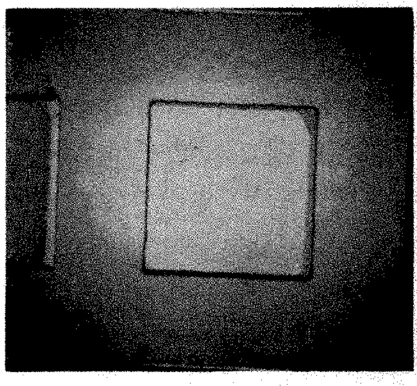

Fig. 2. Selective epitaxial silicon deposit on 0·050 in. × 0·050 in. window in oxide layer

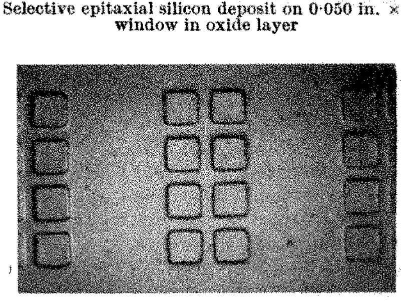

Fig. 3. Selective epitaxial deposits on 0·009 in. × 0·009 in. oxide-layer windows

This latter reaction, however, is not the complete explanation of the masking phenomena. If one assumes that the same quantity of silicon is deposited on the oxide surface as on the silicon surface then, for satisfactory selective deposition, the thickness of single crystal silicon must not exceed the oxide layer thickness. In these experiments it has been possible to deposit selectively silicon layers up to 25μ on oxide layers of thickness 3μ.

The most satisfactory explanation for selective deposition is that, at low flow-rates of gas, the rate of deposition of silicon atoms on a silicon surface is far greater than the rate of nucleation of silicon on a silicon dioxide surface. On increasing the flow-rate, however, the rate of nucleation on the oxide surface will increase considerably and no selective depositions are obtained. This phenomenon is now being investigated. For semiconductor devices this technique offers advantages in that 'mesa'-structures can be produced on a silicon slice to the desired size and at the required place. For multiple devices, leads can be evaporated on to the surface of the oxide and will not come into contact with the bulk of the silicon slice. By a suitable choice of substrate and of epitaxial film resistivities, further heating of the sample will cause diffusion of the impurities so as to bring the junction below the oxide layer, with the consequent advantage of low leakage currents, as is found with planar devices.

We thank Texas Instruments, Ltd., for permission to publish this communication.

B. D. JOYCE
J. A. BALDREY

Research and Development Department,
Texas Instruments, Ltd.,
Bedford, Beds.

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MINERALOGY

Orientation of OH Bonds in Kaolinite

INFRA-RED spectroscopy has been found to be a useful method in establishing the orientation of OH bonds in layer silicates[1]. In micas and related forms of crystallization, the orientation of the OH depends on the occupancy of the octahedral positions. In trioctahedral compositions, the OH bonds are normal to the flakes and these groups are free of association in any supplemental bond (absorption band at 3,700 cm.$^{-1}$). In dioctahedral minerals, the OH are inclined about 20° to the plane of the flakes and directed to the unoccupied octahedra; the absorption band is observed at 3,620 cm.$^{-1}$, which indicates some degree of secondary association.

Previous attempts to establish the orientation of OH groups in kaolinite, with a greater population of OH than the mica substances, were not conclusive[1,2], but data on dickite by Newnham[3] and on kaolinite by Fripiat and Toussaint[4] strongly indicated that oriented specimens could be successfully prepared and analysed. This communication reports a successfully analysed oriented aggregate.

The sample of kaolinite was dispersed in water and after sedimentation the fraction < 1μ was taken. A plate of 'Irrtran-2, disposed horizontally, was immersed in the suspension. After 24 hr., the plate with the sedimented kaolinite particles was removed and allowed to dry at room temperature and afterwards at 100° C.

Fig. 1 presents the infra-red spectra of the oriented kaolinite for different incidence angles. In Fig. 2 the spectrum of an unoriented sample obtained by the potassium bromide disk technique is given for comparison.

The kaolinite shows four absorption bands at 3,695, 3,670, 3,650 and 3,620 cm.$^{-1}$. The band at 3,695 cm.$^{-1}$ presents a marked increase in intensity with increasing angle of incidence and must correspond to OH bonds in a direction near normal to the flakes. These OH's must be almost free; that is, not participating in any significant hydrogen bonding association. However, this band is relatively wide, which suggests that the unassociated OH bonds are of at least two not quite equivalent energies.

The 3,620 cm.$^{-1}$ band, by analogy with the results obtained in micas, must be assigned to OH directed to the unoccupied octahedra. This allocation is substantiated by the observation that the magnesium minerals analogues (serpentines) do not show this absorption (Fig. 3).

The other absorptions at 3,670 and 3,650 cm.$^{-1}$ must correspond to OH which are participating in hydrogen bonding between layers. These bonds will be very weak.

The magnitudes of the absorption coefficients at the four positions where absorption is maximal indicates that free OH with axes near normal to the plane of the flakes comprise about half the total population, bonds directed toward unoccupied octahedra make up only about a fourth, and no more than a fourth

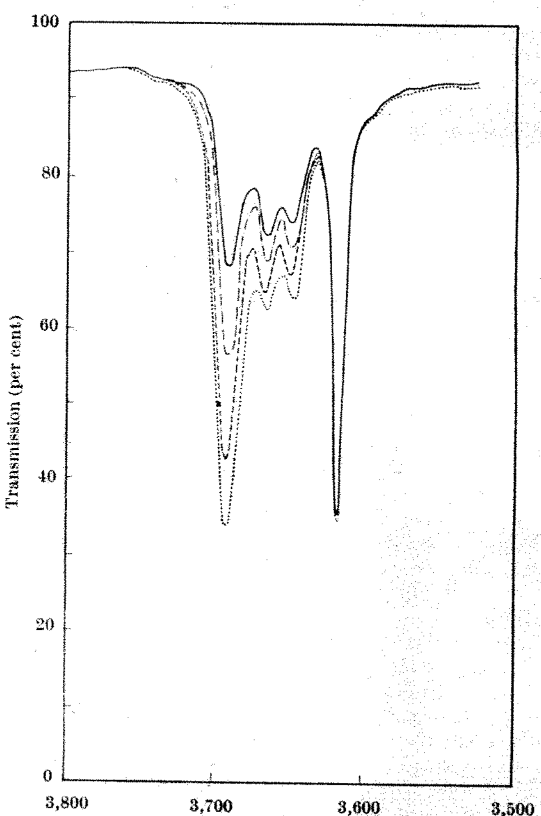

Fig. 1. Infra-red spectra of an oriented sample of kaolinite at various angle of incidence. ——, 0°; —·—, 30°; – – –, 50°; · · · ·, 60°

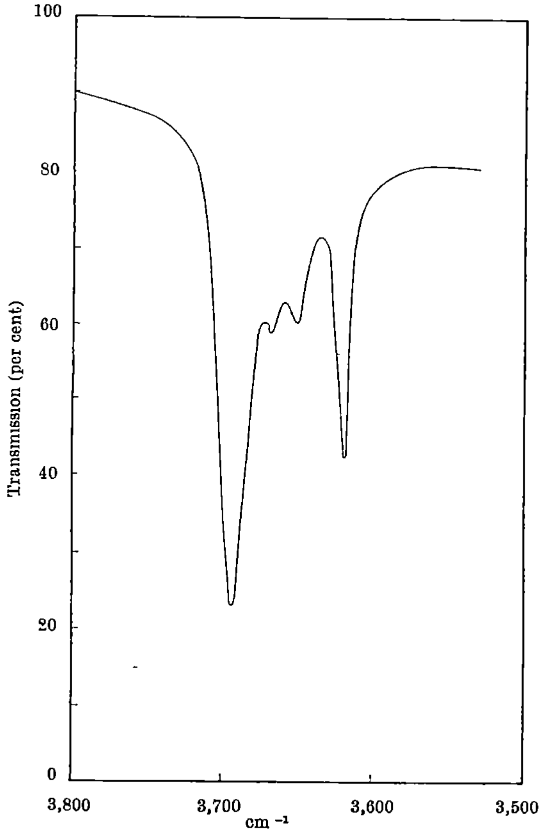

Fig 2. Infra-red spectrum of kaolinite in potassium bromide pellet (unoriented)

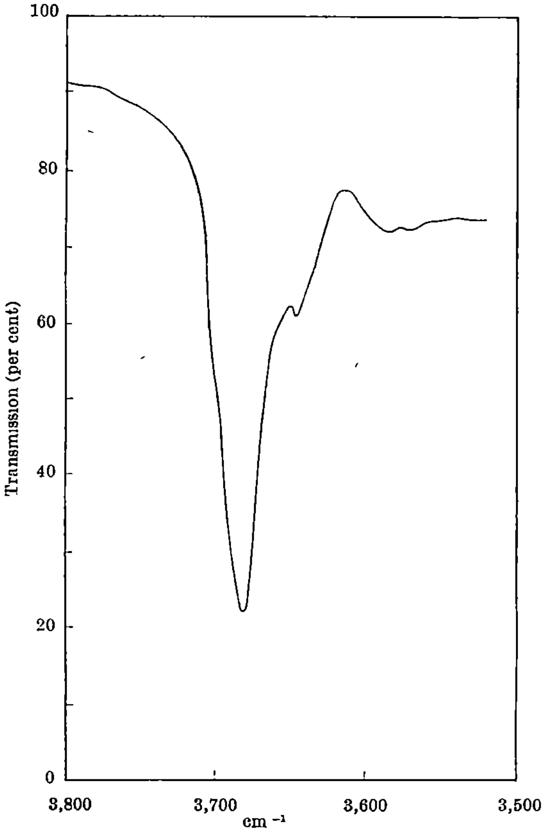

Fig 3. Infra-red spectrum of chrysotile in potassium bromide pellet

can be participating in the weak interlayer hydrogen bonds which are frequently cited as important features in the structure This existence of the two weak maxima presumably indicates that the weak bonds are of two different kinds

The spectra were recorded on a Perkin-Elmer model 221*G* double beam spectrophotometer

We thank Prof W F Bradley, University of Texas, for his valuable suggestions

J M Serratosa
A Hidalgo
J M Vinas

Laboratorio del Transporte y Mecánica del Suelo,
Instituto de Edafologia, and
Instituto de Optica, C S I C ,
Madrid

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RADIATION CHEMISTRY

Radium α-Activity of Cigarette and Pipe-Tobacco Ash

During the course of a series of measurements of the radium content of natural waters and coal in New South Wales[1], we made some analyses of tobacco Two packets of Australian-made cigarettes and one 2 oz tin of pipe tobacco (Australian made, English blend) were taken at random The ash was fused with alkali carbonate the mixture was neutralized and then silica was removed by filtration The filtrate was then analysed for radium by Goldin's radiochemical method[2]

The cigarettes (including paper but excluding filters) and tobacco were ashed over a Meker burner Part of the ash (1 gm) was fused with sodium carbonate (10 gm), the melt was dissolved in distilled water, which had been passed through a mixed ion-exchange bed, and the solution was acidified with hydrochloric acid After evaporation to dryness, and baking, the silica was removed and the filtrate was almost neutralized with ammonia Citric acid was added to the solution and also lead and barium nitrate scavengers Radium isotopes are isomorphously carried with the mixed sulphates obtained by precipitation with dilute sulphuric acid Further purification followed from co-ordination with ammoniacal ethylenediamine tetraacetic acid and preferential decomposition of the barium complex A small residue of barium sulphate, containing radium, was then transferred to a stainless-steel planchette for measurement of its α-activity An argon gas flow-proportional counter of the Sharp Atomic Energy Research Establishment, Harwell, design was used with an efficiency of 25 6 per cent at an operating voltage of 1 05 kV as determined by calibration with 1 mgm natural U_3O_8 (ref 3) Self-absorption corrections were also made from measurements on barium sulphate samples of varying weights The α-activity does not change with time in the same way as can be expected of radium-226 and its daughters and it seems that other α-emitting radioisotopes are present, probably radium-223 and radium-224 (refs 2, 4), although radium-226 is likely to be predominant

Results of the analyses are given in Table 1

We have obtained larger radium activities from analysis of larger samples in the expectation that

Table 1. RADIUM α-ACTIVITY OF TOBACCO

Sample	Total ash (gm.)	Total activity* (c./min.)	Total activity (μμc.)	Activity/gm. ash (μμc.)	Activity per cigarette (μμc.)
Packet *A* (20 cigarettes)	1.75 (8.62)†	5.8 ± 0.02	10.2	5.8	0.5
Packet *B* (20 cigarettes)	1.75 (8.62)	7.2 ± 0.02	12.7	7.3	0.6
Pipe tobacco					
Sample *A* (25 gm.)	2.42 (9.69)	3.8 ± 0.01	6.7	2.7	
Sample *B* (25 gm.)	2.42 (9.69)	4.1 ± 0.01	7.2	3.0	

* Measurements of radium in the reagents gave no α-activity distinguishable from the normal counter background, 0.46 c./min. The total count-rates were constant for one day after the separation of radium on barium sulphate. Ingrowth or decay factors are not included.

† Ash content of tobacco is shown as a percentage in parentheses.

α-pulse analysis will enable us to distinguish the various isotopes present.

We wish to acknowledge the subsidy provided by the Sydney City Council for the provision and operation of the Trace Radioactivity Laboratory, in which this work was done.

E. T. PALLISTER
J. H. GREEN

Department of Nuclear and Radiation Chemistry,
University of New South Wales,
Kensington, Australia.

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CHEMISTRY

Free Electrons with Thermal Energy: their Generation for Chemical Use

FREE electrons with thermal energy react readily with the molecules of many chemical substances, since the free electron may be regarded as the simplest free radical, an unattached valence bond, and indeed as an ultimate chemical particle, this reaction is clearly of interest and importance in chemistry. The lack of a source of uncontaminated free electrons at thermal energy has restricted the observation of free electron reactions to systems where the free electrons were present simultaneously with other reactive species such as free radicals, positive ions, excited atoms and molecules, and ionizing radiation. Some or all of these reactive contaminants are present in the reactions of radiation and electro-chemistry.

This communication describes a simple apparatus for the generation of a flux of uncontaminated thermal energy electrons. The electron source is designed specifically for chemical use and provides an electron flux sufficient to produce reaction products in quantities adequate for chemical measurements.

Primary electrons set free from matter are almost always at a high temperature, this is because their separation requires the application of intense electrical fields, heat or energetic radiation. These relatively severe conditions also tend to liberate other active particles including atoms, radicals, negative and positive atomic and molecular ions. The problem of designing a source of pure thermal electrons is principally that of separating the primary electrons from contaminating particles and then cooling them to normal temperatures. For chemical use it is most convenient to dispense thermal electrons in a flow of inert gas at normal temperature and pressure.

There are two reliable methods of setting free electrons from a gas at N.T.P.: a corona discharge or the application of ionizing radiation. Thermionic or photoelectric electron sources which are frequently used in physical studies are generally unreliable in a chemical environment where the poisoning of the electron-emitting surface can readily occur. The best inert carrier gases for free electrons are the noble gases; in one of these gases the application of a corona discharge or of ionizing radiation produces, as long-lived products, only free electrons and positive atomic ions; these are readily separated from one another by the applied electrical field. Even in the presence of a weak electrical field, however, a mixture of free electrons and noble gas atoms is far from isothermal, since the electrons have little opportunity to lose the energy they gain from the field during their elastic collisions with the relatively massive noble gas atoms. Cooling to thermal equilibrium is achieved by introducing into the noble gas stream, after the primary separation of the electrons, a flow of polyatomic gas such as carbon dioxide or methane; non-elastic collisions between the electrons and either of these gases soon reduces their temperature to thermal equilibrium with the supporting gas mixture.

Fig. 1 illustrates the dimensions and materials of construction of a corona discharge thermal electron source. The corona discharge is maintained at the tip of a No. 0 sewing needle by the application of a negative potential of between 6 and 30 kV. The needle is mounted coaxially inside a polytetrafluorethylene tube of 3 mm. internal diameter through which argon is flowing at a rate of 100 ml./min. Approximately 5 mm. beyond the needle tip the polytetrafluorethylene tube enters a larger tube of the same material (1 cm. internal diameter) into which a gentle stream of carbon dioxide at 1 ml./min. is flowing. After flowing through 2 or more cm. of this larger tubing, the electron stream is allowed to react with the test material. The negative charge conveyed to the test material by the electron stream is led to ground potential either through an inert electrode such as a sheet of platinum foil, or where it is important to avoid the possibility of anode reactions through a well-defined electrode such as silver, silver chloride in 1.0 *M* potassium chloride.

In use the potential required depends on the conductivity of the test material, the electron flux needed and the distance between the needle point and the surface of the test material. Up to a current flow of 30 μamp. a stable corona discharge is easy to maintain. At higher currents up to 200 μamp. care must be taken to ensure that a streamer type discharge does not take place. With such a discharge the test material would be exposed to high-energy electrons and to positive ions.

The detailed results of the use of this apparatus will be described elsewhere; however, a brief account of some of the experiments with the electron source are given as follows to illustrate its potentialities.

Substances such as bromo- or nitro-benzene in the pure dry state have a negligible ohmic conductivity, nevertheless they appear to offer no appreciable resistance to the flow of free thermal electrons. With bromobenzene the reaction with electrons appears to be that of dissociative capture since a stoichiometric yield of bromide ions can be collected from the anode compartment. Alternate intermediates of the Krebs's cycle are known to react vigorously with

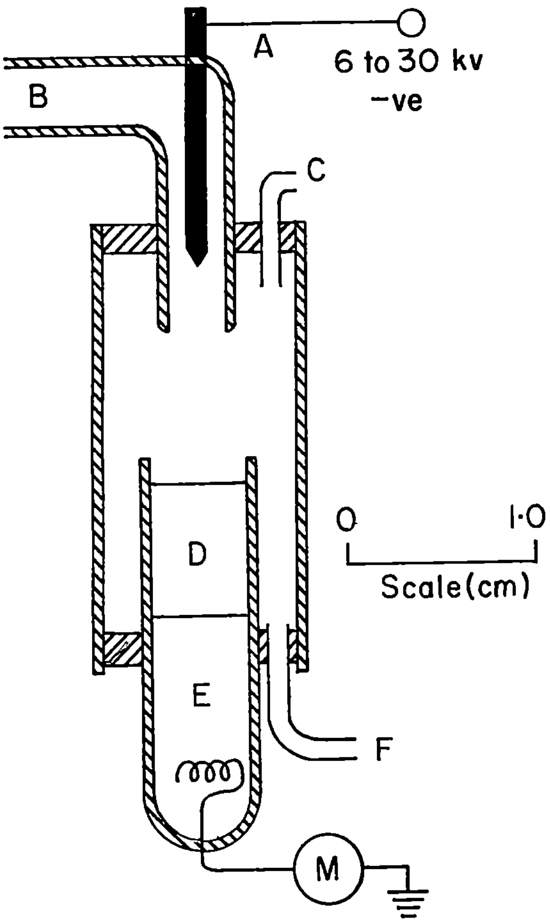

Fig 1 Generator of free electrons with thermal energy The apparatus is constructed from polytetrafluorethylene tubing and the dimensions are indicated by the scale included on the diagram *A*, a No. 0 sewing needle, *B*, inlet for argon gas, *C*, inlet for carbon dioxide, *D*, test material, *E*, 1 0 *M* potassium chloride solution containing a silver, silver chloride electrode, *F*, gas outlet, *M*, 0–100 µamp meter

thermal electrons[1], the determination of the nature and products of this reaction is potentially important to the understanding of the biological process of oxidative metabolism, particularly in connexion with the function, if any, of free electrons in this system A layer of diethyl fumarate was exposed to free electrons in the apparatus illustrated in Fig 1. After several hours the products in the remaining ester layer and in the potassium chloride solution of the electrode compartment were analysed, it was found that, for each electron added, approximately 30 molecules of fumarate were removed and that the principal products were succinate, oxalacetate and malate It is interesting and significant that the injection of free electrons did not lead simply to reduction products, but rather it would appear initiated in the presence of water a radical chain reaction, the products of which were the same as those of the biological process of oxidative metabolism

In addition to reaction studies the apparatus described may also be used for the measurement of electron absorption coefficients and electron affinities in the liquid phase In this type of application it is usually preferable, however, to use soft β-radiation to set free the primary electrons, a corona discharge is normally too 'noisy' for precise measurements

I thank Dr C B Cox, National Institute for Medical Research, London, for his advice This work was begun during a visit to the University of Houston, Texas

J E. Lovelock

College of Medicine,
Baylor University.
Texas Medical Center,
Houston, Texas

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Methylhydroperoxide and the Slow Combustion of Methane

It has generally been assumed that in the uncatalysed slow combustion of methane at below atmospheric pressure (temperature-range ~ 400°–700° C) peroxides, other than hydrogen peroxide, play no part and the reaction between methyl radicals and oxygen is

$$CH_3 + O_2 \rightarrow CH_2O + OH \text{ (ref 1)}$$

We have investigated the oxidation at about 400° C catalysed by the photo-decomposition of acetone With a new quartz vessel (HF-treated) no peroxides were detected in the products However, after the vessel had been in use for about a year they were found to contain very appreciable quantities (~ 10 per cent carbon combusted) of methylhydroperoxide, identified and determined approximately by paper chromatography[2] and vapour-phase chromatography Small amounts were present in the products of a subsequent uncatalysed oxidation, but the hydroperoxide yield fell below the limit of detection after 2–3 experiments, and then was also below this limit in a subsequent photo-catalysed reaction In further catalysed oxidations under standard conditions the yield gradually increased, though it remained small The yield of methanol also rose during this 'conditioning' process (~ 3 times), but in any particular combustion it only became appreciable after the concentration of formaldehyde had built up On addition of formaldehyde to the reactant mixture initially the alcohol was produced immediately

The decomposition of methylhydroperoxide in the vessel at 395° C gave formaldehyde, methanol, water and probably oxygen The half-life was roughly 30 sec when the surface had been 'conditioned' by photo-catalysed oxidations (the CH_2O/CH_3OH ratio in the products being about unity), and 5–10 sec when it had been 'aged' by uncatalysed slow combustions (the CH_2O/CH_3OH ratio being about 4) The half-life of CH_3OOH with respect to homogeneous decomposition must therefore be $\geqslant 30$ sec in between those of hydrogen peroxide and ethyl and higher hydroperoxides at the same temperature[3] This seems not unreasonable

The results indicate that, in these systems at around 400° C , most CH_3O_2 radicals probably abstract hydrogen from formaldehyde, after this has been formed in appreciable amounts, namely

$$CH_3O_2 + CH_2O \rightarrow CH_3OOH + HCO$$

(cf work on the photo-oxidation of azomethane at below 200° C)[4] The hydroperoxide decomposes heterogeneously, namely

$$CH_3OOH \rightarrow CH_2O + H_2O \text{ or } CH_3OH + \tfrac{1}{2}O_2$$

although some methanol may be formed homogeneously, namely

$$CH_3OOH \rightarrow CH_3O + OH$$

$$CH_3O + CH_2O \rightarrow CH_3OH + HCO$$

Thus the mechanism of the slow combustion of methane at relatively low temperatures may be more complex than considered previously[5].

I. P. FISHER
C. F. H. TIPPER

Donnan Laboratories,
University of Liverpool, 7.

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Quantitative Determination of Bilirubin and other Tetrapyrrols

A REAGENT, 3-methyl-2-benzothiazolon-hydrazone hydrochloride (MBTH, supplied by Aldrich Chemicals Co., Inc., Wisconsin), synthesized in 1910 has recently been introduced in quantitative photometry for the determination of aliphatic aldehydes[1], and also for aromatic amines, imino-heteroaromatic compounds, certain azodyes, carbazoles, stilbenes, Schiff bases and pyrrole derivatives[2].

Only the steps involved in the reaction between MBTH and formaldehyde have been settled[1]. First, formaldehyde condenses with MBTH, and secondly, excess MBTH is oxidized by ferric-chloride to a reactive cation which afterwards condenses with the formaldehyde–MBTH complex to a strongly coloured cationic pigment.

Because MBTH reacts with certain pyrrole derivatives, investigations have been carried out to see if this reagent forms highly chromophoric bonds also with bilirubin and other tetrapyrrols. Three different reactions between 'bilirubin' and MBTH have been studied.

(*A*) MBTH reacts with purified bilirubin to give a pigment with a broad maximum at 635 mμ and an extinction coefficient (moles/l./cm.) of 119,000 compared with 60,500 for bilirubin dissolved in chloroform read at 454 mμ, and to 81,000 for bilirubin coupled with diazotized sulphanilic acid read in a strong acid solution at the 570 mμ maximum.

(*B*) Bilirubin is split into two dipyrrols when the tetrapyrrole reacts with diazotized sulphanilic acid. Theoretically, formaldehyde is released when the second dipyrrole couples. However, the excess diazo-reagent necessary to get a complete coupling seems to interfere and formaldehyde could not be detected with MBTH in the distillate.

(*C*) The azo-pigments of bilirubin separated from the reagents by adsorption to alumina oxide react with MBTH to give pigments which have their maximum absorption at 615 mμ with extinction coefficients near 80,000 moles/l./cm.

Oxidation products of bilirubin (biliverdin, urobilin) also react with MBTH to form strongly coloured products. The fraction of oxidized bilirubin present in a solution may, consequently, be determined because bilirubin and its oxidation products react with MBTH, while only bilirubin and mesobilirubin couple with diazotized sulphanilic acid.

Procedure for reaction A. A reaction mixture containing 0·5 ml. of bilirubin in chloroform, 4 ml. of isopropyl-alcohol and 1 ml. of 0·4 per cent MBTH in water is allowed to stand for 5 min at room temperature. The colour turns to blue-green when 1 ml. of 1·3 per cent of ferric chloride in 0·25 *N* hydrochloric acid is added. After 1 hr. the mixture is read in a spectrophotometer at 635 mμ against a blank without bilirubin.

J. FOG

Institute for Medical Biochemistry,
University of Oslo

E. JELLUM

Institute of Aviation Medicine,
Blindern, Oslo

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π-Charge-Transfer Complexes of Hexamethylborazole

BORAZOLE has been described as inorganic benzene. It might be expected[1] that appropriately substituted borazoles would form complexes similar to the well-known π-molecular complexes of benzene and its derivatives[2]. Recently it has been observed[3] that mixtures of hexamethylborazole and tetracyanoethylene in chloroform show a broad featureless absorption band (λ_{max} = 561 mμ) not possessed by either component alone. It was suggested that hexamethylborazole, acting as an electron donor, forms a π-π-charge-transfer complex with the electron acceptor tetracyanoethylene. Other electron acceptors should also form such complexes with hexamethylborazole. Unfortunately complexes with hexamethylborazole are relatively weak: the association constant (K) for the tetracyanoethylene complex is 0·7 l. mole^{-1} in chloroform at 21°, compared with K = 27·6 l. mole^{-1} for the corresponding hexamethylbenzene complex. The only other similar interaction which was reported was with the electron acceptor chloranil. In this case, instability of the complex, and the position of the charge-transfer band which overlapped a transition of chloranil itself, made the definite determination of the charge-transfer band impossible.

We have now observed a broad absorption band in mixtures of hexamethylborazole with 2,3-dicyano-*p*-benzoquinone in chloroform (λ_{max} = 460 mμ), similar mixtures with 2,3-dicyano-5,6-dichloro-*p*-benzoquinone give an absorption with λ_{max} = 487 mμ.

Several workers have demonstrated the empirical linear relationship between the ionization potential (I_p) of the donor molecules and the frequencies of the charge-transfer bands of complexes of electron donors with a given electron acceptor[4]. This result is not immediately apparent from theoretical arguments[5]. Nevertheless the relationship has been shown to give consistent values of I_p for donors from the positions of the absorption maxima of complexes with several different acceptors[6]. Application of such a relationship to a series of complexes of donors of known ionization potential with 2,3-dicyano-*p*-benzoquinone gives I_p = 8·6 eV for hexamethylborazole, similarly from corresponding complexes of 2,3-dicyano-5,6-dichloro-*p*-benzoquinone, I_p = 8·6 eV. The earlier work with the tetracyanoethylene complex yielded a value of I_p = 8·5 eV.

Although it is not claimed that this method gives accurate values of the vertical ionization potential for hexamethylborazole, the consistency of I_p interpolated from λ_{max} for these three complexes of hexamethylborazole at least suggests that the observed optical absorptions are the result of intermolecular charge-transfer complex formation

R Foster

Department of Chemistry,
Queen's College,
University of St Andrews,
Dundee

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Reaction of Fluoro-2,4-dinitrobenzene with Amino-Acids and Peptides

THE reaction of fluoro-2,4-dinitrobenzene with the terminal amino-group of peptides and proteins under mild alkaline conditions has been widely used for the quantitative assay of *N*-terminal amino-acids[1] In the corresponding reaction with amino-acids, it has been shown[2] that some 2,4-dinitrophenyl-peptide is formed, the proportion of the DNP-peptide increasing with the separation of the amino- and carboxyl-functions

A peptide linkage is readily formed between an *N*-substituted amino-acid DNP-ester and an amino-acid[3] and this mechanism could be responsible for DNP-polymer formation through the intermediate DNP-amino-acid DNP-ester

The bimolecular nucleophilic substitution of fluorine by a carboxylate ion should be related to the basicity of the anion present in the alkaline solution[4] The nucleophilic power of the attacking reagent would then increase with increasing value of pK_1 for the substituted amino-acid The reaction-rate is also influenced by the ion-pairing tendency of carboxylate ion in the substrate, following the same order of accelerating power as in the above sequence

O-dinitrophenylation would be more likely to occur in the presence of an excess of fluoro-2,4-dinitrobenzene than when an excess of amino-acid is present Increasing yields of DNP-amino-acid have been obtained by raising the concentration of amino-acid in the mixture[5], or raising the reaction temperature and evaporating the mixture to dryness[2] Once the DNP-amino-acid DNP-ester is formed, the reaction of this 'mixed anhydride' with a free amino-group would occur in competition with the reaction of fluoro-2,4-dinitrobenzene at the same amino-group

The intermediate DNP-amino-acid DNP-ester has now been isolated by paper chromatography of the products of reaction between fluoro-2,4-dinitrobenzene and β-alanine The products from a typical reaction are (R_F values in brackets), ethyl 2,4-dinitrophenyl ether (0 95), *bis*-2,4-dinitrophenyl ether (0·73), 2,4-dinitrophenyl-β-alanine 2,4 dinitrophenyl ester (0·63), 2,4-dinitrophenol (0 53), 2,4-dinitroaniline (0 46), 2,4-dinitrophenyl-β-alanine (0·26), 2,4-dinitrophenyl-β-alanyl-β-alanine (0 06), and 2,4-dinitrophenyl-di-β-alanyl-β-alanine (0 01) The solvent system was 95 per cent propanol, 95 per cent acetic acid and petroleum ether (b p 100–120°), (20 6 100 v/v), moving on Whatman No 1 paper buffered with 0 1 *M* citric acid Elution of the 'active ester' and subsequent reaction with β-alanine gave β-alanyl-β-alanine and 2,4-dinitrophenol A similar intermediate phosphate DNP-ester has been isolated, in high yield, from the reaction between a monophosphate ester and fluoro-2,4-dinitrobenzene in the presence of pyridine[6] This base must be excluded from reactions involving amino-acids, due to the concurrent ring opening of the intermediate pyridinium fluoride in the presence of a primary amino-group

The reaction of fluoro-2,4-dinitrobenzene with a series of ω-amino-acids, including aspartic acid and glutamic acid, also produced considerable amounts of DNP-polymers In the case of glutamic acid the identity of the polymers as γ-glutamyl peptides has been shown by comparison with authentic material prepared by the phthaloylglutamic anhydride procedure[7]. The corresponding α-glutamyl compounds were made from the acid chloride of phthaloylglutamic acid γ-methyl ester

In related reactions with glycine, glycylglycine, and diglycylglycine, the proportion of dimer was only significant with the latter compound, although the formation of DNP-glycylglycine from DNP-glycine has been reported[2] Observations on higher molecular weight starting material are rendered difficult by the low mobility of the DNP-polymers on chromatograms The results of these experiments are shown in Table 1

Table 1 DNP-POLYMERS FORMED BY REACTION OF MONOMER WITH FLUORO-2,4-DINITROBENZENE

Monomer	pK_1	No of monomer units in polymer 2	3	4	5
Glycine	2 34	trace	–	–	–
β-Alanine	3 60	+	+	+	trace
ε-Aminocaproic acid	4 43	+	+	+	+
Glutamic acid	2 19				
	4 25	+	+	trace	–
Aspartic acid	1 88				
	3 65	+	+	trace	–
Glycylglycine	3 12	+	trace	?	?
Diglycylglycine	3 26	+	?	?	?

Reaction of an excess of fluoro-2,4-dinitrobenzene with glutamic acid at *p*H 8 5 and 40°, washing with hexane followed by further reaction with glutamic acid, gave substantially increased yields of the DNP-γ-dimer with little of the higher polymers The free dipeptide was obtained by fast hydrogenolysis using a large excess of platinum catalyst, followed by extraction from the *m*-phenylenediamine produced[8] This reaction is now being applied to the synthesis of peptides containing aminoalkylphosphonic acid residues

BRIAN L TONGE

College of Technology,
Plymouth

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Solid-State Polymerization of Phosphonitrilic Chloride

SINCE the work of Mesrobian *et al* [1] on the polymerization of acrylamide, many monomers have been polymerized in the solid state by γ-rays or electron beam[2–4] Hayashi and Okamura[5] have recently irradiated cyclic monomers and found that the rate of polymerization increases up to the melting point of

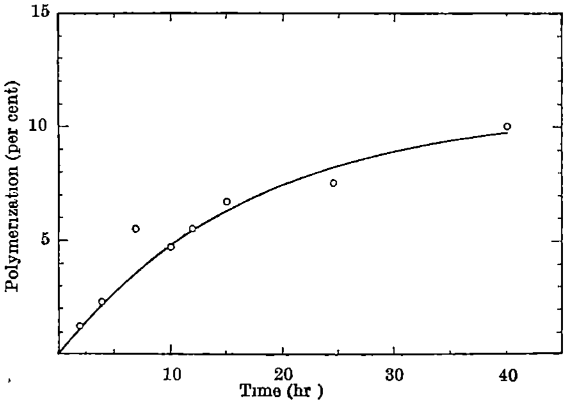

Fig 1 Polymerization of phosphonitrilic chloride at 100° C, induced by X-rays (dose-rate, 1 2 Mr /hr.)

the monomers and that polymerization occurs only in the solid state

Phosphonitrilic chloride has been polymerized thermally with and without initiator at temperatures above 200° C from the melt[6–9] Below this temperature the rate of polymerization is of a few per cent conversion per day An attempt to polymerize this monomer in the solid state by γ-radiation has failed to give any significant amount of polymer up to 10^6–10^8 rads[10]

More recently[11], phosphonitrilic chloride has been irradiated by 4 MeV electrons with doses of 10^9 rads at −70°, 25° and 120° C *in vacuo* In spite of the high dose, only a small amount (1–2 per cent) of an insoluble product was obtained under these conditions

We have now found that phosphonitrilic chloride does polymerize in the solid state when irradiated by 50-kV X-rays The rate of polymerization increases with temperature and has a maximum near the melting point (114° C) Irradiation has been carried out on freshly sublimed phosphonitrilic chloride (Albright and Wilson, Ltd) at a dose-rate of 1 2 Mr /hr The samples after irradiation have been washed thoroughly with petroleum ether to remove any unpolymerized material and the white product thus obtained has the appearance of the elastomer which is obtained thermally

Polymerization conversion at a given dose-rate increases with time of irradiation up to a certain value of the yield where a saturation phenomenon occurs as shown in Fig 1

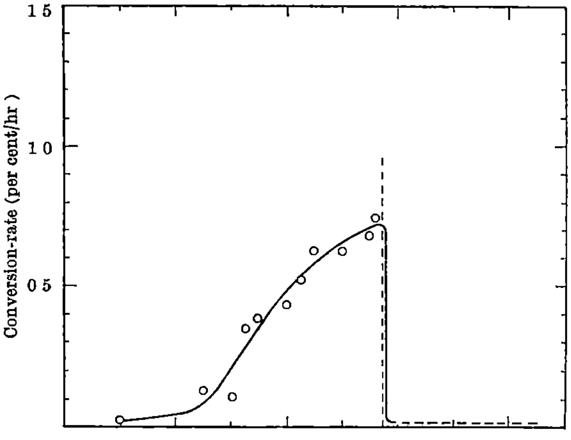

Fig 2 Effect of temperature on the polymerization of phosphonitrilic chloride (dose-rate, 1 2 Mr /hr , irradiation time, 10 hr)

The polymerization-rate is influenced by the presence of oxygen, the purity of the material and the size of the crystals

The increase of reaction-rate with temperature seems to be a common feature of the polymerization of cyclic monomers in the solid state As may be seen in Fig 2, the rate of polymerization reaches a maximum and then decreases almost to zero above the melting point This behaviour has been observed for cyclic siloxane[12] and for some organic monomers[5]

A mechanism which may interpret this behaviour has not yet been worked out It should explain if chains propagate by ions or free radicals and therefore if polymerization is somehow related to the species which may be more or less active near the melting point The drop of the polymerization-rate with fusion could thus be provoked by the fact that the active species may have a longer life-time in the solid state, being the recombination inhibited in these conditions

The structure of the solid may be, however, the determining factor for the polymerizability of the monomer An increase of temperature may, for example, allow favourable positions for the monomer molecules to grow

An investigation of the structure of the polymer in the solid monomer may very likely provide more information about the whole process An examination study of the X-ray rotation diagrams on a single crystal in various stages of polymerization is in progress in this laboratory

V CAGLIOTI
D CORDISCHI
A MELE

Istituto di Chimica Generale ed Inorganica,
Università di Roma
Laboratorio di Chimica delle Radiazioni e
Chimica Nucleare del C N E N ,
Roma, Italy

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BIOPHYSICS

Time-dependence of the Radical State in an X-irradiated Keratin*

THE importance of biological solids as model substances for radiation reactions has grown considerably since the *milieu*-dependence of some radiation effects in solids has been demonstrated[1–5]. In this connexion the electron spin resonance investigation of X-irradiated keratin offers two additional advantages First it gives a broad characteristic signal, which is probably due to a sulphur-containing radical[6]. Therefore it should be possible to follow a single reaction in the solid, independently of other reactions which possibly may occur The second advantage is also based on the fact that the keratins contain a large amount of sulphur[7] Therefore it seems possible to obtain some information about radiation protection mechanisms from radiation experiments on keratins

Dried keratins (4 days over phosphorus pentoxide at 45° C , 6 per cent weight reduction) were irradiated with 2 8 Mrads (half-value layer 10 mm water) for 7 min. and the effect afterwards investigated in a laboratory-built X-band spectrometer[8] at different temperatures of the reflexion cavity. The cavity permanently contained a single crystal of copper sulphate, adjusted for maximum g-value. This permitted an intensity calibration of the spectrometer[9-11]

We found, as already mentioned in a preceding paper[12], a slow increase of the intensity in the low-frequency part of the keratin spectrum (Fig 1) up to saturation values of 130–150 per cent of the initial intensity (Fig 2) The ratio of A^x/B^x, which is a measure for the line shape, remains nearly constant during the observation time of 80 min

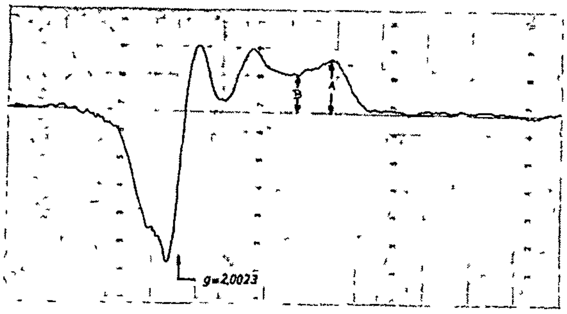

Fig 1 Electron spin resonance of X-irradiated feather quills at 9,523 mc /sec , 13 min after irradiation with 2 8 Mrads, dose-rate, 0 4 Mrad/min The transient line at the position of the free spin value is still present

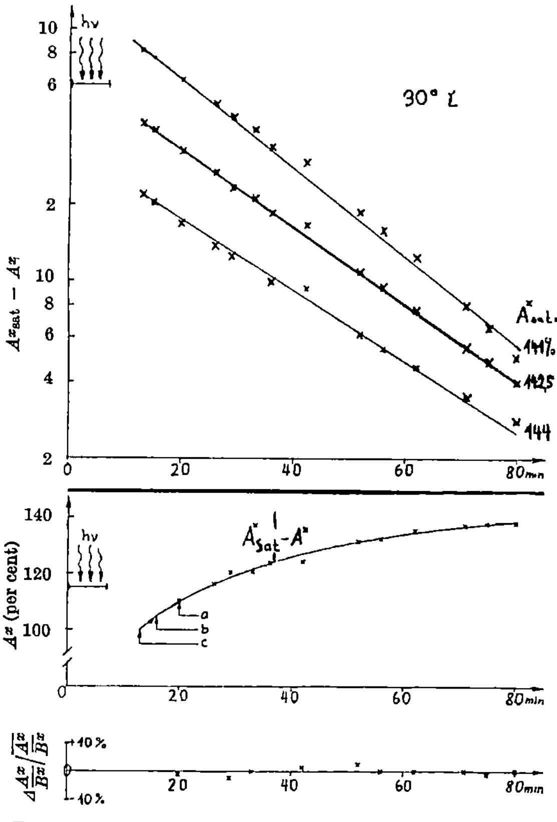

Fig 2 Time behaviour of the sensitivity-corrected value A^x of the amplitude A (cf Fig 1)

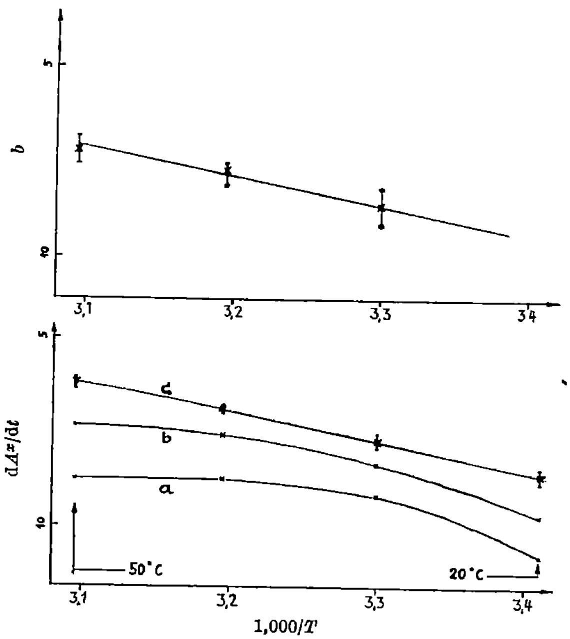

Fig 3 Rate constant b and reaction-rate dA^x/dt at three different times a, b, c versus reciprocal temperature

If A^x denotes the amplitude, A, in the keratin spectrum (Fig 1) related to the spectrometer sensitivity, we found at a first approximation the relation

$$\ln\,(A^x_{\text{sat}} - A^x(t)) = a - bt \qquad (1)$$

For different values of the saturation amplitude A^x_{sat}, this relation is shown in Fig 2 Here a saturation value of 142 5 per cent gives the best match with the experimental points The rate constant b is exponentially related to the cavity temperature T

$$b = b_0 . \exp\left(-\frac{\Delta E}{2kT}\right) \qquad (2)$$

Fig 3, upper part, shows the relation $b(1/T)$ From this the activation energy was shown to be ΔE = 0 64 eV After equation (1) with A^x_{sat} = constant, the rate constant can also be calculated from the reaction rate dA/dt at the time t (Figs 2 and 3) at which the sample was inserted into the heated cavity The activation energy here was ΔE = 0 65 eV , which agrees fairly well with the values stated However, the accuracy of measurement, confined by the small temperature interval investigated, does not allow for exclusion of bimolecular parts of the reaction The observed increase of intensity with time in this intensity could only be found with fresh feather quills, which were sufficiently dried Both influences will be discussed in further investigations

From the constancy of the line-shape parameter A^x/B^x (Fig 2) during the time of measurement, we conclude that there is a slow increase in the concentration of a certain radical Possibly the radical state remains in a fixed position, while the reaction-rate b is determined by solid-state diffusion of gas molecules or lattice defects More probably (cf ref 11), however, it seems that there is a radical state migration to the cystine part of the keratin From experiments in a high vacuum we hope to obtain further information The magnitude of the effect (+30 – 50 per cent) does not explain the absence of the spectra of other amino-acid residues in the keratin In addition, the observed value of ΔE does not correspond to the forbidden gap, which has been calculated for the energy-band model of the protein for a special structure[14] and expanded to other structures[15] Since it was possible to measure dependence on temperature of a transfer process at room temperature we conclude that this radiation reaction is to be defined as indirect Possibly similar indirect actions of radiation may also occur in other dry systems

B RAJEWSKY
A REDHARDT

Max Plank-Institut fur Biophysik,
Frankfurt am Main

* On the occasion of the twenty-fifth anniversary of the Max Planck-Institut für Biophysik

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Structure of *T*-2 Bacteriophage

X-RAY scattering from orientated phages[1,2] togethei with optical measurements[3,4] have shown that the primary orientation of deoxyribonucleic acid (DNA) in the bacteriophage *T*-2 is parallel to the main axis of the phage These data, combined with electron microscopy by Rubenstein[5], and low-energy electron inactivation investigations by Cole[6], enable the construction of a detailed model for the arrangement of DNA in phage head

The model is based on the following experimental observations

(*a*) Cairns[7] reported that the DNA of *T*–2 is 520,000 Å long, as measured by autoradiography Thomas and Berns[8] obtained a value of 550,000 Å by independent methods

(*b*) From the *T*-2 head, Rubenstein[5] extracted DNA with most of its organization still preserved Electron microscopy of this DNA showed a 'bulge' with approximately 80 Å cross-striations The bulge in the DNA was partially uncoiled as a 75 ± 6 Å filament The end of the uncoiled filament showed a stepwise decreasing diameter (Table 1, col 1) The uncoiling of the bulge was from two ends, one from the surface of the bulge, the other from the centre of the bulge The second one corresponded to the end of the bulge located at the tail side of the phage head

(*c*) Slow electron inactivation studies of *T*-2 by Cole[6] showed a central insensitive region in the phage head This is interpreted as representing a DNA free region or a hole

Table 1 COMPARISON OF *T*-2 DNA FILAMENT DIAMETERS

Measured diameters (ref 5) (Å)	Calculated diameter of secondary helix	*n*
27 ± 5	22 7	(DNA molecule)
41 ± 6	44 3	2
57 ± 7	55 1 65 9	3 4
75 ± 6	76 7	5

(*d*) Flow birefringence studies of *T*-2 and *T*-4 bacteriophages by Gellert[4,9] led him to conclude that, for optical effects, about 9–16 per cent of DNA is preferentially aligned parallel to the main axis of the phage Ultra-violet dichroism confirmed the birefringence results[4]

(*e*) X-ray scattering[1,2] of *T*-2 showed preferential alignment of DNA along the main axis of the phage

Model of DNA arrangement in the head of T-2 bacteriophage Combining the foregoing information, we have arrived at the following model The DNA molecule, of length 520,000 Å (ref 7) and diameter 22 7 Å (ref 10), is coiled into a secondary helix This is done in such a way that each turn of the secondary helix will be a multiple of the 34 Å repeat unit of DNA Such coiling allows interdigitation of neighbouring loops and permits the greatest stability The secondary helix will then have a pitch of 20 Å To fit the 80 Å striations observed by Rubenstein[5], a secondary helix loop will consist of five 34 Å repeat units This will give an outer diameter of 76 7 Å for the secondary helix which is 61,000 Å long (Fig 1)

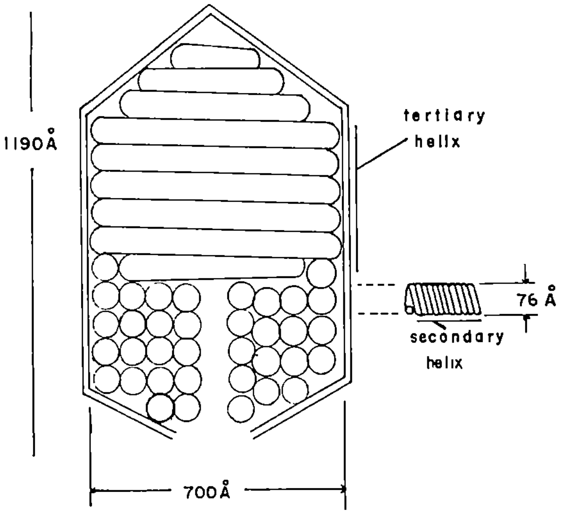

Fig 1

As the next step, the secondary helix is wound into a 'tertiary' helix in the fashion that wire is wound up on a spool Four layers of such tertiary helix are arranged in the phage head (Fig 1) The pitch of the tertiary helix is 76 7 Å , which is equal to the diameter of the secondary helix A phage head, 1190 Å long and 700 Å wide[11], could accommodate a secondary helix 60,000 Å long There would then be an 80 Å. hole in the centre, in agreement with slow electron inactivation studies

The secondary helix ridges on the surface of the final coiling will give the striations on the DNA bulge as observed by Rubenstein[5] The stepwise decreasing diameter of the uncoiled filament is explained by the stretching of the secondary helix so that the helix will have a smaller number (*n*) of DNA repeat units of 34 Å per individual loop (see Table 1 for comparison) Such organization of the DNA would presumably be formed in a fashion as proposed by Kellenberger and

Sechaud[12], according to their 'condensation principle' They showed experimentally that the phage DNA was organized in the infected cell before the assembly of the protein coat[12,13]

Some consequences of the model (1) This arrangement of DNA will give an X-ray diagram with the preferential DNA pattern along the main axis of the phage[1,2]

(2) Detailed calculations of the optical birefringence[14] of the proposed model agree with the quantitative measurements[4]

(3) This model has the additional advantage that the DNA molecule can be easily uncoiled starting with the innermost layer at the tail entrance The only motion required is that the segment already unravelled would be free to rotate around its own axis This requirement is needed since, as the molecule unwinds from the centre of the innermost tertiary helix, two rotations are to be performed (*a*) a 360° turn for the uncoiling of each loop of the secondary helix, (*b*) a 360° turn for each loop of the tertiary helix The hole in the tail of the phage is adequate to accommodate the passage of the 22 7 Å diameter DNA molecule

The most reliable data at present available were used to construct the model, but the exact dimensions must still be considered as approximate

This work was supported by a research grant (*E*–3399) from the U S Public Health Service.

R Kilkson
M F Maestre

Biophysics Department,
Yale University,
New Haven, Conn

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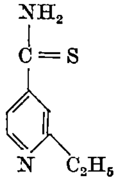
BIOCHEMISTRY

Identification of a Metabolite of the Antituberculous Drug Ethionamide

Ethionamide (2-ethyl-4-thiocarbamoylpyridine) (I) is a drug which has been introduced recently for the treatment of tuberculosis and which is undergoing widespread clinical trials

The polarographic method for the determination of concentrations of ethionamide in biological fluids is the simplest and most sensitive method available[1] It was noted that, in both human and rabbit serum, in addition to the ethionamide wave, a further polarographic wave was obtained, the half-wave potential of which was about 200 mV more positive than that of ethionamide This wave was ascribed to a metabolite of unknown nature Recently, a sample of the compound (II)[2]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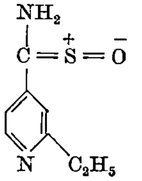
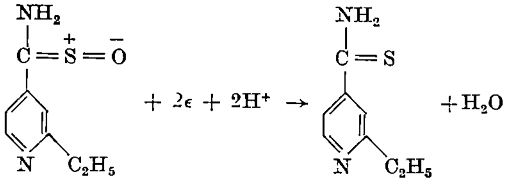

(II)

hereinafter referred to as ethionamide sulphoxide, was made available to us under the reference 1405*Th* by the Société Theraplix, Paris Its polarographic properties were investigated both in aqueous solution and in normal horse serum These properties, with those of ethionamide in the same media for comparison, are given in Table 1

Ethionamide sulphoxide gave two waves with a separation of half-wave potentials of about 200 mV , and with wave-heights in the ratio 1 2 The half-wave potential of the second wave of ethionamide sulphoxide was so close to that of the single wave of ethionamide that, when both were present in solution, these waves coalesced At equimolar concentrations the heights of the ethionamide wave and the second ethionamide sulphoxide wave were very similar Since it is known that the ethionamide wave is a 4-electron/molecule reduction[3], it follows that the sulphoxide is yielding 2- and 4-electron/molecule waves It seems likely that the first sulphoxide wave is attributable to the reaction



Although the half-wave potential of the first wave of the sulphoxide was very similar to that of the unknown metabolite, this fact by itself was not considered a positive identification It was felt, however, that, if the metabolite could be separated from ethionamide, a positive identification from polarographic properties of the two waves could be made

Partition coefficients of the two compounds between horse serum and ether measured by a polarographic method gave these results

Ethionamide K (ether/serum) = 2 7

Ethionamide sulphoxide K (ether/serum) = 0 093, and from these measured values it was calculated that if a sample of serum containing both substances were extracted four times with an equal volume of ether, the concentrations left in the serum would be

Table 1 Polarographic Properties of Ethionamide and Ethionamide Sulphoxide

Medium	Compound	Concentration	Wave-heights (μamp)			Half-wave potentials (V vs S C E)		
			Wave (1)	Wave (2)	Ratio	Wave (1)	Wave (2)	ΔE
*p*H 9 buffer	Ethionamide	0 40 m*M*	—	6 8	—	—	0 99	—
*p*H 9 buffer	Ethionamide sulphoxide	0 40 m*M*	3 0	5 9	2 0	0 74	0 99	0 25
*p*H 9 buffer	Both	0 40 m*M*	3 1	12 7	—	0 76	1 00	0 24
Horse serum	Ethionamide	0 04 m*M* = 6 6 μgm /ml	—	0 45	—	—	0 94	—
Horse serum	Ethionamide sulphoxide	0 04 m*M* = 7 2 μgm /ml	0 20	0 46	2 3	0 71	0 92	0 21
Horse serum	Both	0 04 m*M*	0 20	0 86	—	0 72	0 92	0 20

0 5 per cent for ethionamide and 70 per cent for ethionamide sulphoxide of the amounts originally present A serum sample taken from a rabbit 90 min after oral administration of 400 mgm /kgm of ethionamide was examined polarographically before and after four-fold ether extraction, the results being as in Table 2.

Table 2 POLAROGRAPHIC PROPERTIES OF A SAMPLE OF RABBIT SERUM TAKEN 90 MIN AFTER ORAL ADMINISTRATION OF 400 MGM /KGM ETHIONAMIDE

Sample	Wave-heights (μamp)			Difference of half-wave potentials (V)*
	Wave (1)	Wave (2)	Ratio	
Before ether extraction	0 97	7 3	—	0 21
After 4-fold ether extraction	0 65	1 6	2 5	0 17

* Owing to the small volume of serum the polarograms had to be recorded against a silver wire internal anode In these circumstances, absolute values of half-wave potential are not significant although the differences are significant

It is obvious from Table 2 that the polarographic properties of the metabolite, after 99 5 per cent of the ethionamide had been separated, are virtually identical with those of ethionamide sulphoxide in horse serum, slight differences being attributable to pH differences or the presence of dissolved ether It is also noteworthy that from the values of the height of wave (1) before and after extraction, 67 per cent of the metabolite was left in the serum after extraction, compared with 70 per cent required for ethionamide sulphoxide

This treble characterization of the metabolite, namely, ratio of wave-heights, difference of half-wave potentials and partition coefficient, makes its identification as ethionamide sulphoxide certain

The fact that this metabolite gives a polarographic wave having a half-wave potential identical with that of ethionamide means that concentrations of ethionamide in serum and cerebrospinal fluid as measured by the polarographic method will be incorrectly high whenever this metabolite is present[4] The extent of the error can be calculated from the known polarographic properties of the metabolite On results published to date, this error has not exceeded 50 per cent of the reported 'ethionamide' concentrations

P. O KANE

Research Laboratories,
May and Baker Ltd.,
Dagenham, Essex

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Inactivation of Staphylococcal Penicillinase by Reaction with Resistant Penicillins

WHILE studying the kinetics of penicillinase inactivation of various penicillins, it became evident that a penicillinase-susceptible penicillin, such as benzylpenicillin, behaved differently from resistant penicillins, such as methicillin (2,6-dimethoxyphenyl penicillin) or oxacillin (5-methyl-3-phenyl-4-isoxazolyl penicillin) (The trade name of Bristol Laboratories, Division of Bristol Myers Co , for methicillin is 'Staphcillin' and for oxacillin is 'Prostaphlin') The former was decomposed at a constant rate for the greater part of the reaction while the rate of decomposition of the resistant penicillins progressively decreased and only a small fraction of the penicillin was finally destroyed under the conditions of this experiment

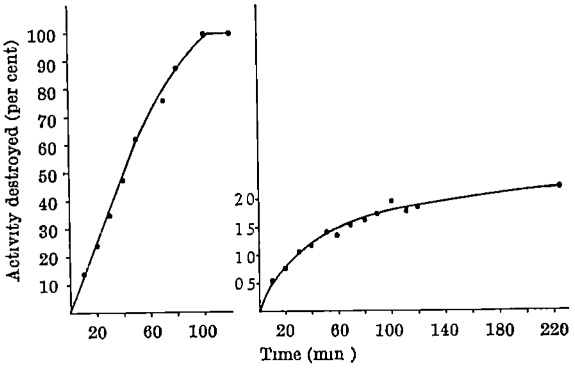

Fig 1 Destruction of different penicillins by staphylococcal penicillinase. 0 06 M penicillin was reacted with 100 μgm /ml. staphylococcal penicillinase in 0 25 M phosphate buffer at pH 6 5 Left, Benzylpenicillin, right, methicillin Note different scales for percentage of destruction

This phenomenon was investigated using Tucker's iodometric method[1] to determine penicillin decomposition The staphylococcal penicillinase used in this work was prepared using the method of Gilson and Parker[2] and methicillin-induced *Staphylococcus aureus* cells (M-2 strain, kindly supplied by Dr Steinman) Fig 1 illustrates the different pattern of behaviour of benzylpenicillin and methicillin already described

It was at first thought that the results may be explained by inhibition of the reaction by the reaction products This hypothesis was tested by degrading methicillin with penicillinase, extracting the penicilloic acid thus formed and adding it to the reaction mixture No effect was observed The penicilloic acid of methicillin did not influence the degradation

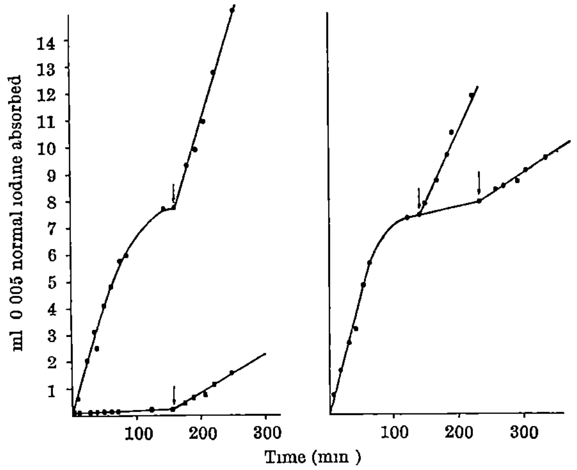

Fig 2 Effect of different penicillins on penicillinase Left, 0 06 M solution of benzylpenicillin or methicillin were incubated with 100 μgm /ml staphylococcal penicillinase The amount of decomposition was measured by iodometric titration At the times indicated by the arrows, the reaction mixtures were diluted ten-fold with 0 01 M solution of benzylpenicillin, and the incubation and titration continued The amount of iodine absorbed is reported per ml of final solution ●, Benzylpenicillin, ■, methicillin Right, a mixture of 0 06 M benzylpenicillin and 0 06 M methicillin was incubated with 100 μgm /ml. staphylococcal penicillinase, and the decomposition followed by iodometric titration At times indicated by the arrows, aliquot portions were diluted ten-fold with 0 01 M benzylpenicillin and incubation and titration continued Note that (a) the decomposition of the mixture followed benzylpenicillin pattern until complete destruction of this compound, then it followed the methicillin pattern, (b) the reaction with benzylpenicillin (until the first arrow) did not appreciably affect the enzyme, (c) the reaction of penicillinase with methicillin (after destruction of benzylpenicillin—between the two arrows) inactivated the enzyme in a manner similar to the experiment with methicillin alone

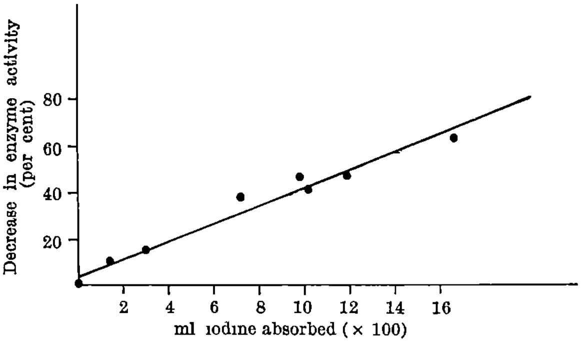

Fig 3 Relationship between penicillinase activity and the amount of methicillin destroyed

of either benzylpenicillin or methicillin when added at the same molarity as the penicillins (0 01 *M*)

Another possibility that could account for the effect is that the enzyme is inactivated in the course of reaction with the resistant substrates This hypothesis was tested in the following way

Equivalent amounts of benzylpenicillin and methicillin and also a mixture of the two were treated with equal amounts of staphylococcal penicillinase The course of the reaction was followed by iodometric titration After some time, the reaction mixtures were diluted 1 10 with a solution of benzylpenicillin in 0 25 *M* phosphate buffer *p*H 6 5 and the course of the reaction again followed iodometrically As benzylpenicillin is 200 times more susceptible to decomposition than methicillin[3], the rate at which it decomposes after being added to the reaction mixture is indicative of the amount of non-inactivated enzyme

The results of this experiment are indicated in Fig 2 It is apparent from this figure that the decomposition of benzylpenicillin does not affect the enzyme since the decomposition of the second portion of benzylpenicillin proceeds at essentially the same rate as that of the first In contrast to this, contact of the enzyme with methicillin markedly reduces the activity of the enzyme That these effects are not due to a competitive inhibition of the enzyme is shown by the behaviour of the mixtures of the substrates The benzylpenicillin component is decomposed at the same rate as if it were the only penicillin present, and its decomposition does not change the activity of the enzyme When all benzylpenicillin is degraded, the methicillin begins to affect the enzyme and the reaction-rate decreases Similar results were obtained when oxacillin was used in place of methicillin

In order to determine whether the degree of enzyme inactivation is a direct function of the amount of the resistant penicillin destroyed, the following experiment was conducted methicillin was incubated with penicillinase under the same conditions as in previous experiments, and at various times samples were taken to determine the amount of methicillin decomposed and the degree of inactivation of the enzyme The latter was determined by measuring the rate of decomposition of benzylpenicillin In Fig 3 is plotted the decrease of enzyme activity against the amount of methicillin destroyed It will be seen that there is a direct relationship between the two

The experiments thus indicate that the reaction of staphylococcal penicillinase with penicillinase-resistant penicillins, methicillin and oxacillin, leads to an inactivation of the enzyme Thus, these penicillins are biologically active not only because of their resistance to hydrolysis by the penicillinase, but also by virtue of their ability to inactivate the enzyme that would ordinarily destroy them

After this work was completed, it came to our attention that Crompton *et al* [4] had made similar observations However, they did not provide any experimental details

A Gourevitch
T A Pursiano
J Lein

Research Division,
Bristol Laboratories,
Syracuse,
New York.

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Effect of Dilution on the Lactic Dehydrogenase Isozyme Pattern obtained in the Starch Gel

The demonstration of the heterogeneity of lactic dehydrogenase (LDH) activity in human serum and red cells[1] was followed shortly by a report describing LDH heterogeneity in tissues of other mammals[2] Multiple molecular forms of LDH have been utilized in clinical medicine[1,3–5], embryology[6] and tissue culture[7] Markert and Møller directed attention to the general phenomenon of multiple enzymatic forms exhibiting similar substrate specificities within a cell by designating them isozymes[6] Recent reports have indicated that tissue homogenization[8] and enzyme purification[9] may alter the pattern of isozymes observed in the starch gel The present communication shows that the LDH isozyme pattern obtained may be altered by changing the concentration of enzyme activity applied to a starch gel prior to electrophoretic separation The distribution of total LDH activity among the 5 LDH isozymes of human leucocytes and muscular tissue varies according to the amount of activity applied (Fig 1) Since relative activities of the serum LDH isozymes have been used in the diagnosis of disease states[1,3–5], it is important to specify the total amount of activity applied prior to electrophoresis

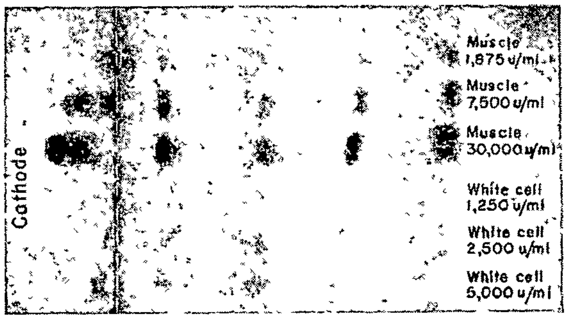

Fig 1 Photograph of a starch gel in phosphate–citric acid buffer *p*H 7 0, 0 01 *M*, stained histochemically for LDH activity (ref 5) Human leucocytes and muscle prepared as previously described (ref 5) were diluted prior to application to produce the units of LDH activity indicated Note that with increasing dilutions isozyme 5, the cathodally migrating band, changes electrophoretic mobility and disappears, whereas the anodally migrating isozymes are visualized at all dilutions Note also that the intensity of staining of the anodal bands decreases approximately in proportion to the dilution in LDH units of leucocyte and muscle extract

Human liver and muscle obtained at autopsy and leucocytes prepared by dextran sedimentation were disrupted by sonic oscillation, centrifuged, the supernatants assayed spectrophotometrically for LDH activity[5] and then applied in varying dilutions to starch gels prepared as previously described[5] in a phosphate–citric acid buffer *p*H 7·0, 0·01 *M*. After vertical starch-gel electrophoresis for 12 hr. the gels were sliced and stained histochemically for LDH activity[5]. The results show that isozyme 5, the cathodally migrating band, present in human liver, muscle and leucocytes, is intensely stained only at high total LDH activities (Fig. 1) and that at lower activities isozyme 5 may not be observed although the anodally migrating isozymes are visualized (Fig. 1). The dilutions were made with distilled water or with phosphate-citric acid buffer *p*H 7·0, 0·01 *M*. With increasing dilutions the mobility of isozyme 5 is regularly altered until it fails to migrate into the gel and probably remains at the origin as suggested by the increased staining observed at the origin in very dilute samples. Differential loss of LDH activity in isozyme 5 with dilution also occurred in borate starch gels at *p*H 8·9, 0·01 *M* or at *p*H 9·5, 0·01 *M*, but was not observed when either filter paper in barbital buffer *p*H 9·5, 0·053 *M* or a starch block in barbital buffer *p*H 8·6, $\mu = 0·01$ was used. With filter paper or starch as a supporting medium all LDH isozymes behaved identically on dilution. Therefore, the phenomenon in the starch gel of differential loss of activity in LDH isozyme 5 on dilution appears to be artefactual. Since the starch gel has been utilized by certain workers for diagnostic purposes[4], it should be emphasized that material low in LDH activity may be unsuitable for application to the gel because isozyme 5, frequently not observed or observed only in trace amounts at low total activities, may become very prominent at higher total activities.

ELLIOT S. VESELL

The Rockefeller Institute,
New York.

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Appearance of Fructose-1,6-Diphosphatase in Post-Natal Rat Liver

MANY liver enzymes are known to increase in activity after the birth of mammals[1–4], but the biological significance of such changes is often difficult to assess. Mitochondrial enzymes concerned with the metabolism of carbohydrate[4] and microsomal enzymes[1,2] show especially marked changes in the liver of early post-natal animals. In the rat, sheep, pig and guinea pig, glucose-6-phosphatase a microsomal enzyme, makes its first appearance at parturition[1]. This enzyme is an obligatory component of the system for the conversion of liver glycogen to blood glucose, and its rapid appearance at parturition reflects the changed circumstances of the new-born, in that the animal is now obliged to regulate its own blood glucose-levels.

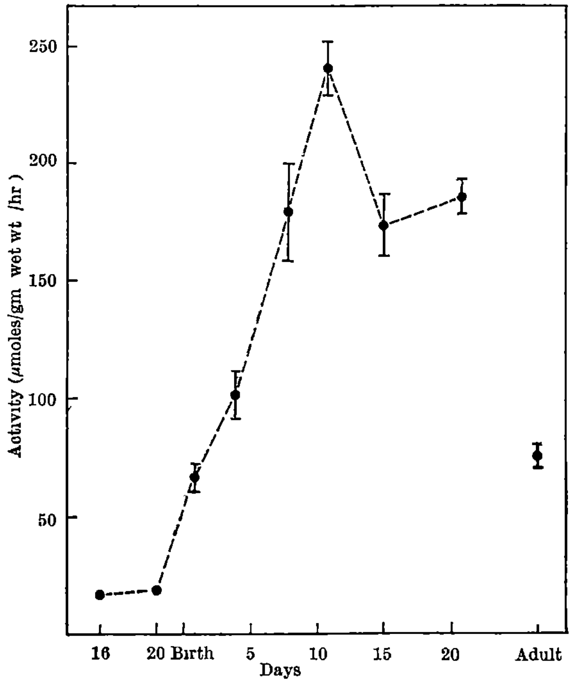

Fig. 1. Appearance of fructose-1,6-diphosphatase in post-natal rat liver. Enzyme determinations in liver homogenates by the method of Pogell and McGilvery. Temperature of assays, 30°. Each point represents the mean of determinations on 4–7 animals. The vertical bars represent ± 1 *S.E.*

This communication describes the appearance of fructose-1,6-diphosphatase in the post-natal rat liver and the changes which occur in its activity during subsequent growth of the animal.

Young of the Wistar albino strain of *Rattus norwegicus* were killed by decapitation. The livers were removed and homogenized in borate buffer *p*H 9·3 in chilled coaxial homogenizers. Fructose-1,6-diphosphatase was assayed in these homogenates at 30° C. by the method of Pogell and McGilvery[5]. Pregnant rats were killed by a blow on the head. The embryos were removed immediately and the livers treated in the same way as the post-natal livers. The stage of pregnancy was determined by the time after the appearance of a vaginal plug.

The activity of fructose-1,6-diphosphatase throughout the period studied is shown in Fig. 1. The assay method also detects non-specific alkaline phosphatase, and although the activity of this enzyme is low compared with fructose-1,6-diphosphatase in the adult rat liver[6], it is a characteristic enzyme of embryonic liver[7]. The apparent activity of fructose-1,6-diphosphatase present in the fœtal liver may therefore be due to alkaline phosphatase, but with present assay procedures the activities of these two enzymes cannot be distinguished in homogenates. Notwithstanding this fact, the rate of hydrolysis of fructose-1,6-diphosphatase by the liver increases sixteen-fold during the first 11 days after birth, and it should be noted that during this period glycogen is resynthesized in the post-natal liver[8]. Fructose-1,6-diphosphatase is an obligatory enzyme in the synthesis of glycogen from glycerol, lactate or amino-acids, and the liver of the rat thus acquires this ability only near the time of birth of the animal. On this evidence it is suggested that the large glycogen deposits formed in the fœtal liver prior to parturition[8] are synthesized from hexose units obtained from the maternal circulation. This contention is further supported by a report that hexokinase, which is required for hexose phosphorylation, has a high activity in fœtal livers[9]. In the embryonic chicken liver, on the other hand, a very high activity of fructose-1,6-diphosphatase occurs as early as 10 days[10,11] and this organ is thus capable of the synthesis of glycogen from varied metabolic sources during the later embryonic development of the animal.

F. J. BALLARD
I. T. OLIVER

Department of Biochemistry,
University of Western Australia,
Nedlands, Western Australia.

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Effects of Insulin on Turnover of Carbon-14 in HeLa Cells

AN *in vitro* assay which could be adapted to a routine determination of some physiological effect of insulin would be useful in a search for new compounds having insulin-like activity. These preliminary investigations were designed to determine whether insulin would influence the turnover of carbon-14 labelled HeLa cells.

HeLa cells were labelled with carbon-14 by growing them in tubes in Eagle's medium containing L-methionine-methyl-^{14}C (6.55 μc./μmole). The glass-attached labelled cells were washed with 'Lactalplus' medium[1] containing 10 per cent (v/v) calf serum, incubated for 4–6 hr. in a fresh portion of this medium, rewashed with medium and finally incubated at 37° in fresh medium with and without insulin (1 I.U. per ml.). (A clinical preparation (Iletin^R, Lilly) was used in these experiments. The results have since been confirmed with crystalline insulin (California Corporation for Biochemical Research).)

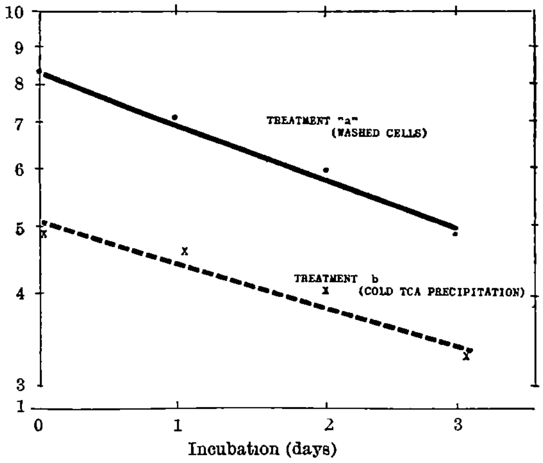

Fig. 1. Turnover of carbon-14 labelled HeLa cells

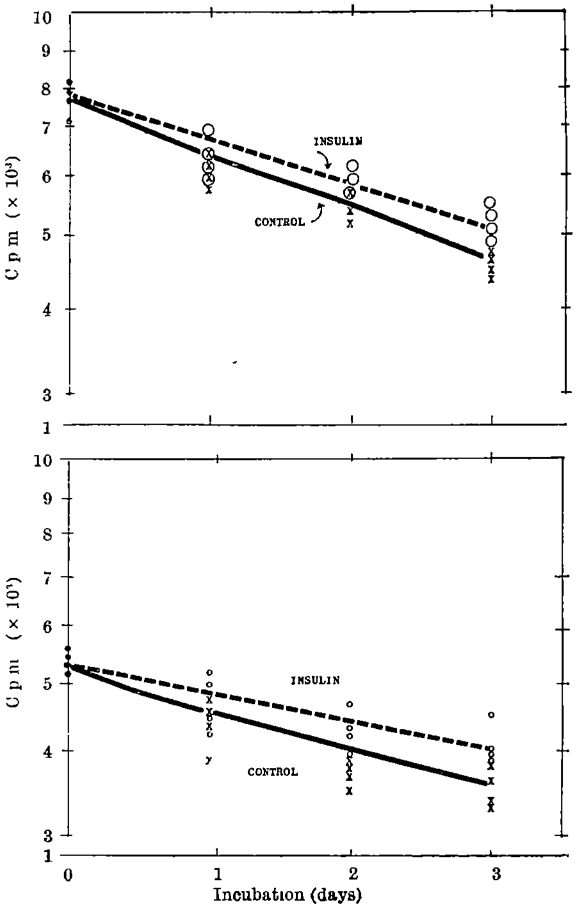

Fig. 2. Effect of insulin on the turnover of carbon-14 in labelled HeLa cells. *a* (above), treatment *a* (washed cells); *b* (below), treatment *b* (cold trichloroacetic acid precipitation)

At various time intervals the cells were washed with warm balanced salt solution and then treated as follows: (*a*) no treatment; (*b*) treated with 5 per cent cold trichloroacetic acid and then extracted with ethanol and ether. Cells were prepared for counting by dissolving them in a solution of 2 per cent Difco '1: 250' trypsin in 0.2 per cent sodium carbonate. Aliquots of the dissolved cells were dried in nickel-plated planchets and counted for a total of 6,400 events. Corrections were made for self-absorption. As measured by treatment (*a*) approximately 50 per cent of the added label was incorporated into the cells.

In Fig. 1 the turnover of carbon-14 can be seen to occur linearly over the three-day period. From the slope of these curves a turnover-rate of about 0.65 per cent per hour was calculated. This value is somewhat lower than the 1 per cent per hour reported by Eagle[2] for HeLa cells, and is slightly higher than that reported by Jordan *et al.*[3] for Walker 256 carcinoma.

A lower rate of turnover was observed for cells treated with insulin (Fig. 2*a* and *b*). In order to assess the consistency of these results, experiments were carried out and the data were treated statistically. Decay curves were plotted by the method of least squares and were found to correspond to the following equations:

Insulin added, treatment (*a*) $y = 3.88 - 0.041x$
Insulin added, treatment (*b*) $y = 3.71 - 0.041x$
Control treatment (*a*) $y = 3.88 - 0.074x$
Control treatment (*b*) $y = 3.71 - 0.060x$

With both treatments the difference in slopes between insulin-treated and control cultures was significant at the level of 5 per cent, showing that insulin retarded the rate of turnover of carbon-14

We thank Dr. W Kwolek for his help in the statistical treatment of the data

G Melnykovych
P A Miller

Biochemical Research Section,
Lederle Laboratories,
American Cyanamid Co ,
Pearl River, New York

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Effect of 6-Mercaptopurine on Carbohydrate Metabolism of Human Cancer Cells

In an investigation directed toward the evaluation of the relative effects of various chemotherapeutic chemicals or drugs on the carbohydrate metabolism of intact human cancer cells[1], an unpredicted and unusual disappearance of glucose was noted when cells were incubated with 6-mercaptopurine This effect was discernible under a variety of test conditions, incubation time varied from 1 to 3 hr , cell concentrations varied from 60 × 10^6 to 230 × 10^6, chemical concentration varied from 5 to 50 μgm /10^6 cells It was also discernible in all human cell types investigated, acute and chronic lymphatic leukæmia, acute and chronic myelogenous leukæmia, and in ascites cells from patients with carcinoma of the ovaries It is also readily demonstrated with tumour cell suspension obtained from a transplantable (in Sprague–Dawley rats) chloromyeloid leukæmia[2] In all, this effect has been found without exception in some 30 separate examinations of human cancer cells

In Table 1 are summarized the results of a typical experiment which demonstrates this unusual effect The cells were obtained from a patient with sub-acute lymphatic leukæmia The glucose utilized is the difference between the glucose at zero time and the glucose in the flask at the end of the experiment as determined by the glucose oxidase test[3] The glucose dissimilated is the sum of glucose equivalents used in respiration and glucose equivalents of lactic acid produced, the latter was determined by a chemical method[4] The excess of glucose dissimilated over the control value is due entirely to the stimulation of aerobic lactic acid production by the chemicals, except for nitrogen mustard which produced inhibition of lactic acid production The quantitative effects caused by changes in respiration are almost negligible

Table 1 Balance of Glucose Utilization of Human Leukæmic Leucocytes in Homologous Plasma

Condition	Chemical concentration (μgm /10^6 cells)	Glucose utilized* (mgm)	Glucose dissimilated† (mgm)	Differences (mgm)
Control	0	0 20	0 22	0 02
Diazouracil	22	0 57	0 51	0 06
8-Azaguanine	21 6	0 66	0 75	0 09
6-Mercaptopurine	21 9	*2 56*	0 46	*2 10*
Nitrogen mustard	21 8	0 20	0 16	0 04
Triethylene thiophosphoramide	10 9	*1 21*	0 71	*0 50*
Vinleucoblastine	3 3 ,	0 60	0 59	0 01

* Glucose in flask at zero time minus glucose in flask at end of experiment

† Sum of glucose equivalents used in respiration and glucose equivalents of lactic acid produced

The broader aspects of these chemical effects will be published elsewhere[5]

In the last column can be seen the unusual effect produced by 6-mercaptopurine This amount of glucose represents more than 50 per cent of the glucose initially present The effect of triethylene thiophosphoramide is worthy of note However, we have not observed this effect in all our work as we have with the 6-mercaptopurine

It has been reported[6] that 6-mercaptopurine causes an increased deposition of glycogen in the cells of Ehrlich ascites tumour obtained from mice given intraperitoneal injections at 100 mgm /kgm for 4 days followed by intraperitoneal glucose injections This group[7] also report a concomitant inhibition of lactic dehydrogenase, under their experimental conditions

However, under the conditions of our experiments, short term and *in vitro*, lactic dehydrogenase inhibition is not evidenced based on the stimulation of aerobic lactic acid production

It is possible that the immediate effect is on the hexokinase reaction with subsequent disposition of the glucose-6-phosphate dependent on intracellular enzymatic conditions. The quantitative localization of this 'missing' glucose is under investigation in human and animal cancer tissues[8]

Not to be overlooked is the fact that the specific stimulation of glucose uptake by 6-mercaptopurine offers a new approach to the control of diabetics

This work was supported by grant *T*–125 *B* of the American Cancer Society

Bernard J Katchman
Robert E Zipf
James P F Murphy

Department of Research,
Miami Valley Hospital,
Dayton 9, Ohio

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Effect of Pyridine Nucleotides on the 1-^{14}C-Glucose Utilization of Normal Human Red Blood Cells

A number of investigators have shown that disphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) enhance the metabolism of glucose in liver preparations via the Embden–Meyerhof and pentose-phosphate (hexosemonophosphate) pathways, respectively[1-3] We have found that TPN and reduced triphosphopyridine nucleotide (TPNH) stimulate the 1-^{14}C-glucose metabolism of normal human red blood cells as measured by the increased production of $^{14}CO_2$ with increasing amounts of the pyridine nucleotides Since normal human red blood cells contain an Embden–Meyerhof pathway but cannot oxidize pyruvate, the only source of carbon dioxide is from the decarboxylation of carbon-1 in the pentose-phosphate pathway Therefore, $^{14}CO_2$ production is a measure of the amount of 1-^{14}C-glucose metabolized via the pentose-phosphate pathway

Blood was obtained by venipuncture from normal fasting subjects, placed in a flask containing heparin, the plasma removed by centrifugation at 2,500 r p m for 10 min , and the cells washed three times in Krebs–Ringer-phosphate buffer, *p*H 7 3 The buffy coat and top layer of the packed red blood cells were discarded One-half ml of packed red blood cells weighing 0 5 gm was placed into a 25 ml Erlenmeyer flask equipped with a centre well, containing 2 ml of Krebs–Ringer-phosphate buffer with 22,200 c p m of 1-^{14}C-glucose (0 03 μgm) and incubated for 1 hr at 37° C in an oxygen atmosphere in a metabolic shaker at 45 cycles per min At the end of incubation 1 ml of 1 *M* hyamine base was injected into the centre well and 0 3 ml of 0 4 *N* sulphuric acid was injected into the red cell suspension The flask was incubated for 1 hr to allow for complete trapping of carbon dioxide The hyamine was then transferred to a vial containing 15 ml of scintillation fluid (2,5-diphenyloxazole) and counted in a Packard liquid scintillation counter[4] DPN, reduced DPN (DPNH), TPN, and TPNH (obtained from Sigma Chemical Co , St Louis, Missouri) were added in varying amounts (0 1, 1 0, and 2 0 m*M*) to the appropriate flasks

The typical response of normal human red blood cells to the various pyridine nucleotides is shown in Fig 1 Both TPN and TPNH stimulated the metabolism of 1-^{14}C-glucose as measured by the increased production of $^{14}CO_2$ The greatest increase was seen at levels of 2 0 m*M* DPN and DPNH were only mildly stimulatory

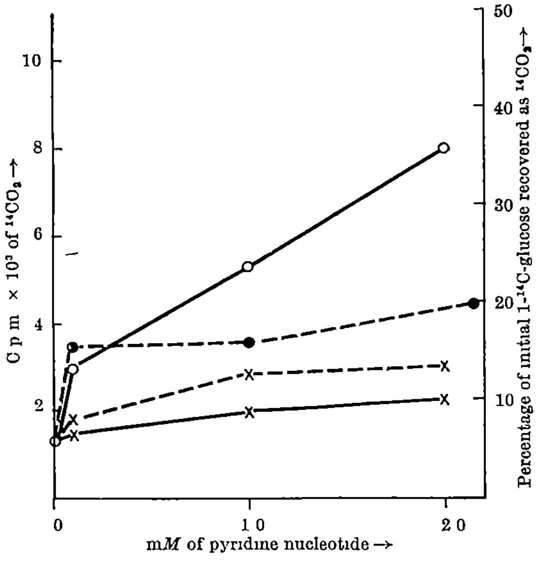

Fig 1 Effect of pyridine nucleotides on the 1-^{14}C-glucose metabolism of human red blood cells Normal human red blood cells, 0 5 gm., added to 2 0 ml of Krebs–Ringer-phosphate buffer, *p*H 7 3, containing 22,200 c p m of 1-^{14}C-glucose, and incubated for 1 hr at 37° C in an oxygen atmosphere There were 100 white blood cells per mm^3 in the red blood cell suspension O, TPN added, ●, TPNH added, x——x, DPN added, x - - - x, DPNH added

By providing the proper coenzyme (TPN) in greatly excess amounts for the enzymes of the pentose-phosphate pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase) the glucose is increasingly metabolized via the pentose-phosphate pathway as reflected by an increase in the $^{14}CO_2$ production The TPNH could become stimulatory by being converted to TPN by reacting with DPN in a transhydrogenase reaction[5] as follows

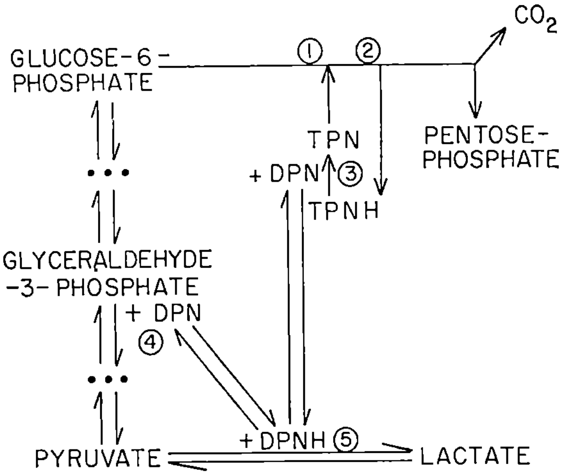

Fig 2 Schematic diagram depicting the interrelationships between TPN, TPNH, DPN and DPNH in the red blood cell The enzymes involved are (1) glucose-6-phosphate dehydrogenase, (2) 6-phosphogluconic acid dehydrogenase, (3) transhydrogenase, (4) glyceraldehyde-3-phosphate dehydrogenase, (5) lactic acid dehydrogenase

$$\mathrm{TPNH+DPN = TPN+DPNH}$$

The DPN is regenerated with the formation of lactate from pyruvate and is available again to react with TPNH

$$\mathrm{DPNH+pyruvate = DPN+lactate}$$

Only slight stimulation occurred with DPN and DPNH alone In each case both could eventually react with endogenous TPNH, but since the amount of endogenous TPNH is necessarily limited only a slight degree of stimulation would be expected Fig 2 depicts the sequence of events in a simplified fashion.

Robert H Herman*
Jesse Berkowitz†
Lloyd E Clayton
Jacques L Sherman, jun ‡
Department of Metabolism,
Division of Medicine,
Walter Reed Army Institute of Research,
Washington 12, D C

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* Major, Medical Corps, U S Army

† Captain, Medical Corps, U S Army Reserve Present address, Mount Sinai Hospital, New York

‡ Lieut -Colonel, Medical Corps, U S Army

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Kinetics of Synthesis of Fibroin in the Posterior Division of the Silk-gland of the Silkworm, *Bombyx mori*, L.

The synthesis of fibroin and secretion in the posterior division of silk-gland can be divided into three phases (1) absorption of amino-acids, (2) synthesis of fibroin, (3) secretion of fibroin We have tried to show that such a complex reaction is an enzyme system, by analysing the phenomenon of the incorporation of glycine labelled with carbon-14 into fibroin *in vitro* A piece of the posterior division of silk-gland, 200 mgm wet weight, 1–2 cm long, was removed from the larva of the silkworm, *Bombyx mori*,

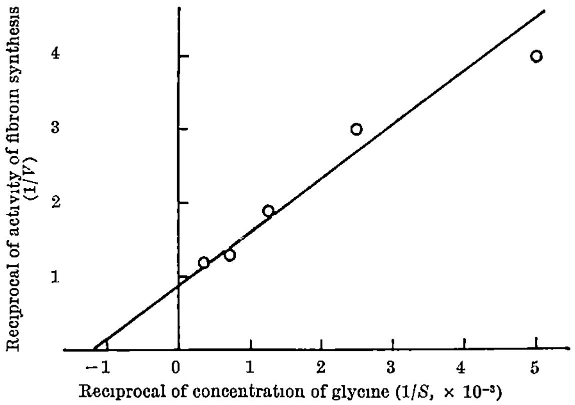

Fig 1 Relation between concentration of glycine (S) and the activity of synthesis of fibroin (V)

during the middle period of the fifth instar, and incubated in a medium consisting of 0 5 ml of saline solution (sodium chloride, 2 0, potassium chloride, 0 2, calcium chloride, 0 08, magnesium chloride, 0 04 per cent), 0 2 ml of 0 2 M phosphate buffer (pH 7 4), 5 μmoles of α-ketoglutaric acid, 210 μgm of glycine, 140 μgm of L-alanine, 75 μgm of L-serine, 70 μgm of L-tyrosine, 0 025 μc of ^{14}C-glycine (4 1 mc /mM) and filled with distilled water to 2 0 ml at 31 7° C, and mildly shaken for 1 hr After the incubation, fibroin in the gland cavity was separated from gland cells by the trichloroacetic acid method[1]

The relation between the concentration of glycine in the medium and the activity of synthesis of fibroin is shown by calculating from the value of ^{14}C-glycine incorporation into fibroin at respective concentration of glycine varying from 30 to 500 μgm / 2 ml (Fig 1) This result showed that the reaction of synthesis of fibroin in the posterior division of silk-gland nearly followed the Michaelis–Menten law, and the Michaelis constant K_{Mg} of the reaction concerning glycine was 6 2 × 10^{-4} M Moreover, it was made clear that the activity of fibroin synthesis varied with the concentrations of tyrosine between 0 and 10 μgm /2 ml, by calculating the value of ^{14}C-glycine incorporation into fibroin with co-existence of tyrosine at above concentrations In this case, the reaction nearly followed the Michaelis–Menten law as the case of glycine and the Michaelis constant K_{Mt}, so far as tyrosine was concerned, was 1 4 × 10^{-5} M

The ratio of the Michaelis constant of glycine-enzyme complex to that of tyrosine-enzyme complex in the reaction of fibroin synthesis may be 7 5 1, representing the constructive molar ratio of these two amino-acids in fibroin[2] However, the ratio of K_{Mg} to K_{Mt} presented from the foregoing experiments was 44 3 1, which did not agree with the expected ratio Therefore, K_{Mg} and K_{Mt} seem to be apparent values It was assumed that the true concentration of the substrate, such as glycine or tyrosine, for the reaction of fibroin synthesis was the sum of the concentration of the substrate which was added to the reaction mixture before the incubation and that of the substrate which existed in the gland cells The concentrations of the substrates existing in the gland cells were calculated as 7 0 × 10^{-5} M of glycine and 1 2 × 10^{-5} M of tyrosine, by comparison of the rate of ^{14}C-glycine incorporation into fibroin after pre-incubation under the condition of glycine-deficiency and presence of glycine The actual Michaelis constants concerning glycine and tyrosine were 1 0 × 10^{-3} and 1 3 × 10^{-4} M, respectively

Table 1 EFFECT OF CO-EXISTENCE OF ALANINE, SERINE AND TYROSINE ON THE INCORPORATION OF ^{14}C-GLYCINE INTO FIBROIN OF THE POSTERIOR DIVISION OF SILK-GLAND *in vitro*

Amino-acids present	Relative activity Exp 1	Exp 2	Exp 3	Average
Glycine	100*	100*	100*	100
Glycine + alanine	106	113	—	110
Glycine + alanine + serine	64	61	—	63
Glycine + alanine + serine + tyrosine	316	437	—	377
Glycine + tyrosine	—	301	300	301
Glycine + alanine + tyrosine	—	516	326	421

* Activities were 441 in experiment 1, 88 in experiment 2 and 63 c p m /mgm fibroin in experiment 3

The effects of the co-existence of amino-acids other than glycine on the incorporation of ^{14}C-glycine into fibroin were as follows alanine did not show any remarkable effect, serine retarded the reaction but tyrosine was apparently effective in acceleration (Table 1) Such an effect of tyrosine emerged from the fact that this amino-acid was essential for the reaction of the composition of the amino-acids in fibroin, since there were no effects of the related compounds of tyrosine, that is, phenylalanine, tyramine, phenol, p-cresol, DL-fluorophenylalanine and tryptophan, on the reaction of incorporation of ^{14}C-glycine into fibroin Moreover, it was shown that glycyl-tyrosine accelerated the incorporation of ^{14}C-glycine into fibroin through decomposition of the peptide by dipeptidase in the gland cells, while glycylglycine showed no effect on the reaction

HAJIME SHIGEMATSU
TOMOKO KOYASAKO*

Sericultural Experiment Station,
Koenji, Suginami-ku,
Tokyo

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* Present address Biological Institute, Faculty of Science, Nagoya University

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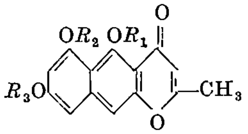
Fonsecin, a Naphthopyrone Pigment from a Mutant of *Aspergillus fonsecaeus*

IN 1953 Raper and Fennell[1] described an ultraviolet mutant of *Aspergillus fonsecaeus* (N R R L 67), which was characterized by a rich deposit of yellow pigment in the vesicles and primary sterigmata We became interested in this material as a possible precursor of the brown and black pigments of the parent strain and, accordingly, isolated the crude pigment by ethyl acetate extraction of the dried mycelial mat Fractional precipitation and recrystallization from ethyl acetate–petroleum ether mixtures gave a pure substance in the form of bright yellow irregular prisms (m p 198° decomposition) to which the name fonsecin was assigned λ_{max} (ethanol) 233, 275, 321, 332, and 400 mμ (ε, 26,400, 31,400, 7,300, 8,025, and 8,775, respectively) (Found C, 62 3, 62 1, H, 4 94, 4 79, OCH_3, 11 4, CH_3, 6 2 $C_{15}H_{12}O_5$ - H_2O requires C, 62 06, H, 4 82, 1 OCH_3, 10 7, 1 CH_3, 5 2)

Fonsecin is isomeric with rubrofusarin, a red pigment first isolated by Ashley, Hobbs and Raistrick[2] from *Fusarium culmorum* and *Fusarium graminearum* Like these investigators, as well as Mull and Nord[3], we first concluded on the basis of general properties that fonsecin was a dihydroxymethoxy-methylxanthone; recently, however, Dreyer[4], Stout, Dreyer and Jensen[5], and Tanaka and Tamura[6] have established that rubrofusarin is a naphtho-γ-pyrone and is represented by structure I Using this new work as a guide, we have been able to show that fonsecin has structure II



I $R_1 = R_2 = H, R_3 = CH_3$
II $R_1 = R_3 = H, R_2 = CH_3$
III $R_1 = R_2 = R_3 = H$
IV $R_1 = H, R_2 = R_3 = CH_3$
V $R_1 = R_3 = CH_3, R_2 = H$
VI $R_1 = R_2 = R_3 = CH_3$

When fonsecin was demethylated with hydriodic acid, nor-rubrofusarin (III) was obtained (analysis, identical ultra-violet and infra-red curves, m p and mixed melting point with authentic nor-rubrofusarin obtained from rubrofusarin from two different sources, supplied by Drs H Raistrick and J H Birkinshaw and by Dr H G Stout)

On methylation with diazomethane for a short time, fonsecin gives a monomethyl ether (IV) of m p 176° Rubrofusarin monomethyl ether (V) is reported[2,4] to have a m p of 203°–204°, which is the same as that reported by Lund, Robertson and Whalley[7] for asperxanthone, a yellow pigment isolated from *Aspergillus niger* A direct comparison of these two compounds apparently has not been made

Prolonged treatment of fonsecin with diazomethane gives a product (VI) identical in all respects with dimethyl rubrofusarin

When fonsecin monomethyl ether (IV) was oxidized with potassium permanganate[8], 3,5-dimethoxyphthalic acid was detectable by paper chromatography, this acid could not be found when rubrofusarin monomethyl ether (V) was oxidized under the same conditions These findings confirm previous observations[4]

Our yields of fonsecin (4 5 per cent of the dried mycelium) are much higher than those reported by Ashley *et al* [2] for rubrofusarin and by Lund *et al* [7] for asperxanthone Our work makes these natural naphtho-γ-pyrones more readily available now for study

OSCAR L GALMARINI*
FRANK H STODOLA
KENNETH B RAPER†
DOROTHY I FENNELL‡

Northern Utilization Research
and Development Division,
Agricultural Research Service,
U S Department of Agriculture,
Peoria, Illinois

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* Acknowledgement is made of the sponsorship of the John Simon Guggenheim Memorial Foundation that made it possible for Dr Galmarini to collaborate in this work Present address Catedra de Quimica Organica, Facultad de Ingenieria, Buenos Aires

† Present address University of Wisconsin, Madison, Wisconsin

‡ Present address Pioneering Research Division, Quartermaster Research and Engineering Center, Natick, Massachusetts

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Amino-acids in Corn-steep

LACTIC acid fermentation during the steeping of corn influences the biological value of corn-steep for penicillin fermentation[1–4] Until now no attention has been paid to the changes in the amino-acid content of corn-steep during lactic acid fermentation after the completion of the steeping process

For the quantitative determination of some amino-acids we have used the method of descending electrophoresis according to Mikeš[5] and Bode's evaluation method[6] The methods of cultivation of the strain of *Penicillium chrysogenum NG* on a rotary shaker and the analytical methods used have been described[4]

The fermented samples were prepared in the following way 20 litres of the oldest steeping liquor were withdrawn into a glass flask from the end of the counter-current steeping battery of a corn starch factory After thorough homogenization, 5 litres were withdrawn from the sample and concentrated *in vacuo* in a glass distillation apparatus to contain about 60 per cent dry matter The rest of the steeping liquor was kept at a temperature of 50° C , where it was subject to spontaneous fermentation At 24-hr intervals samples of the fermenting steeping liquor were collected for concentrating *in vacuo* Samples of concentrated corn-steep prepared in this way on a laboratory scale were tested biologically and analysed chemically

Under our experimental conditions, no substantial changes in the amino-acid content were observed in the course of the supplementary fermentation of corn-steep Noticeable differences in the content of aspartic acid were observed only in those batches where this amino-acid was already present prior to the process of fermentation In some of the batches the content of aspartic acid increased during fermentation (Fig 1), in others the content decreased (Fig 2). In batches where this amino-acid was absent prior to fermentation, aspartic acid did not emerge in the course of fermentation It is evident that the increase or

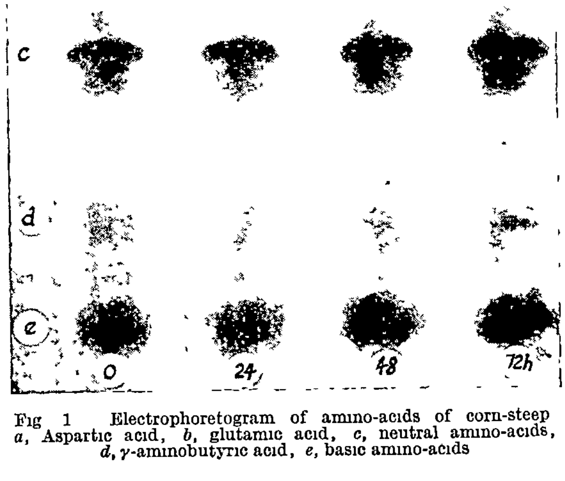

Fig 1 Electrophoretogram of amino-acids of corn-steep *a*, Aspartic acid, *b*, glutamic acid, *c*, neutral amino-acids, *d*, γ-aminobutyric acid, *e*, basic amino-acids

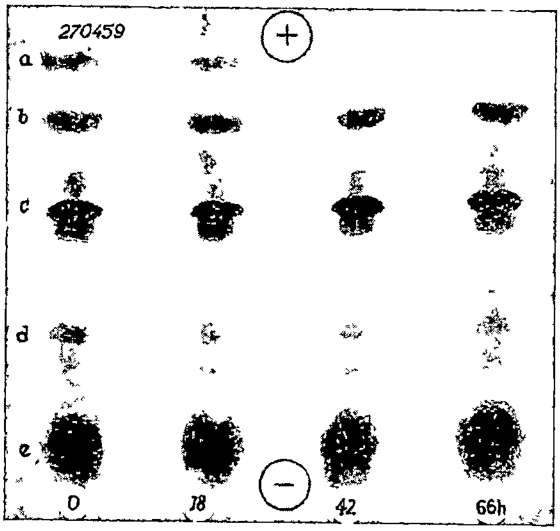

Fig 2 Electrophoretogram of amino-acids of corn-steep *a*, Aspartic acid, *b*, glutamic acid, *c*, neutral amino-acids, *d*, γ-aminobutyric acid, *e*, basic amino-acids

decrease of the content of aspartic acid is caused by a specific microflora

In Table 1 the results of amino-acid assays of some batches of corn-steep and the results of biological tests are presented The yields of penicillin on media prepared with corn-steeps without the supplementary fermentation are considered as 100 per cent The presented results of amino-acid assays are means of results of 15 assays and the biological tests are means of 2–4 assays

The chemical composition of these batches is presented in Table 2 After a 24-hr fermentation the yield of the antibiotic did not decrease in any of the assayed samples, on the contrary, a tendency to increase can be observed From the technological point of view it is important that after a 24-hr fermentation a decrease of foaming of fermented corn-steeps is observed

Table 1 AMINO-ACID CONTENT AND BIOLOGICAL TESTS OF FERMENTED CORN-STEEPS

Batch no / hr of fermentation	Amino-acids (per cent of dry matter)					Biological test (per cent)
	Aspartic acid	Glutamic acid	Neutral amino-acids*	γ-aminobutyric acid	Basic amino-acids†	
270459/ 0	0 84	1 08	6 14	1 81	5 69	100
270459/18	0 53	1 16	6 27	1 99	5 52	100
270459/42	—	1 19	6 18	1 72	5 35	98
270459/66	—	1 30	6 15	1 55	5 88	83
190559/ 0	0 67	1 43	6 89	1 91	6 18	100
190559/24	0 57	1 66	7 30	2 10	6 57	119
190559/48	0 69	1 68	7 71	2 26	6 22	110
190559/72	0 69	1 30	7 10	1 88	5 72	107
300659/ 0	0 43	1 22	6 45	2 43	6 29	100
300659/24	0 64	1 18	6 50	1 93	5 98	105
300659/48	0 54	1 23	7 09	1 92	5 79	98
300659/72	0 64	1 17	6 57	1 55	6 20	102

* As alanine
† As arginine hydrochloride

Table 2 CHEMICAL COMPOSITION OF FERMENTED CORN-STEEPS

Batch no / hr of fermentation	Dry matter (per cent)	Results of assays (per cent of dry matter)						$\frac{\text{N-NH}_2}{\text{N-total}}$
		Reducing substances	Lactic acid	N-NH$_2$	N-total	Ash		
270459/ 0	57 30	1 16	19 00	1 83	7 24	20 98		0 25
270459/18	54 00	0 57	22 70	2 07	7 40	21 28		0 29
270459/42	60 60	0 52	25 40	2 06	7 48	20 04		0 28
270459/66	62 60	0 35	24 50	2 29	7 31	21 28		0 31
190559/ 0	59 95	12 70	21 60	2 01	8 30	18 64		0 24
190559/24	59 00	5 90	—	2 04	8 01	18 23		0 25
190559/48	63 00	0 62	25 50	2 07	8 03	17 70		0 25
190559/72	61 60	0 85	25 60	2 20	7 72	18 81		0 28
300659/ 0	56 80	10 60	18 46	1 86	7 84	17 71		0 24
300659/24	61 45	4 15	23 19	1 95	7 58	17 23		0 26
300659/48	63 60	0 75	22 85	2 25	7 71	17 41		0 29
300659/72	63 50	0 40	21 34	2 27	7 64	17 12		0 30

JÁN ZELINKA
MARIUS HUDEC
LIBUŠE PELCOVÁ

Department of Technical Microbiology,
Biological Institute,
Slovak Academy of Sciences,
Boleráz, Czechoslovakia

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A Quantitative Cytochemical Technique for Measurement of Glycogen

THERE seems to be a need for a quantitative cytochemical technique for assessment of glycogen within intact cellular structures In histochemical detection of glycogen, only the fraction of total cellular glycogen soluble in trichloroacetic acid takes part in the staining reaction[1] To overcome this difficulty, an attempt has been made to establish the present technique for measuring the absolute dry mass of glycogen of a cell Danielli[2] recorded that after fixation of the tissue in absolute alcohol mainly protein, nucleic acid and carbohydrate remain in the cellular material

A small piece of liver from a normal adult albino rat was quickly fixed in chilled absolute alcohol inside a refrigerator, sectioned at 5μ thickness and mounted on glass slides without application of water and care taken not to overheat in order to prevent the formation of diffusion artefacts, as described before[3] Paraffins were removed from the unstained sections by treatment with xylol, dehydrated in absolute alcohol and some hepatic cells of the same microscopic field of a section were measured in double distilled glycerine before and after digestion in malt diastase solution at 1/1,000,000 under a Baker interference microscope (× 100 objective, numerical aperture 1 30, × 8 binocular eyepieces, mercury green filter) At this higher dilution malt diastase shows only pure glycogenase action[4] without any ribonuclease action Table 1 shows phase difference (in degrees) proportional to dry mass of glycogen per unit area within normal rat hepatic cells

Table 1

Organelles	Average phase difference (300 cells)		Average phase difference proportional to dryness of glycogen per unit area
	Pre-digestion	Post-digestion	
Cytoplasm	44 ± 16	16 ± 4	28
Heterochromatins	41 ± 3	36 ± 4	5
Nucleoli	53 ± 5	49 ± 3	4

The results exhibited in Table 1 show a considerable change in phase difference proportional to dry mass of glycogen per unit area within the hepatic cell cytoplasm of rats Phase changes are insignificant in the nuclei and nucleoli of these cells The present quantitative cytochemical technique, which is quite new, may be utilized also with other tissue fixatives for studying altered cellular functions where glycogen plays an important part This method will also be useful for measuring interferometrically the dry mass of protein in cells where considerable amounts of carbohydrate are present

P De
R Chatterjee

Cell Research Department,
Chittaranjan National Cancer Research Centre,
37 Shyama Prosad Mookerjee Road,
Calcutta 26

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PHYSIOLOGY

Diffusion Coefficient of Oxyhæmoglobin

In work described elsewhere[1] we showed how to understand the steady-state transport of oxygen through hæmoglobin solution by the application of Fick's law of mass transport The oxygen flux was shown to depend not only on the pressure difference across the diffusion cell but also on the absolute partial pressures of oxygen at the cell boundaries This behaviour is a direct consequence of the non-linear relation between oxyhæmoglobin concentration and oxygen partial pressure We pointed out, for example, that if the hæmoglobin is saturated with oxygen at both ends of the diffusion path it cannot contribute to the transport of oxygen and all the oxygen transport must therefore take place through the plasma

Hemmingsen[2] recently reported a series of experiments which he interprets as showing that although the hæmoglobin is oxygen saturated at both ends of the diffusion path it still contributes to oxygen transport His conclusions are, however, largely incorrect and the experiments prove instead to be an ingenious way of determining the diffusion coefficient for oxyhæmoglobin

Hemmingsen measured the transport of oxygen-18 through a hæmoglobin solution under conditions where the total oxygen pressure was identical at both ends of the diffusion path and maintained large enough to ensure that the hæmoglobin was in the oxygenated form A difference was established, however, in the partial pressures of ^{18}O at the diffusion cell boundaries which caused a transport of ^{18}O across the cell In comparing results for identical experiments with hæmoglobin, methæmoglobin, and water, Hemmingsen found the ^{18}O flux through the hæmoglobin to be about 2 6 times that through methæmoglobin or water

Fick's law is again quite applicable to understanding these experimental results It must be stressed, however, that the law applies only to the ^{18}O, since it is only the flux of this component of the system which has been measured Now although the total pressures of oxygen are maintained equal at each end of the diffusion path, the partial pressures of ^{18}O are not Similarly, though the concentrations of oxyhæmoglobin are the same at each cell boundary, the concentrations of the ^{18}O-hæmoglobin are not equal This is an important point because it is true whether or not all the hæmoglobin is in the oxygenated form In fact a simple equilibrium argument shows that the fractional amount of ^{18}O-oxyhæmoglobin is just equal to the fractional partial pressure of dissolved ^{18}O One can easily picture exactly what must happen in the diffusion cell The concentration gradient of dissolved ^{18}O causes an ^{18}O flux through the plasma while the gradient in ^{18}O-oxyhæmoglobin causes an additional flux of the ^{18}O-oxyhæmoglobin There must also be a flux of ^{16}O-hæmoglobin in the opposite direction to preserve a mass balance on the hæmoglobin We can now use our previous results to write a mathematical expression for the ^{18}O flux

$$J(^{18}O) = \frac{D_A k(P_i - P_f)}{x_f} + \frac{D_E\{f(kP_i) - f(kP_f)\}}{x_f} \qquad (1)$$

where P_i and P_f are the initial and final partial pressures of ^{18}O respectively, k is a Henry's law constant, D_A and D_E are the diffusion coefficient of ^{18}O through plasma and the diffusion coefficient of ^{18}O-oxyhæmoglobin respectively, $f(kP_i)$ is the initial concentration of ^{18}O containing hæmoglobin and $f(kP_f)$ the final concentration, and x_f is the length of the diffusion path

The first term in equation (1) is the oxygen flux through the plasma, the second is an additional flux due to the presence of a gradient in the oxyhæmoglobin concentration In Hemmingsen's experiments on water or methæmoglobin the entire flux is described by the first term of equation (1) In the experiments on hæmoglobin the second term is also important and accounts for the enhanced ^{18}O flux through this solution Using a value of 20–21 vol per cent for the oxygen capacity of the hæmoglobin solution, the data from Fig 2 in ref 2, and applying equation (1), we obtain $1\ 9 \times 10^{-7}$ cm ^{2}/sec for the diffusion coefficient of oxyhæmoglobin, D_E We should point out that one must be very careful in using the numbers in Hemmingsen's Fig 2, since he has not reported the ^{18}O flux but has scaled that flux up by the experimental $^{16}O/^{18}O$ ratio This is a spurious correction and must be removed to obtain the data The previous experiments of Scholander and Hemmingsen[3] wherein the hæmoglobin was oxygen saturated at one end of the path and kept at zero oxygen partial pressure at the other end also give a value of $1\ 9 \times 10^{-7}$ cm ^{2}/sec for D_E This value was chosen by us in our treatment of the steady-state oxygen transport in whole blood[4] and leads to an adequate description of the experimental data on whole blood obtained by Scholander[5]

The ^{18}O experiments might profitably be done as a function of hæmoglobin concentration to determine the concentration dependence of D_E As it stands they merely show once again that Fick's law is applicable to any component of a system providing the equilibrium with the other components is fast compared with diffusion-rates

This work was supported by U S Public Health Service grant No *H*6796

I Fatt

Miller Institute for Basic Research
in Science,
Department of Mineral Technology,

R C La Force

Department of Mineral Technology,
University of California, Berkeley

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Effect of an Anæsthetic on the Heart and Respiration of Teleost Fish

The properties of Sandoz '*M.S.* 222' compound, a meta-amino-benzoic acid ethyl ester in the form of a soluble salt, as a general anæsthetic[1,2] have led to its widespread use in studies of fish physiology. The general effects of this, and other anæsthetics on fish, have been examined by McFarland[3], but, in the course of experiments on some aspects of the respiratory and circulatory physiology of teleosts, it became clear that '*M.S.* 222' caused changes of considerable significance not hitherto described.

In these experiments the electrocardiogram, respiratory rate, and respiratory amplitude were recorded in tench (*Tinca tinca*, L.) held in a tank of 1,750 ml. capacity. Handling the fish caused a considerable rise in the heart-rate, often to three times the value found in a fish that had remained inactive and undisturbed in the tank for periods of more than 1 hr. The usual range of heart-rate in an undisturbed tench was found to be 15–30 beats/min. When the heart-rate had reached this resting value, a known concentration of '*M.S.* 222' was passed through the tank for periods up to 90 min. in duration. Six concentrations of the anæsthetic were used, 25–200 mgm./l., and the experiments were carried out on 15 fish weighing 38–144 gm.

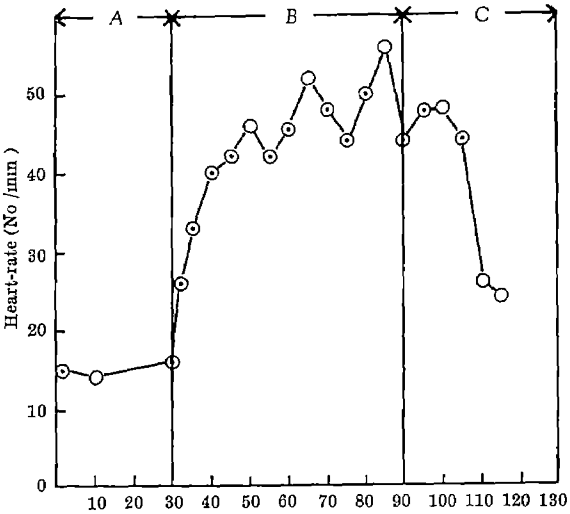

Fig. 1. Effect of '*M.S.* 222' on the heart-rate of a 57-gm. tench (temperature, 17° C.). *A*, Freshwater; *B*, '*M.S.* 222' 33 mgm./l.; *C*, freshwater

The effect of '*M.S.* 222' was to produce an increase in heart-rate (Fig. 1), the increase being more marked at higher concentrations. The respiratory-rate and amplitude were also increased, though in a somewhat variable way as the relationship between these two parameters of the breathing movement is complex. '*M.S.* 222' probably acts on the heart via the parasympathetic nervous system (there being no sympathetic innervation) since the direct effect of the substance on the isolated and perfused hearts of tench, trout and roach was to decrease the frequency of beat. This conclusion was substantiated when it was found that '*M.S.* 222' in the ventilation stream of a tench in which the tenth vagi had been sectioned bilaterally resulted in a decrease in the animal's heart-rate.

The increase in heart-rate caused by '*M.S.* 222' in the intact animal was paralleled by a simultaneous increase in respiratory rate and, though there was no strict correlation between the heart beat and any part of the respiratory cycle, it is suggested that some connexion exists between cardiac and respiratory control systems within the brain. In concentrations of 100–200 mgm./l. '*M.S.* 222' respiratory collapse was common, the breathing becoming irregular and ultimately stopping. Simultaneously the heart-beat would also become irregular and the frequency would fall. Recovery in the breathing was always accompanied by a similar recovery in the regularity and frequency of the heart beat.

D. J. Randall

Department of Zoology,
The University,
Southampton.

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Effects of Mammalian Growth Hormone on *Cottus scorpius* Blood

It is now well established that the teleost pituitary contains a growth-promoting hormone, the evidence coming from injection of fish pituitary preparations and from the effects of hypophysectomy[1]. Although growth effects have been studied, the metabolic responses of either fish or mammalian somatotrophin in teleosts, such as possible diabetogenic action, have not been investigated. Both the nitrogen retention and diabetogenic effects of somatotrophin in mammals are well known and it has now been shown during work on hypophysis-pancreas relations in teleost fish that mammalian growth hormone affects blood glucose, protein and urea in *Cottus scorpius*.

Armour 'Somar *A*' was injected intraperitoneally into twelve *Cottus scorpius* weighing 300–500 gm. at a 1 mgm./kgm. dose-level every third day for fifteen days. Blood was obtained from the ductus *Cuvieri* running subcutaneously immediately beneath the operculum and blood sugar determined spectrophotometrically by a glucose oxidase method ('Blutzucker Farb-Test', Biochemica Boehringer). The blood glucose, determined every third day for ten days prior to growth hormone, was 6.6 ± *S.E.* 0.34 mgm./100 ml. blood. There was no rise in blood sugar for the first three days after injection of 'Somar *A*', but within six days the blood glucose-level had risen to 12.6 ± *S.E.* 1.11 mgm./100 ml. This marked elevation of blood glucose was maintained for the duration of the injections. This effect is in contrast to fish growth hormone injections, which exert no metabolic effect in mammals (nitrogen retention, cardiac glycogen)[2] and to experiments which indicate that hypophysectomy of *Cottus scorpius* does not result in any lowering of blood glucose-levels over a period of a month[3].

After fifteen days, blood samples of the growth hormone treated fish were taken and the plasma total protein, urea and amino-acid nitrogen determined by biuret, urease-nesslerization and β-naphthoquinone-4-sulphonate methods respectively. The total plasma protein of the treated fish was lowered, being 24.5 mgm. ± *S.E.* 2.45/ml. compared with an untreated fish value of 34.1 mgm. ± *S.E.* 1.79/ml. The plasma urea was also reduced to 65.8 μgm. ± *S.E.* 5.32/ml. plasma compared with untreated fish of 109.0 μgm. ± *S.E.* 14.5/ml. plasma. The nitrogen retention effect of mammalian growth hormone on fish is thus demonstrated, both results being significant at the 5 per cent levels. The plasma and amino-acid values were not, however, significantly different, individual valuations showing wide variation. A similar wide scatter of the amino-acid values have been found also in this laboratory in the blood and muscle of trout and goldfish.

It has been postulated that fish growth hormone is antigenic towards mammals but that mammalian preparations are not antigenic towards fish[4], and these observations, showing as they do metabolic responses by a fish to mammalian growth hormone, help to support this view.

At the termination of this growth hormone investigation the adenohypophysis was examined using a variety of histochemical methods in order to determine any effect of exogenous growth hormone treatment on specific cells which might then be designated somatotrophs. No effects were observed and thus the identification of the teleost somatotroph remains still unsolved.

A. J. Matty

The Wellcome Laboratories of Pharmacology,
The Gatty Marine Laboratory,
St. Andrews, Fife, Scotland.

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HÆMATOLOGY

Serum Globulin Polymorphism in Pellagra

In recent work on the serum iron and total iron-binding capacity of groups of healthy and diseased Africans, we found the total iron-binding capacity of pellagra patients to be approximately 60 per cent of healthy controls[1]. Zone electrophoresis on filter paper in an earlier work[2] did not show that the concentration of the β-globulins in pellagra was any lower than healthy controls and this suggested that there may be a relationship between transferrin polymorphism and incidence of this disease, in view of the findings of Gibbett *et al.*[3] that the faster-migrating transferrins take up more iron.

21 sera from frank pellagra patients attending Harare and Mpilo Central Hospitals were compared with 25 samples drawn from African blood bank donors by the Smithies vertical starch-gel electrophoresis method[4]. $^{59}FeCl_3$ and hæmoglobin were added to the sera and autoradiographs prepared with one half of the gel on Kodak 'No Screen' X-ray film[3]. The other half was stained with *o*-dianisidine-hydrogen peroxide reagent[5] to establish haptoglobin types and finally counter-stained with naphthalene black. This opportunity was taken to establish the haptoglobin types, as no figures were available for Rhodesian Africans.

Table 1. Haptoglobins and Transferrins in Africans suffering from Pellagra compared with Healthy Controls

	No. of samples	Hp^{0-0}		Hp^{1-1}		Hp^{2-1}		Hp^{2-2}		TfCC		TfCD_1	
Pellagra		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
	21	2	9·5	6	28·6	11	52·4	2	9·5	18	85·7	3	14·3
Control	25	1	4	8	32	13	52	3	12	22	88	3	12

The results obtained are shown in Table 1, and there is no significant difference between the two groups for any of the phenotypes listed. The respective haptoglobin gene frequencies for pellagra and controls sera are as follows: Hp^0 0·095 and 0·04; Hp^1 0·55 and 0·58 and Hp^2 0·36 and 0·38. This suggests that the presence of a slower moving TfD does not predispose an individual to pellagra and the reason for the low serum total iron binding capacity in this condition remains obscure.

The frequency of Hp^2 in the Rhodesian African is much lower than that for a number of different population groups quoted by Harris[6], although higher than that for the West African Fulani[7], who have a very much higher frequency of Hp^0.

I thank Dr. Elizabeth Robson for supplying a reference sample of TfCD_1 serum; Dr. B. J. Baldachin and Mr. N. Gregory of Mpilo Central Hospital; and Dr. M. Gelfand and Mr. A. Seymour of Harare Central Hospital for arranging the blood samples.

W. R. Carr

The Government Analyst's Laboratory,
Salisbury,
Rhodesia, Nyasaland.

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Analogies and Differences between Human and Horse Hæmoglobin

In a previous paper[1] I reported two similar and two different histidine-containing peptides isolated from the high-basic fraction of the tryptic hydrolysate of human and horse hæmoglobin. This communication deals with some peptides isolated from the middle basic fractions of the tryptic hydrolysate of human and horse hæmoglobin.

About 1 gm. of the neutral and middle basic group of tryptic digest peptides of human and horse hæmoglobin was fractionated by ion-exchange column chromatography[2]. The individual peptides were obtained from fractions collected after purification of the peptides by high-voltage paper electrophoresis[3] using pyridine–acetate–buffer system *p*H 6·5 and *p*H 3·5 (ref. 4). Each peptide isolated from tryptic hydrolysate of horse hæmoglobin was compared with that isolated from the tryptic digest of human hæmoglobin[2]. Some individual peptides were split by enzymes or partial acid hydrolysis. The peptides Nos. 5, 6 and 7 were partially hydrolysed with 6 *N* hydrochloric acid at 105° for a period of 20–30 min.[5]. The products of partial acid hydrolysis were fractionated by high-voltage paper ionophoresis using pyridine–acetate buffer *p*H 3·5 (ref. 4). Peptide No. 10 was digested with chymotrypsin to yield two fragments. Both fragments were, after ionophoretic separation, degraded by Edman's method of paper strip modification[6]. The estimation of the N-terminal amino-acid of certain peptides was carried out by Fraenkel-Conrat and Harris paper strip technique[6]. For analysis of amino-acids the high-voltage ionophoresis[3] (2·5 per cent formic acid, 4,000 V./cm. for a period of 40 min.[7]) and paper chromatography in the *n*-butanol–acetic acid–water (4 : 1 : 5) as solvent system was used. Peptides summarized here represent those with identical electrophoretic, chromatographic pattern and other similar characteristics as, for example, both terminal amino-acid and amino-acid composition.

By comparison of these results with the recently published total structure, and chains, of human hæmoglobin[8–10] it is possible to indicate these places in the chains of human hæmoglobin, which are identical with those of horse hæmoglobin. It seems that most of the aforementioned peptides are identical in both hæmoglobins (Table 1). The different peptide No. 6 occupies the same place in the chains of horse hæmoglobin as peptide No. 5 in human hæmoglobin[11]. Peptides No. 7 and No. 13 do not occur as a product of tryptic digestion in any chains of human hæmoglobin[8–10].

Table 1

Peptide No.	Amino-acid composition and sequences	Found in tryptic digests of hæmoglobin *M*	*H*	Place in chains of hæmoglobin
1	Lys.Lys	+	−	
2	Leu.Arg	+	+	91–92α
3	Tyr.Arg	+	+	140–141α
4	Tyr.His	+	+	145–146β
5	Ala.Try.Gly.Lys	+	−	12–16α
6	Ala.Try.Ser.Lys	−	+	12–16α
7	Phe.Gly.Lys		+	118–120β
8	Val.Lys	+	+	60–61β
9	Lys	+	+	61,66α,β
10	Val.(Gly,Ala,His,Ala,Gly,Glu).Tyr. Gly.Ala.(Glu,Ala,Leu,Glu).Arg	+	+	17–31α
11	Val.His.Leu.Thr.Pro.Glu.Glu.Lys	+	−	1–8β
12*	Ala.His.Gly.Lys.Lys	+	+	62–66β
13*	Ala.His.Gly.Glu.Lys		+	

* Peptides discovered previously (ref. 1).
M, Human hæmoglobin.
H, Horse hæmoglobin.

My results are in agreement with those of Braunitzer[11] and Kaufmann *et al.*[12]. Analysis of the weak basic and neutral fraction will be published later.

I thank Dr. F. Sanger and Dr. J. I. Harris for their interest in this work, and for Dr. Sanger's permission to carry out some experiments in his laboratory during my stay in Cambridge in 1959.

PAVEL MÄSIAR

Department of Biochemistry,
Faculty of Medicine,
University of P. J. Šafárik,
Košice, Č.S.S.R.

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ANATOMY

Tetracyclines in Human Dentine

WITH the development of new techniques during the past few years, much information has been obtained on the nature of the dentinal tubules and the mode of calcification of the matrix[1].

With the observation that tetracycline drugs are incorporated in tissues which are calcifying at the time of its administration[2], another means of studying these phenomena is now available. The tetracycline is bound in the calcifying zone of the dentine and with ultra-violet light fluoresces bright yellow.

In the present investigation, several deciduous teeth from two children who had received numerous large doses of tetracycline drugs during the first few months of life have been studied. The teeth were yellow in colour, with hypoplastic enamel, most of which had worn off the occlusal surfaces, exposing the dentine.

A longitudinal section was cut from one of these teeth and the areas which fluoresced determined macroscopically or under low power. The tooth was then reorientated on the cutting machine and further sections cut to show the dentinal tubules in cross-section[3].

The preparations were examined with transmitted and ultra-violet light and micro-radiographs were prepared; the appearance under these three conditions was examined.

Longitudinal sections viewed with transmitted light appeared normal except that in some areas a greater number of interglobular spaces was seen than is usually observed in such sections. With ultra-violet light, numerous distinct yellow fluorescent bands were seen, running approximately parallel to the dentino-enamel junction.

In the fluorescent bands it was observed that the outline of the 'calcospherites' was marked by a fluorescent ring. Under high power, within and beyond these areas numerous tubules were seen in which the peritubular zones fluoresced as bright yellow rings around a central dark spot (Figs. 1 and 2). At the peripheral surface of a band, the fluorescence was confined entirely to the peritubular zone and was not detected in the matrix. Microradiographs of these

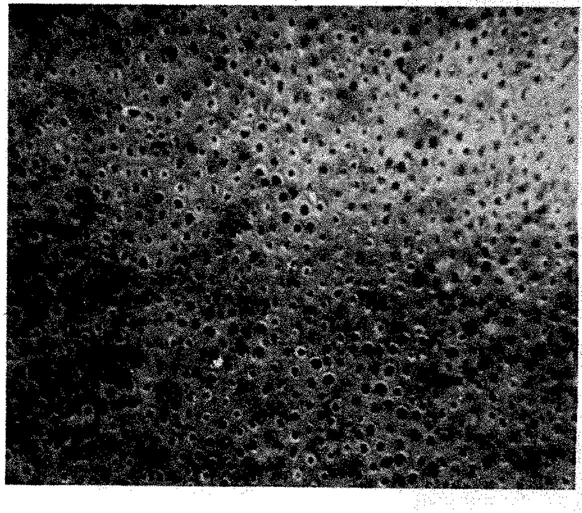

Fig. 1. Photomicrograph (× 500) of transversely cut dentinal tubules, showing well-defined peritubular zones

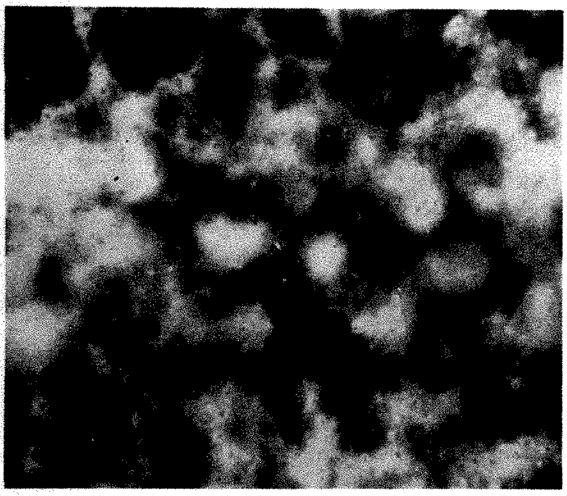

Fig. 2. Photomicrograph (× 500) of the same area as in Fig. 1, showing fluorescence with ultra-violet light. Note fluorescent circular and semi-circular areas in the matrix, and also fluorescence in isolated peritubular zones

areas showed a typical hypermineralized peritubular structure.

Since tetracycline is incorporated in tissue that is calcifying at the time of administration, the distribution of fluorescence in the matrix indicates that calcification occurs in a globular fashion. Furthermore, as the tubules with fluorescent peritubular zones are further from the pulp than the fluorescent matrix, this indicates that the laying down of the hypermineralized peritubular zone occurs at a later stage in dentine development than the mineralization of the matrix. This work tends to confirm earlier observations that the peritubular translucent zone is a manifestation of maturation of the dentine after the mineralization of the dentine matrix proper.

H. F. Atkinson
J. K. Harcourt

Dental School,
University of Melbourne.

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Morphological Changes in Red and Pale Muscles following Tenotomy

In recent experiments one of us[1] has shown that in rabbits the cutting of hind limb tendons produces changes in the pattern of electromyograph activity in a red muscle—soleus—at a time when no change in electromyograph activity can be observed in pale muscles. In conscious rabbits continuous electromyograph activity was recorded from the normal soleus, no matter whether the animal was standing, sitting, or jumping. After tenotomy no electromyograph activity could be recorded when the animal was sitting or standing. Only when the position of the animal was suddenly changed were short bursts of action potentials observed.

We have now carried out experiments to determine some of the structural changes accompanying these functional alterations. After cutting all the tendons around the ankle joint, the animals were killed at varying intervals after the operation, and cross-sections of the soleus and peroneus longus muscles were examined. It is known[2,3] that muscle fibres are only slightly reduced in size three months after tenotomy. In the experiments recorded here we were able to confirm this in the pale peroneus longus, whereas in the red soleus at a similar time after tenotomy most of the fibres have undergone complete degeneration, and those that remain are of very small calibre lying among a mass of fatty and fibrous tissue (Figs. 1 and 2). The degenerative changes in soleus are well advanced by the end of 2–3 weeks (Fig. 3).

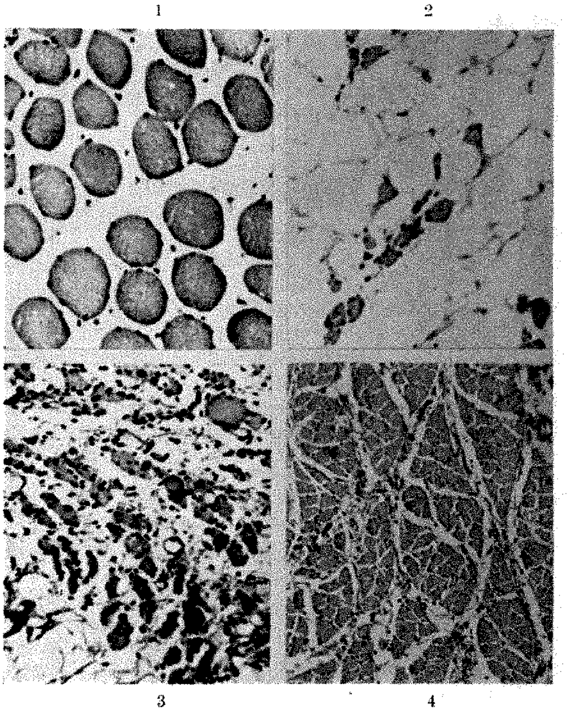

Fig. 1. Normal soleus

Fig. 2. Soleus three months after tenotomy

Fig. 3. Soleus three weeks after tenotomy

Fig. 4. Soleus three weeks after tenotomy and spinal cord section. Apart from the atrophy due to cord section, there are no other degenerative changes

(All stained by Van Gieson. × *c.* 100)

This cannot be explained by suggesting that mechanical stretch is more important for maintaining the structural integrity of red muscles than of pale, as we have also found that when the spinal cord is sectioned at the same time as tendon section the degenerative changes in soleus are negligible (Fig. 4). The finding that much of the degenerative change in the tenotomized soleus can be prevented by simultaneous section of the spinal cord suggests that impulses from supraspinal structures are responsible for the dramatic changes seen in soleus following tenotomy.

R. M. H. McMinn
Gerta Vrbová

Departments of Anatomy and Physiology,
King's College,
Strand, London, W.C.2.

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RADIOBIOLOGY

Differential Autoradiography of Tritium and Another β-Emitter by a Double Stripping Film Technique

CERTAIN investigations on cellular biochemistry may require examination of the simultaneous incorporation of two metabolites, or of the incorporation of the same metabolite at different times, by the same group of cells. These investigations may be carried out by administering metabolites labelled with two different isotopes—tritium and any other β-emitter—and then distinguishing their distributions on the same autoradiograph, by processing the respective photographic 'grains' to different colours.

In principle the desired result might be achieved by the use of a two-layer emulsion with incorporated colour couplers, the layer adjacent to the histological sample being sufficiently thick to prevent the penetration of the second layer by tritium β-particles. However, this method is likely to lack sufficient resolution and operating flexibility. An alternative method is to superimpose two autoradiographs and to colour the silver 'grains' in one or both emulsion layers by a dye-coupling technique. Coating uniformity and rigid control of coating thickness in the first emulsion layer are essential with this procedure. Initially attempts were made to coat samples by dipping in liquid emulsion, but the irregular surface of the samples (single cells or clumps of cells) interfered with the draining of the emulsion and caused non-uniform coating. It was, therefore, finally decided to use a thin layer of autoradiographic stripping emulsion without any gelatin supporting layer.

The practicability of such a procedure was demonstrated with HeLa cells grown as mono-layers on glass coverslips. The cells were exposed for a short period to tritiated thymidine, then transferred to fresh medium and, after a suitable interval of time, for a second short period to medium containing thymidine labelled with carbon-14. This procedure was designed to provide unlabelled cells, as well as cells labelled with tritium alone, with carbon-14 alone and with both isotopes, on the same coverslip. The specific activities were adjusted so that the time required to produce an autoradiograph of the tritiated compound was about one-tenth that needed for the carbon-14. The cells were histologically fixed with methanol and were stained by the Feulgen technique.

After thorough washing the cells were coated with a uniform layer of autoradiographic emulsion by the stripping-film technique. This was obtained from an experimental coating made without a gelatin supporting layer and having an overall dry thickness before stripping of $3 \cdot 4$–$4 \cdot 0 \mu$. The processed and dried emulsion was $2 \cdot 1$–$2 \cdot 5 \mu$ thick—sufficient to prevent penetration of tritium β-particles. The fragility of this emulsion necessitated some modification of the normal handling technique. An exposure of 24 hr. sufficed to record the tritium distribution, and the emulsion was then processed to give blue dye-coupled 'grains', by bleaching the developed and fixed image and redeveloping in a colour developer. As a result of the lower specific activity of the carbon-14 present, this autoradiograph was produced predominantly by the tritium.

The first autoradiograph was next coated with a thin ($0 \cdot 1 \mu$) layer of celloidin[1] to insulate it chemically from a second stripping emulsion, a Kodak *AR* 10 plate, applied over it. The second autoradiograph, which was produced exclusively by the carbon-14, was processed in the usual way after ten days exposure, to give black grains.

The first emulsion layer contained a well-defined tritium autoradiograph consisting of 'grains' of dye, whereas in the second, *AR* 10 emulsion, there was some loss of resolution due to the separation produced by the first layer. However, despite this loss of resolution, the autoradiograph was sufficiently well defined to distinguish cells containing carbon-14 from other cells. On examination with an oil immersion objective, the two images could be separated by differential focusing. Fig. 1 shows the degree of discrimination observed between cells labelled with one or other isotope, or with both. With

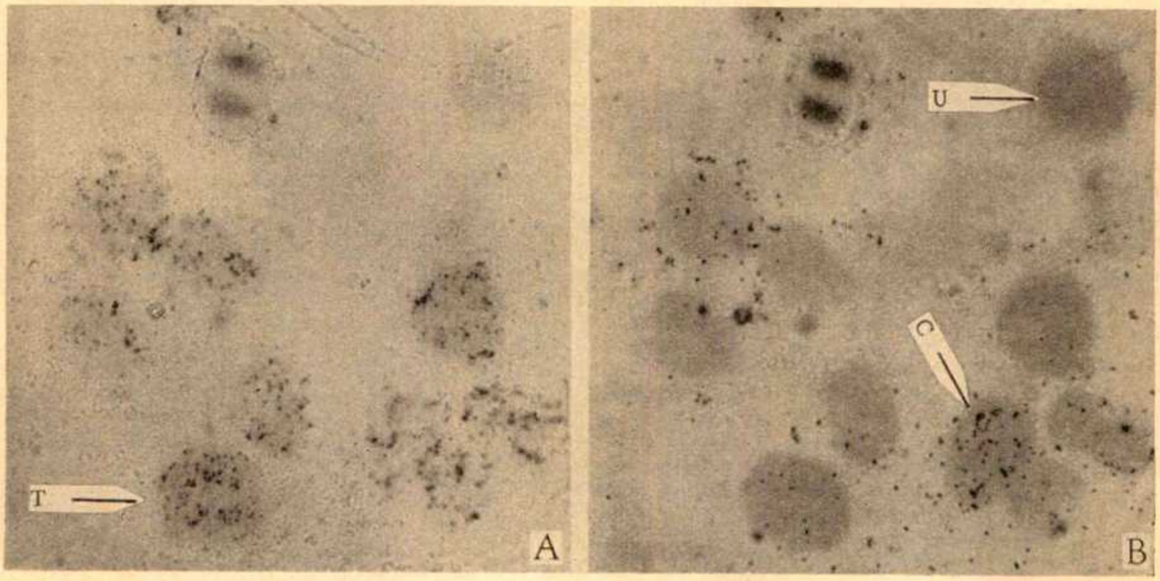

Fig. 1. Autoradiograph of a HeLa cell colony exposed to tritiated and carbon-14-labelled thymidine. *A*, Focused on first emulsion layer (tritium: blue 'grain'); *B*, focused on second emulsion layer, with deep blue filter (carbon-14: black 'grains'); *T*, cell containing tritium only; *C*, cell containing predominantly carbon-14; *U*, unlabelled cell. (Oil immersion, × *c*. 1,125)

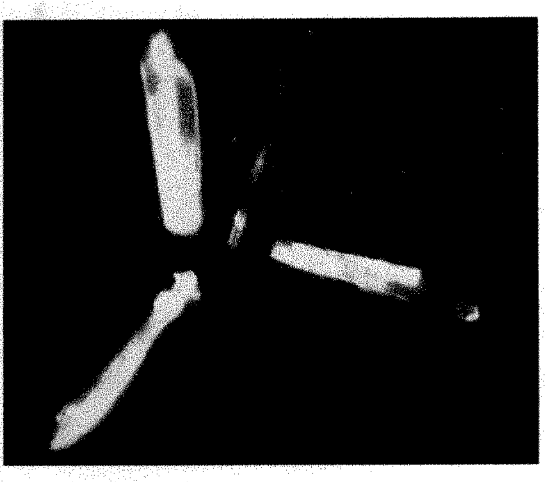

Fig. 2. Crystals from ethanol of the 'brain' hormone. (× c. 17)

obtained from the colourless, active solution in the process of cooling to $-18°$ C.

Using one of the afore-mentioned three parts as material, recrystallization was repeated with acetone and ethanol. 4 mgm. of a crystal was obtained from 3,539 mgm. of crude but active extract of the 'brain' hormone after these treatments (Fig. 2). The melting point of the crystal was 142°–143° C. Both ultra-violet and infra-red spectra were examined.

Thirty-seven days old 'dauer-pupæ' (artificially diapausing, 'brainless' pupæ) of *Bombyx mori* were prepared for use in the bioassay. When 0·02 µgm. of the crystal which was dissolved in 0·01 ml. of ethanol was injected into each 'dauer-pupæ' of *Bombyx mori*, five injected animals emerged respectively as moths on the 22nd (♀), 24th (♂), 28th (♀), 29th (♀) and 35th (♂) day following injection. However, no moth emerged from 38 control animals which were either not injected or mock injected with 0·01 ml. of ethanol without the crystal, for 100 days following pupation.

These results show that the crystal is the most important factor of the active principle of the 'brain' hormone.

We thank Prof. S. Hara, Tokyo College of Pharmacy, Tokyo, for giving us laboratory facilities, and also Y. Ishitoya, H. Akai and Miss K. Tani, Sericultural Experiment Station, Tokyo, for help with the extirpation of pupal brains.

Masatoshi Kobayashi
Jiro Kirimura
Mieko Saito

Sericultural Experiment Station,
Suginami-ku, Tokyo.

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A Change of Phototaxis and a Tropotactic Response by Larvæ of the Mustard Beetle

De Ruiter and van der Horn[1] have reported on changes in phototaxis during the larval life of *Smerinthus ocellata* L. and mentioned the problem of functional significance. Preliminary work by me on the olfactory responses of the larvæ of the mustard beetle, *Phaedon cochleariae* Fab., were hampered for a time by complete reversals of results obtained in earlier replicate olfactometer tests. The cause proved to be a response to light, and experiments similar to those of de Ruiter and van der Horn gave results comparable with those obtained by these authors, with larvæ generally positively phototactic at the beginning of an instar and negatively phototactic at the end. Observations of larval behaviour in the rearing cages showed that larvæ about to moult moved to the darker areas of the food-plant, mainly the underside of the leaf, so that the negative phototaxis shown by the larvæ is probably a response which gives protection during moulting.

After selection for equal aged larvæ in the olfactometer tests an additional response, this time to touch, was observed. In the experimental procedure two larvæ were used at a time, one at each side of a test airstream which occupied the centre of the chamber and contained the odour. A score of 0 or 1 was given, depending on whether the larvæ moved into, or away from, the test air-stream. It was found that a strong bias could be given to the results, for, when the larvæ were transferred to the chamber with a moist camel hair brush, they tended to move away from the side on which they were picked up by the brush. To overcome this, pairs of the 200 larvæ used were transferred in individual tests with the brush alternately on the opposite side of the body.

M. T. Tanton *

Imperial College of Science and Technology,
London, S.W.7.

* Present address: Nature Conservancy, Monks Wood Experimental Station, Abbots Ripton, Huntingdonshire.

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MICROBIOLOGY

A General Structure for Cell Walls of Gram-Negative Bacteria

The cell walls of a number of Gram-negative bacteria have been shown to contain a mucopeptide component similar in composition to that which forms a major fraction of the cell walls of Gram-positive bacteria[1]. We have recently analysed the cell walls of an *Escherichia coli* mutant 26–26 requiring lysine for growth. A mucopeptide was isolated from cell walls by the procedure described by Mandelstam[2] which contained glucosamine, muramic acid, alanine, glutamic acid, diaminopimelic acid, serine, lysine and aspartic acid as major components. An ethanol-insoluble fraction from trichloroacetic acid extracts of cell-wall preparations contained on hydrolysis all the amino-acids found in the mucopeptide and also ribitol, phosphate and glucose. These results suggest that the cell wall of this organism contains the characteristic mucopeptide and in addition a ribitol teichoic acid[3].

E. coli 26–26, grown in a minimal medium supplemented with sub-optimal amounts of lysine, accumulated inside the cell various precursors of these cell-wall components including uridine nucleotide mucopeptides. It also released into the growth medium uridine and cytidine nucleotides, diaminopimelic acid and lipomucoprotein[4].

The mucopeptide component represents the rigid layer described by Weidel, Frank and Martin[5] in *E. coli B*. They concluded that the cell wall consists of three layers of which the innermost is the rigid mucopeptide and the middle lipopolysaccharide with lipoprotein forming the outermost layer. The uridine nucleotide mucopeptides accumulated by *E. coli*

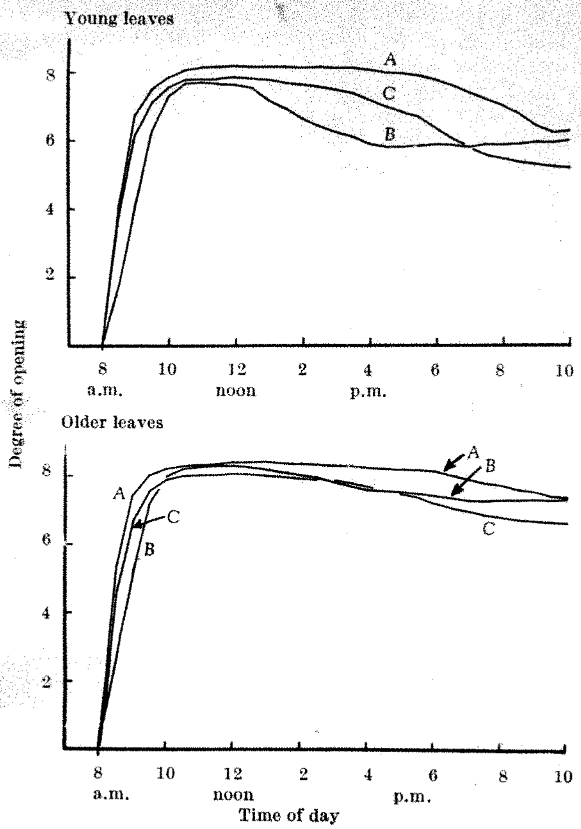

Fig. 1. Stomatal behaviour in illumination of 15,000 lux from 8 a.m. to 10 p.m. Each curve is based on observations on four leaves of different plants. Treatments prior to 8 a.m.: *A*, 8 hr. 15 lux + 8 hr. darkness; *B*, 8 hr. darkness + 8 hr. 15 lux; *C*, 16 hr. darkness. Ordinate scale: arbitrary units of total pressure minus pressure across the leaf, in a resistance porometer, that is, increasing values indicate opening

there was no response to the light in terms of stomatal opening at the time when it was given. It is clear from these and the previous results[1,2] that the 'normal' light responses of stomata, that is, day-time opening in high-intensity light, can to some extent be modified according to the nature of the immediate pretreatment. This leads to the belief that there are two kinds of response to light; first, there is the 'high-intensity' response which operates through changes in carbon dioxide level brought about by photosynthesis[4]; secondly, there is a 'low-intensity' response which is recognizable in after-effects—the opening controlled by carbon dioxide can apparently be modified by a preceding 'low-intensity' treatment; this is probably due to light-induced changes in an endogenous rhythm.

In many species the rhythms of stomatal aperture in light are more pronounced than in *X. pennsylvanicum* (some unpublished experiments have shown that it does not exhibit a true persistent rhythm), and the closure during the first cycle is considerably greater, cf. soybean 'Hernon'[3]. In these the effects described may prove to be of greater importance.

Changes in the pattern of stomatal behaviour involving early 'afternoon closure' could perhaps result in differences in the degree of transpiration and so affect water economy. It might be possible to exert some control over transpiration by suitable manipulation of low-intensity light treatments given at night.

I thank Prof. O. V. S. Heath for very valuable discussion. This work is supported by a grant from the Agricultural Research Council.

T. A. Mansfield

University of Reading,
Horticultural Research Laboratories,
Shinfield Grange,
Shinfield, Berks.

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ENTOMOLOGY

Crystallization of the 'Brain' Hormone of an Insect

The extraction of the 'brain' hormone from the dissected pupal 'brain' in the silkworm, *Bombyx mori*, was carried out by Kobayashi and Kirimura[1]. Since then about 500,000 'brains' dissected out free of corpora cardiaca and corpora allata of pupæ have been used as material in the purification of the hormone.

We have succeeded in isolating and crystallizing the 'brain' hormone by several treatments of a fraction which is separated by the counter-current distribution from a crude but active extract of the 'brain' hormone as follows.

Crude 'brain' hormone extract was prepared from 220,000 'brains' dissected from *Bombyx* pupæ by the method as shown in Fig. 1.

Several active fractions were separated by means of the counter-current distribution, using the same solvent system as the method of Cole *et al.*[2]. Colourless, active solution was separated from one of the active fractions with petroleum ether (b.p. 40°–60° C.). Three kinds of non-purified parts were separated by organic solvents from the precipitate which was

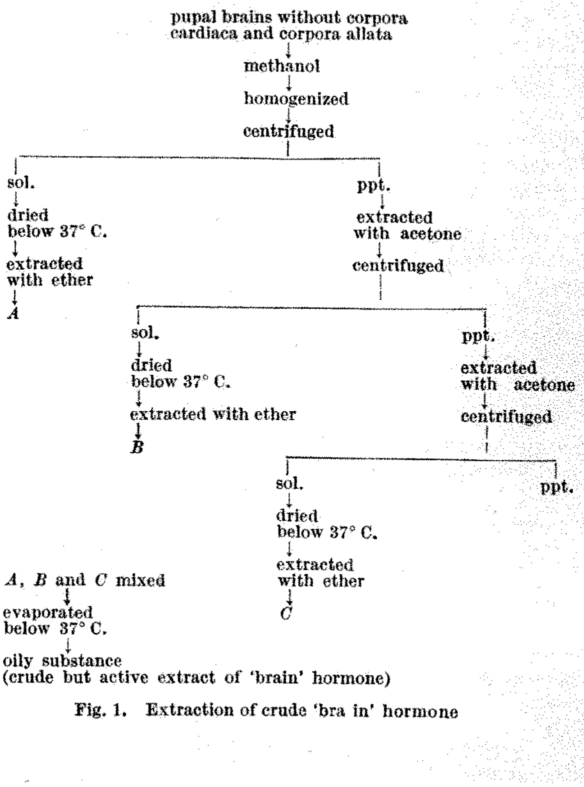

Fig. 1. Extraction of crude 'bra in' hormone

close to 30,000 lb./in.^{2} at the ultimate stress. Although little is known about the mechanical properties of apatite, this is surely more than it could bear in bulk.

In two-phase materials such as fibreglass[5,6] there are fibres having a high modulus of elasticity and a high ultimate tensile strength embedded in a matrix having a low modulus of elasticity and, usually, a low strength. This produces a material with a stiffness intermediate between those of the two components, and with a strength higher than either of the two components. The increased strength is attained because any cracks forming in the stiff fibres, which would normally spread right through the whole structure and cause it to fail, run into the flabby matrix, which will not transmit the crack, but will merely deform.

At first sight, bone appears to be built on a plan contrary to that adopted for man-made two-phase materials: the fibrous collagen is strong, but has a low value for E, and the apatite matrix is weak, but has a high value for E. However, the essential feature of bone from the mechanical point of view is that the apatite does not occur in bulk, with fibres of collagen running through it, but that it is instead in the form of very small crystals. These crystals are of the order of size 500 Å. by 40 Å. (ref. 7), and they can be considered to be very short fibres embedded in a collagenous matrix. This being so, the chances of any particular crystal being large enough to contain a dangerous Griffith crack are small. What cracks there are will spread only under the influence of very high stresses. If they do spread they will immediately run out of the apatite crystals into the collagen, which will deform under their influence, but will not rupture. It is probable, therefore, that the apatite in bone is very strong in tension.

This arrangement requires that the apatite and the collagen should be bound fairly firmly together. At the moment it is not certain what forces are binding the two components. It is possible that it may be simple hydrogen bonding or, though less likely, the small amount of mucopolysaccharide in bone may be acting as a cement.

If this scheme is correct, the great advantage of bone over most of the other heavily calcified skeletal materials found in the animal kingdom is apparent. In other groups the calcifying material is usually in the form of large crystals of calcium carbonate, and so dangerous Griffith cracks can easily form in them. This tendency is carried to an extreme in some echinoderms, in which the crystals may have dimensions of several centimetres, although in this group the crystals have a porous structure that may help to interrupt cracks. Bone combines high strength, particularly tensile strength, with a high modulus of elasticity. The superiority of bone over most other skeletal materials is probably one of the main foundations of the success of the vertebrates.

J. D. CURREY

Department of Zoology,
University Museum,
Oxford.

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Stomatal Sensitivity to Low-Intensity Illumination in *Xanthium pennsylvanicum*

EVIDENCE was found in previous work with *X. pennsylvanicum*[1,2] that an endogenous rhythm which influenced the behaviour of the stomata occurred in darkness. The rhythm was shown to be highly sensitive to light and, at 27° C., 10 lux given instead of darkness with sufficient to delay its progress[1].

A recent experiment has demonstrated a somewhat different effect of low-intensity illumination. *Xanthium* plants grown to the eight-leaf stage in the glasshouse in 16-hr. days, consisting of 8 hr. daylight + 8 hr. of low-intensity supplementary light, were placed in controlled condition cabinets (temp. 27° C.) and after an 8-hr. day of high-intensity light were subjected to one of the following treatments: *A*, 8 hr. of 15 lux + 8 hr. of darkness; *B*, 8 hr. of darkness + 8 hr. of 15 lux; *C*, 16 hr. of darkness; these were followed by a period of high intensity light (15,000 lux) during which measurements of stomatal opening were made using an automatically recording porometer[3]. Illumination was from incandescent lamps with a screen of 1 cm. of water, and the intensity was reduced to 15 lux by means of white paper. For each treatment the measurements were replicated on two leaves (5th and 6th) on each of four plants.

There were significant effects of the treatments on the course of stomatal behaviour during the subsequent period of high light. These effects differed according to the age of leaf and consequently the curves of stomatal behaviour in younger and older leaves are presented separately (Fig. 1). It will be seen that in the younger leaves after treatment *B* the stomata began to close early in the afternoon and by 4 p.m. the degree of opening, on the scale used[3], was less than 75 per cent of that in leaves that had received treatment *A* (significant at $P < 0{\cdot}001$). After 4 p.m., however, there was no further closure in *B*, but in *A* the stomata began to close and continued until about 9.30 p.m. After treatment *C* the 'afternoon closure' occurred at a time more or less mid-way between that in the other treatments. A further difference was in the rates of opening after the high light was switched on at 8 a.m.: that in *B* was significantly slower than in *A* and *C*.

In older leaves the effects were less marked; opening after full illumination at 8 a.m. was again significantly slower in *B* than in *A* and *C* but differences in levels of aperture at 4 p.m. were not.

A possible explanation of these effects is that in *A* and *B* a 'light rhythm' commenced when illumination was given after the 8 hr. period of darkness, namely, in 15,000 lux at 8 a.m. in treatment *A*, and 8 hr. earlier, at the beginning of the period of 15 lux, in treatment *B*. Thus the rhythm was out of phase in the two. The fact that the behaviour in *C* differed from both *A* and *B* may have been because the 'dark rhythm', in which the phase is determined mainly by the time of onset of darkness[2], had proceeded for 16 hr. and at 8 a.m. had already reached the 'night opening' stage during which the guard cells had presumably passed into a condition similar to that brought about by light. They would have been in this condition for about 4 hr.[2] prior to 8 a.m., which would explain the finding that the closure in *C* occurred at a time mid-way between *A* and *B*.

These observations are interesting because they provide additional evidence of stomatal sensitivity to low-intensity light. This sensitivity has been detected as after-effects; so far as could be measured brood the eggs to a varying degree, to the single known viviparous species.

This communication records the brooding habit for one additional species, adds some details to the process in another and lists all the occurrences which I have been able to trace in the literature, in the hope that more attention will be given to this aspect of the biology of the Amphineura and that additional cases may be brought to notice.

While examining specimens of *Ischnochiton constanti* (Vélain) collected by the *William Scoresby* off Gough Island in 40–60 fathoms, it was noted that one specimen had a clump of eggs fixed to the branchial groove on each side. The use of the above name for this species, now known from St. Paul and Amsterdam, southern South Africa and Tristan da Cunha and Gough Islands, has been discussed elsewhere[2]. A specimen of *Sypharochiton nigrovirens* (Blainville) from Saldahna Bay, South Africa, collected during the *Discovery* Investigations in the southern hemisphere, proved to have fully developed young clustered under the girdle edge, some 33 on the left side and 22 on the right.

In most species of the Amphineura which have been investigated, eggs and sperm are shed into the surrounding sea water where fertilization and subsequent development occur. There has been some confusion as regards the method of reproduction in the European *Trachydermon cinereus* L., apparently because the details of the process may differ in individuals. From Thorson's[3] summary of the position it now appears that, in this species, eggs and sperm may be released freely into the water, or the fertilized eggs may be extruded in groups which adhere to the substrate, or the fertilized eggs may be retained between the mantle and the gills until they develop into embryos. Thorson believes that the varied method of egg deposition is of slight significance since the embryos all hatch at the same stage in all cases, but in view of the sequence outlined here, this case may perhaps demonstrate how a more persistent brooding habit could develop.

Several species in widely different families have now been recorded as retaining the fertilized eggs within the branchial groove. In some cases the method of subsequent development has not been recorded and it is therefore possible that the developing young may also be brooded by the adult in some of these species.

The brooding of eggs within the branchial groove has been recorded in the following species: *Middendorffia capreanum* (Scacchi) (= *Chiton polii* Philippi)[4], *Ischnochiton imitator* (Smith)[5], *Lepidochitona raymondi* (Pilsbry)[6], *Trachydermon cinereus* L.[3], *Heterozona subviridis* (Iredale and May)[7], *Ischnochiton constanti* (Vélain)[1], *Chiton barnesi* Gray[5], *Nuttalina thomasi* (Pilsbry)[6].

In some cases it has been observed that the eggs and young are retained in the branchial groove until the young have eight well-developed valves. This is true for: *Sypharochiton nigrovirens* (Blainville)[1,8,9], *Hemiarthrum setulosum* Dall[10,11]. Plate[5] has given an account of reproduction in the only known viviparous member of the group, *Callistochiton viviparus* Plate.

The species listed here as demonstrating some stage in the development of viviparity are distributed in four families, the Hanleyidae (*Hemiarthrum*), Ischnochitonidae (*Ischnochiton*, *Heterozona*, *Trachydermon*, and *Lepidochitonida*), the Chitonidae (*Chiton* and *Sypharochiton*) and the Callistoplacidae (*Callistochiton*, *Middendorffia* and *Nuttalina*). It would therefore appear to be a habit which is widely spread systematically in the Amphineura. There is also a wide geographical range for the known occurrences, embracing Europe, the Mediterranean, western North America, southern South America, South Africa, the Subantarctic Islands and southern Australia. It will probably be found to be much more common when the method of reproduction is known for more species. That the habit is no necessary barrier to dispersal is shown by *Hemiarthrum*, which has achieved very wide distribution in the Southern Hemisphere.

R. K. Dell

Dominion Museum,
Wellington,
New Zealand.

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Strength of Bone

SURPRISINGLY little attention has been paid to the way in which collagen and apatite, the major components of bone, combine to produce the mechanical properties shown by bone. These properties are not, in fact, what might be expected if the properties of collagen and apatite are the same in bone as they are when considered separately, in bulk.

Ceramic materials are characteristically very much stronger in compression than in tension. Thus heavy-duty electrical porcelain has a compressive strength of about 100,000 lb./in.^{2}, but a tensile strength of only 5,000–8,000 lb./in.^{2} (ref. 1). The reason for this[2] is that minute Griffith cracks in the material cause intense stress concentrations, and so will tend to spread under the influence of tensile stresses, which tend to open up the cracks, but not under the influence of compressive stresses, which will tend to force the sides of the crack together. It is most likely, therefore, that the ceramic component of bone, apatite, would have a low tensile strength in bulk. Collagen, on the other hand, can in certain circumstances have a very high tensile strength, about 80,000 lb./in.^{2} (ref. 3). Collagen has a low modulus of elasticity, E (the modulus of elasticity) being about 200,000 lb./in.^{2}. The value for E for bone is about $2 \cdot 5 \times 10^6$ lb./in.^{2} (ref. 4), which is more than ten times the value for collagen. Therefore apatite must have an elastic modulus that is higher still. Collagen and apatite combine to produce a third material, bone, with a tensile strength of about 15,000 lb./in.^{2}. The compressive strength of bone is about 25,000 lb./in.^{2}.

In an ordinary compound bar, if the two components have the same volume and one has a modulus of elasticity ten times that of the other, then the forces acting on the bar as a whole will be distributed in the ratio 10 : 1 between the components, with the component with a higher value for E bearing the greater part of the force. Therefore, at an ultimate tensile stress of 15,000 lb./in.^{2}, the component with the higher value for E will be bearing a tensile load of 27,300 lb./in.^{2}. If bone is considered to be a compound bar, the apatite should be bearing a load in tension round herrings raises interesting problems concerning the origin of the Dussumieriidae and their relationship to the Clupeidae. The Dussumieriidae can be split into two sub-families (accepting the round herrings as a whole as a distinct family), the more primitive Dussumieriinae and the more clupeid-like Spratelloidinae. From the evidence of pelvic scutes, the latter sub-family could now usefully be divided into two tribes, those with modified and those with unmodified pelvic scutes, for in the latter group, comprising the southern African forms and *S. malabaricus*, the species all share several further characters distinguishing them from *Spratelloides* and *Jenkinsia* (notably the rather advanced pelvics, the lack of a second supra-maxilla, possession of a toothed premaxilla, and a rather different postero-ventral outline to the gill-opening). But except for the first of these differences, the remainder suggest affinities as much with the Dussumieriinae, and especially *Etrumeus*, as with the Clupeidae. It may be that the southern African species are derived from forms which lay close to the original separation of the clupeids and the dussumieriids.

A systematic account of the round herrings is in preparation.

P. J. P. Whitehead

Department of Zoology,
British Museum (Natural History),
London, S.W.7.

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Effect of Abrasion on the Cuticular Scale Pattern of some Mammalian Fibres

The observation that halo-hairs from lambs 2–4 months old showed a waved, crenate cuticular scale pattern not previously described in sheep, and not shown by halo-hairs taken at birth, suggested that the scale pattern had been drastically changed by weathering. Halo-hairs which had developed the crenated scale pattern stained much more readily with methylene blue than did those with smooth scale margins, indicating that gross scale damage may be a factor in the greater affinity for dyes shown by weathered wool.

Fibres from a number of species were treated by rubbing with abrasives (sand paper, jewellers' rouge) and the following alterations were brought about: halo-hairs taken at birth developed the pattern seen in those taken from older lambs; the pattern of the basal region of human hairs (irregular waved mosaic, smooth margins) was converted to a good approximation to the pattern typical of the distal region (wave with ripple to crenate margins); the smooth-margined regular mosaic or diamond petal pattern of the basal part of rat monotrichs and of kolinsky guard hairs was converted to, or towards, a waved, near, crenate pattern. In working on fibres with abrasives it appeared that possibly the lateral edges of the scale wore away first and became invisible, creating a waved pattern, and then the distal margins chipped and wore down, so that the pattern changed towards "near, crenate".

Examination of numerous other fibres and photomicrographs[1,2] confirmed that in all fibres which showed the waved crenate type of pattern this tends to occur distally, the basal or root end almost invariably showing smooth margins and mosaic or petal patterns. Faint traces of the original lateral divisions between scales can often be discerned in the more distal parts. Scale margins of fine wool fibres near the tip in weathered fleeces have been found to show chipping similar to that which produces crenate patterns on coarser fibres, and the scale pattern of sheep face hairs is often waved, near, crenate, except close to the root.

Kassenbeck[3] attributes differences in scale pattern to biomechanical forces within the follicle, and correlates crenate and mosaic scale patterns with ortho- and para-cortex respectively. The facts reported here are not incompatible with his theory that forces within the follicle determine such differences as those between mosaic and petal patterns, but is at variance with his explanation of waved crenate pattern types. If the latter are, as now seems probable, due to external friction acting on the fibre above the skin surface it seems unlikely that they can be associated only with orthocortex as Kassenbeck suggests. Hausman[4] considered that the change in form of the cuticular scales along the length of mammalian fibres is due to greater external friction towards the tips, but did not suggest that crenations and similar irregularities of the scale margins can be produced by wear.

It is concluded from these observations that probably all mammalian fibres have smooth-margined cuticular scales when they emerge from the follicle, and very likely that the patterns are always some form of mosaic or petal; all rippled and crenate, waved patterns being derived from the original patterns by wear. The smooth scale margins of wool and adult kemp of sheep are believed to be due to protection within the fleece. These conclusions are of significance for fibre identification and also in connexion with dyeing and other commercial processing of mammalian fibres; they give further justification for the customary use of the proximal parts of fibres for electron microscopy and chemical tests, and suggest that comparative work on distal regions and on fibres known to have suffered abrasion would be valuable.

Marca Burns

Wool Biology Unit,
Department of Textile Industries,
University of Leeds.

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Stages in the Development of Viviparity in the Amphineura

The Amphineura are usually considered to demonstrate many primitive features among the Mollusca. It is therefore surprising that more attention has not been given to a study of methods of reproduction in the group. Reference to the reproduction of various species is scattered through the literature; but there have been few attempts since Simroth[1] to give a general account for the group. The sexes are separate, and it is usually stated that fertilization is external, although one truly viviparous species has been long recognized and is usually quoted. It does not appear to be generally recognized that a complete series can be traced within the group from species in which fertilization is external through those which the high-power dry objective, owing to the greater depth of focus, cells containing tritium alone, carbon-14 alone, and both isotopes together are readily distinguished from one another and differential counts can be rapidly carried out.

It was found that even thinner, unsupported emulsions can be handled by the stripping film technique; these would cause less loss of resolution in the second emulsion but are more likely to be penetrated by tritium β-particles. Full details of the above technique and its experimental applications will be published elsewhere.

K. B. Dawson
E. O. Field

Radiobiological Research Unit,
Institute of Cancer Research,
Royal Cancer Hospital,
Fulham Road, London, S.W.3.

G. W. W. Stevens

Research Laboratory,
Kodak Ltd.,
Wealdstone,
Harrow, Middlesex.

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BIOLOGY

Abdominal Scutes in the Round Herrings (Dussumieriidae)

The Dussumieriidae are usually distinguished from the Clupeidae or true herrings at familial (or subfamilial) level by their lack of abdominal scutes, although one or two modified pelvic scutes have been described (for example in *Etrumeus*[1]). There have been records of fully scuted round herrings; but these all prove erroneous on closer examination. Thus Fowler[2] questioned the inclusion in *Spratelloides* of *S. madagascariensis* Sauvage because the original figure[3] shows the ventral profile with unmistakable scutes. But Bertin[4], in re-describing this species, is emphatic: "Cette carène n'existe pas". Smith[5], evidently accepting Sauvage's figure rather than Bertin's description, made *S. madagascariensis* the genotype of *Pellonulops* Smith, which he described as having 19–20 scutes (that is, both before and behind the pelvic fins). I have now examined one of the two syntypes of *S. madagascariensis*, and there are in fact no abdominal scutes other than the single pelvic scute found in all Dussumieriidae. The "hard, sharp keel on the thoracic region in front of the pectorals" in *Gilchristella aestuarius* (Gilchrist) mentioned by Barnard[6] is not due to scutes, but is formed of the lower edges of the two cleithra meeting in the mid-line; in some specimens it is quite pronounced.

In revising the round herrings, I have found a useful division can be made according to the form of the pelvic scute. In the first, or modified, type there is a W-shaped scute immediately anterior to the pelvic fins, the arms of the W extending laterally round the base of the fin rays. This type is described and figured by Chapman[1] and is shown in Fig. 1. It is found in *Etrumeus*, *Dussumieria*, *Spratelloides* (except *S. malabaricus*—see below) and in *Jenkinsia*. In *Etrumeus*, but apparently not the other genera, there is also a second, oval scute lying immediately behind the pelvic fin base.

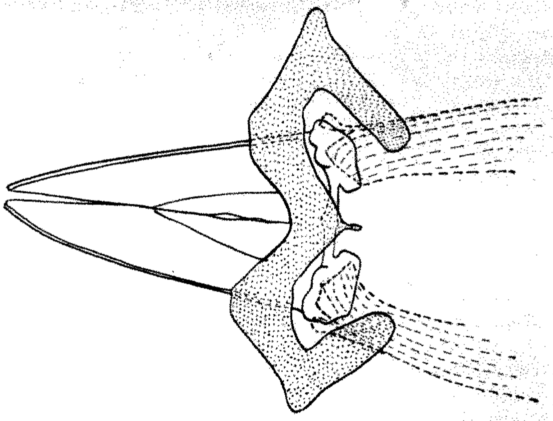

Fig. 1. Ventral view of the modified pelvic scute (stippled) in *Spratelloides delicatulus*. Pelvic fin-rays indicated by broken lines

The second type of pelvic scute, here termed the unmodified type, closely resembles the normal clupeid scute. The base lies immediately anterior to the pelvic fins, but laterally there is a vertical ascending process which is flat and pointed at its tip (Fig. 2). The unmodified type is found in the South African and Madagascar species, *Spratellomorpha bianalis* (Bertin), *Gilchristella aestuarius* and *G. madagascariensis*, and also occurs in *Spratelloides malabaricus* Day of India. The unmodified pelvic scute of *S. bianalis* was figured and mentioned by Bertin[4], but he described it as "l'écaille qui précède cette nageoire"; the scales in fact overlie the scute.

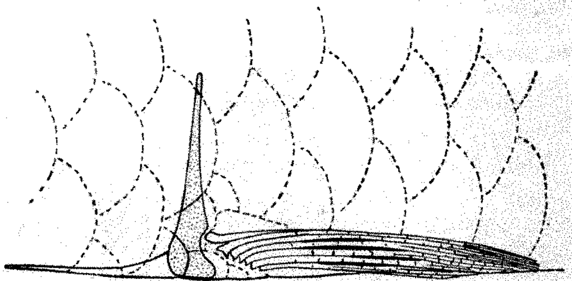

Fig. 2. Left lateral view of the unmodified pelvic scute (stippled) in *Spratelloides malabaricus*. Scales indicated by broken lines

Hitherto, confirmed records of scutes in the Dussumieriidae have been confined to a single pelvic scute, with one case of a second pelvic scute behind this fin. But in two specimens of *G. aestuarius* in this Museum, both from Durban (B.M.Reg. No. 1915.7.6.3 and 1919.9.12.3), there are 6 or 7 unmodified scutes lying in series from just behind the pectoral fins to about four-fifths the distance to the pelvic base (Fig. 3). These scutes, like the unmodified pelvic scutes, have thin, pointed ascending arms and are not keeled but rounded ventrally. Their presence is not easily detected until the specimen has been allowed to dry out somewhat.

If these two specimens are not merely chance mutations, then the presence of true scutes in the

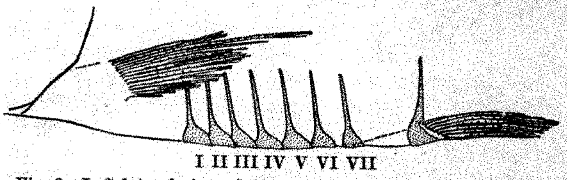

Fig. 3. Left lateral view of the unmodified pelvic and abdominal scutes (stippled and numbered I–VII) in *Gilchristella aestuarius*

26–26 are most likely to be precursors of the rigid layer and we believe the lipomucoprotein found in the growth medium to be fragments of the two outer layers

The Gram-positive bacterial cell wall is clearly visible in electron micrographs as an outer layer distinct from the cytoplasmic membrane It has always been more difficult to distinguish between the cell walls and cytoplasmic membranes of the Gram-negative bacteria

We should like to suggest that our findings and those of Weidel are compatible with the hypothesis that the surface layers of these organisms consist of two 'unit' membranes with a rigid layer in between This is analogous to the double membranes formed by the folding back of the 'unit' membranes to form myelin sheaths in nerve fibres[6] Each membrane would have the 'unit' membrane structure of protein–lipid–lipid–polysaccharide This would allow for a 'cytoplasmic membrane' of this structure, surrounded by a 'rigid wall' composed of mucopeptide and probably teichoic acid, and an 'outer membrane' with the membrane layers reversed to give polysaccharide–lipid–lipid–protein

This structure is consistent with the layers to be seen in electron micrographs of *E coli* by Kellenberger and Ryter[7] and more recently by Murray[8] It is suggested that the rigid layer could be linked to either the inner or outer membrane, giving either but not both membranes the appearance of rigidity

Recently, Brown, Drummond and North[9] have shown that two distinct membranes can be seen in electron micrographs of whole cells and spheroplasts of the marine pseudomonad N C M B 845 We would therefore suggest that a double membrane surface structure may be characteristic of all Gram-negative bacteria

PATRICIA H CLARKE
M D LILLY

Department of Biochemistry,
University College, London.

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Transmissible Drug-resistance Factors and Formation of Multiple Resistance

IN earlier reports several types of drug (chloramphenicol, *Cm*, tetracycline, *Tc*; streptomycin, *Sm*, sulphanilamide, *Da*) resistant *Escherichia coli* and *Shigella* were described[1-3] It was also found that seven types of drug resistance are transmitted by cell-to-cell contact[4]

According to the facts that these types of drug resistance are infectively transmitted and eliminated spontaneously or by treatment with acriflavin[5], the presence of transmissible drug-resistance factor (*R*) has been assumed

We are concerned in this communication with the linkage of *R* (*Tc*) and *R* (*Cm Sm Sa*) and formation of *R* (*Cm Tc Sm Sa*) factor *E coli K*–12 substrain *PA*200 F^- auxotroph resistant to (1) tetracycline, and (2) chloramphenicol, tetracycline, streptomycin and sulphanilamide, and *Shigella flexneri* 3*a* 86 resistant to (1) chloramphenicol, streptomycin and sulphanilamide, and (2) chloramphenicol, tetracycline, streptomycin and sulphanilamide were used All the strains used were able to transmit their drug resistance by mixed cultivation

E coli K–12 strain F^+ prototroph were used as the recipient of the drug resistance by mixed cultivation The temperate phage *P1kc* which adapted to *E coli K*–12 F^+ prototroph was used in the transduction of *R* factor

The cells were collected after over-night culture in Difco brain-heart infusion broth at 37° C One ml of this culture was inoculated into 9 ml brain-heart infusion broth at 37° C in Monod's shaker for 3 hr When the growth reached the middle of logarithmic phase, 1 ml of the donor cell culture was inoculated mixing in 9 ml of the recipient cultures at 37° C in a Monod's shaker The ratio of the donor to recipient of the drug resistance was approximately 1 10 The total number of cells in mixed cultivation was c 10^9/ml , and the increase in number of bacteria was not observed during the incubation of mixing culture (Fig 1)

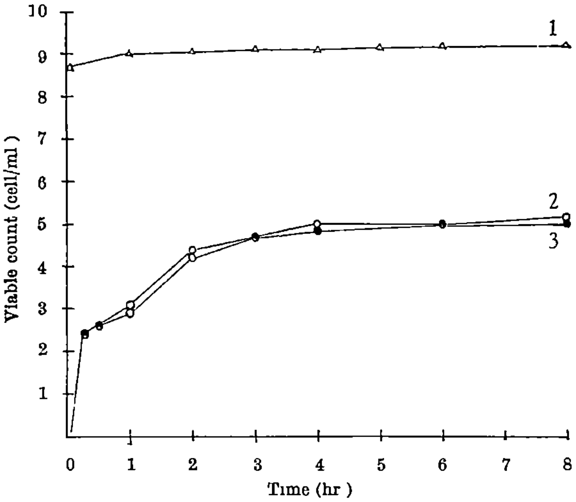

Fig 1 The time course of the transmission of tetracycline resistance and formation of multiple resistance *E coli K*-12 *PA* 200 F^- *R* (*Tc*) and *E coli K*-12 F^+ prototroph *R* (*Cm Sm Sa*) were mixed at time 0 At different intervals of time samples were plated on *ALGa* agar containing tetracycline or chloramphenicol and tetracycline to select the recipient *E coli K*-12 F^+ prototroph, which acquired tetracycline resistance 1, Total number/ml of cells in mixed cultivation, 2, number of cell/ml of *E coli K*-12 F^+ prototroph on *ALGa* agar containing 25 μgm tetracycline, 3, number of cell/ml of *E coli K*-12 F^+ prototroph on *ALGa* agar containing both 25 μgm /ml of chloramphenicol and tetracycline

At desired intervals, an aliquot of the sample was withdrawn, diluted with buffered saline (*p*H 7 2) and streaked on selective media to obtain the recipient cells which acquired drug resistance by mixed cultivation

Medium *ALGa* agar containing drugs was used for the selection of *E coli* from the mixed culture, while *AGGa* agar containing drugs was used for the selection of *Shigella flexneri* 3*a* The *AGGa* agar consists of 1 l of medium *A* agar (ref 6), 5 gm of glucose and 10 gm of sodium glutamate The *ALGa* agar has the same composition with the exception of 5 gm of lactose instead of glucose

The transmission frequency of drug resistance was calculated by dividing the number of recipient cells per ml which acquired drug resistance by the number of donor cells per ml input

For the transduction of the drug-resistance factor *R*, the method described by Lennox[7] was adopted, and for the selection of the transductants which acquired drug resistance, the heart infusion agar plates containing chloramphenicol (25 mcgm /ml), tetracycline (25 mcgm /ml) and streptomycin (25 mcgm /ml), respectively, and synthetic agar plate containing sulphanilamide (100 mcgm /ml) were used

The time course of the transmission of tetracycline resistance to *E coli K*–12 F^+ prototroph *R* (*Cm Sm Sa*) is shown in Fig 1 The transmission frequency of tetracycline resistance to *Sh flexneri* 3*a* 86 *R* (*Cm Sm Sa*) after 4 hr incubation was 10^{-4} [2]

A single colony of the recipient *Sh flexneri* 3*a* 86 which acquired tetracycline resistance and became resistant to four drugs (R_4), namely chloramphenicol, tetracycline, streptomycin and sulphanilamide, was purified by three successive single colony isolations from plates containing both chloramphenicol and tetracycline, followed by three successive inoculations in brain-heart infusion broth

The brain-heart infusion cultures were streaked on normal agar plate The drug resistance of 875 single colonies thus obtained was determined, and all were proved to be resistant to four drugs By the acridine treatment, resistance to four drugs was eliminated and no segregated loss was found

The resistance of *Sh flexneri* 3*a* R_4 to four drugs (chloramphenicol, tetracycline, streptomycin and sulphanilamide), obtained from the mixed cultivation of *Sh flexneri* 3*a* R_3 (chloramphenicol, streptomycin and sulphanilamide) with *E coli K*–12 R_1 (tetracycline), was also transmitted by mixed cultivation and made the recipient cells resistant to the four drugs

The recipient cells showed no segregation of resistance to the four drugs regardless of the difference of selective media The transmission frequency of resistance to the four drugs thus obtained was identical to that of the *Shigella* strain resistant to four drugs obtained from the fæces of humans by mixed cultivation with *E coli K*–12 F^+ prototroph, and no segregated transmission was found in retransmission of resistance to the four drugs thus formed by agitation with blender treatment

The resistance to four drugs which was produced by mixed cultivation of *Shigella* with *E coli* was transmitted to *K*–12 F^+ prototroph The multiple resistance of *K*–12 prototroph thus obtained was transduced by the phage *PIkc* to *K*–12 F^+ prototroph

From these results it would seem possible that R_1 (*Tc*) and R_3(*Cm Sm Sa*) factors become linked when the *R* (*Tc*) factor is infectively transmitted to the cells containing *R* (*Cm Sm Sa*) factor and the cells become resistant to the four drugs

S Mitsuhashi
K Harada

Department of Microbiology,
School of Medicine,
Gunma University,
Maebashi, Japan

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GENETICS

A Body Colour Mutation in the German Cockroach

For many years various cockroach species have been used extensively as experimental animals, particularly in physiological investigations and, more recently, in studies on insecticide resistance Generally speaking, however, there is little known concerning the genetics of cockroaches For some time we have carried out a search for heritable traits in the German cockroach, *Blattella germanica* (L), along a somewhat similar line to that conducted in the mosquito, *Aedes aegypti*, by Craig and VandeHey[1,2] An autosomal recessive, balloon-wing[3], and a tergal abnormality[4] have already been described The genetics of an orange-body colour mutation (*or*) is presented here, as well as the results of tests for linkage of this trait with balloon-wing (*ba*) Crosses with a DDT-resistant strain were also made, but the results are presented elsewhere[5]

Phenotypic expression of the orange-body mutation appears to resemble most closely that of brown body (*bwb*) in *Musca domestica*[6] and yellow in *Drosophila melanogaster*[7] It occurred spontaneously and was first detected in a laboratory strain from Orlando, Florida It was later noted to occur with varying frequency in a number of other laboratory strains, some originating from widely separated geographical localities in the United States *Or* nymphs were separated from these strains and a pure-breeding colony was established. Crosses were then made with wild-type and balloon-wing individuals for genetical study The sexes were separated as nymphs by the method described by Ross and Cochran[8], and matings were made soon after the insects matured

The *or* mutants are easily distinguished from wild-type cockroaches in all post-embryonic stages, although the difference in colour is most striking in nymphs The mutant nymphs are a bright, orange-brown colour Wild nymphs appear black to the naked eye, except for a small, yellowish area on the thorax *Or* adults are lighter and more of an orange-brown colour than wild-type adults They are most readily distinguished by brown, rather than black, longitudinal bands on the pronotum Both *or* and wild-type females have a darker body colour than the corresponding males Hybrids are indistinguishable from wild-type in body colour

In the crosses with wild-type cockroaches, it was found that *or* acts as a simple recessive The results are summarized in Table 1 There was no significant difference between the reciprocal crosses and no significant deviation from a 1 1 sex ratio Consequently, neither reciprocal crosses nor sexes are listed separately Statistical deviations from the expected 1 1 ratio for *or* × hybrid (Table 1, III) and 3 1 ratio for hybrid × hybrid (Table 1, IV) were well within the expected range of variation ($\chi^2_1 = 0\ 31$, $P > 0\ 50$, and $\chi^2_1 = 0\ 44$, $P > 0\ 50$, respectively)

Table 1 Results of Various Crosses involving Orange-Body and Wild-Type German Cockroaches

	Mating	No of crosses	No of wild-type of offspring	No of *or** offspring
I	*or* × *or*	13	0	400
II	*or* × wild	7	255	0
III	*or* × hybrid	10	189	200
IV	hybrid × hybrid	20	441	156
V	hybrid × wild	6	227	0

* *or*, Orange body colour

In the F_2 from a single cross between *or* and wild-type, the results were so peculiar as to warrant special attention There were 337 wild-type and 7 *or* progeny, an approximate ratio of 48 1 instead of the expected 3 1 In an attempt to investigate this occurrence, a series of 56 crosses (largely backcrosses with *or*) covering 4 generations were made The deficiency of *or* offspring proved to be heritable Evidence was obtained that the deficiency is expressed only when the male parent is the carrier A ratio of 28 1 was common in these crosses The female parent also transmits the deficiency, which is then expressed again through the male offspring

The results of these crosses do not allow a satisfactory explanation of the deficiency of *or*, but its occurrence may indicate the presence of a factor(s) epistatic to *or*, or an instability in the *or* locus The frequent appearance of these peculiar ratios would, of course, detract from the value of *or* as a genetic marker Thus far, however, the occurrence is limited to the offspring from a single mating of an *or* female × a wild-type male It appears, therefore, that if care is used this orange-body mutation will be a useful marker

A series of crosses between *or* and *ba* roaches were made to test for linkage In the F_1 all offspring were wild type in appearance, as would be expected since both traits are recessive When the F_1 hybrids were mated, the resulting F_2 did not deviate significantly from a theoretical 9 3 3 1 ratio The results are as follows 524 wild-type wing and body colour 167 wild-type wing, *or* 179 wild-type colour, *ba* 55 *or, ba* The χ^2 goodness of fit test gave a $\chi^2_3 = 0\ 62$, $P > 0\ 80$ These results indicate that *ba* and *or* are independent factors A true-breeding colony of balloon-wing, orange body colour cockroaches has been established from the F_2 individuals showing these traits

This work was partially supported by a grant (No *RG*–6365) from the U S Public Health Service, National Institutes of Health, Division of General Medical Sciences

MARY H ROSS
DONALD G COCHRAN

Department of Entomology,
Virginia Polytechnic Institute,
Blacksburg, Virginia

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VIROLOGY

An Equine Respiratory Virus with Enterovirus Properties

A VIRUS was isolated in monkey kidney tissue cultures from the fæces of 13 of 290 horses held in the stables of the Wellcome Research Laboratories One of these strains was selected for investigation Rabbit hyperimmune serum prepared against it and possessing a neutralizing titre of 1/4,000 neutralized all 12 remaining isolates to the same titre

The virus grew readily in kidney cultures from several monkey species and rather less readily in dog kidney and *HEp*-2 tissues Titres of 6 5 to 7 5 $\log_{10}$ TCD_{50}/1 ml of tissue culture fluid were attained The cytopathic effect was indistinguishable from that of poliovirus, no inclusion bodies were found The virus was resistant to ether, chloroform and fluorocarbon ($C_2Cl_3F_3$) Ultracentrifugation, and electron microscopy of phosphotungstate negatively contrasted concentrates indicated a particle diameter of 30 mμ, there was a similarity to poliovirus in the general shape No detectable drop in titre resulted from 12 months storage in a serum-free medium at − 20° C One week at 22° C caused the titre to fall from 7 5 to 6 8 $\log_{10}$ $TCID_{50}$/0 5 ml No hæmagglutination or hæmadsorption by infected tissue occurred at 4° C room temperature or 37° C , using erythrocytes from rhesus or cynomolgus monkeys, and from man, cat, dog, horse, ox, rat, ferret, guinea pig or chick

No neutralization of 100 $TCID_{50}$ of any of the *ECHO* viruses 1–28 nor of Coe virus by a rabbit antiserum having a titre of 1/800 against the equine virus was demonstrable No relationship with polioviruses I, II, and III was detected by cross-neutralization tests Intracerebral or subcutaneous inoculation of suckling mice produced no pathological effect Virus could not be isolated in tissue culture from brain or muscle of mice inoculated 13 days previously These results suggest that the virus is not a member of the Coxsackie group

It has been demonstrated[1] that the virus is common among horses and reproduces in experimentally infected horses in the tissues of the pharynx, causing a mucoid or mucopurulent reaction, accompanied by a nasal discharge A viræmia, lasting 4–5 days and often accompanied by pyrexia, regularly occurred No evidence was found for reproduction in the tissues of the gut The virus was found in the fæces of infected animals in only small quantities and probably originated from the pharyngeal region

High antibody titres (1/512–1/1,024) to the virus were present in the sera of 3 of 12 stable workers, indicating that the virus is able to infect man None of 59 non-stable workers or 100 young Swiss military recruits possessed antibody Attempts to infect 5 cynomolgus monkeys by means of a stomach tube were unsuccessful, as indicated by a lack of antibody development However, each of 5 young adult cynomolgus monkeys, including one from the previous experiment, were found to have developed antibody titres of 1/1,024 or greater one month following intranasal administration of the virus These animals showed no respiratory symptoms Two older monkeys developed slight mucoid discharge from the nostrils, accompanied by nasal râles, on the third day following intranasal inoculation, lasting 3 days

The properties of this virus are apparently rather similar to those of Coe virus (Coxsackie *A*21) and the rhino-viruses of man In contrast to the common cold viruses, however, the equine virus is able to grow equally well at the general body temperature of its host, the horse, as at the somewhat lower temperature of the upper respiratory tract Temperature therefore cannot account for the seeming inability of the virus to reproduce in the gut

The physical properties of this agent and its behaviour in tissue culture indicate that it is a member of the enterovirus group, despite the localization of its reproduction to the respiratory region rather than the gut The naming of this virus is not easy, as 'equine respiratory enterovirus' is apparently self-contradictory, although the 'enteron' does include the pharynx This difficulty emphasizes the need for reconsidering the classification and nomenclature of the small ether-resistant viruses and the adoption of a system which will embrace the non-human enteroviruses and the 'respiratory enteroviruses'

I thank Dr A P Waterson of the School of Pathology, University of Cambridge, for carrying out the electron microscopy

G PLUMMER

Wellcome Research Laboratories,
Beckenham, Kent

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Apple Virus Inactivation by Heat Therapy and Tip Propagation

THE presence of many viruses latent in apple varieties and rootstocks has been shown by the use of indicator plants such as Virginia Crab, Spy 227, and *Malus platycarpa* Three of these viruses which on *M platycarpa* produce symptoms of line pattern (= chlorotic leaf spot), dwarfing and scaly bark have been shown to be widely distributed No virus-free source was found of many of the apple varieties and rootstocks grown commercially in England

Experiments were therefore started in 1958 to try to inactivate these viruses by heat therapy, a method which had proved successful in similar work with some strawberry and raspberry viruses

A cabinet was used in which the air temperature was controlled at 37° ± 2° C and young apple trees were grown at this temperature for 10, 15, and 20 days Buds from the extension shoots, produced while the trees were in the cabinet, were afterwards retested on *M platycarpa*, using the double-budding method of virus indexing The results shown in Table 1 suggested that the viruses in the deep-seated tissues were not inactivated by any of these treatments, for although some initial improvement was seen, this was not maintained and systemic reinfection was rapid At the end of the test year the virus content of the treated plants was the same as that of the controls

Table 1

	Heat treatment (days)	No of clones treated	No of clones virus-inactivated
No tip propagation	10	12	—
	15	9	—
	20	7	—
Tip propagation	20	22	7
	23	25	10
	30	9	4

A method of isolating the growing tip from the heat-treated plant as soon as possible after treatment was therefore developed The extreme tip (1 cm) of the young shoot was wedge-grafted into a young apple seedling of the same diameter The tiny graft was held in position by self-adhesive crepe bandage and enclosed in a small polythene bag Propagation is possible in this way at any time of the year but most success was achieved with grafts made in the spring, when about 90 per cent grew

The results after heat treatment and tip propagation are also shown in Table 1

It can be seen that the introduction of tip propagation after heat treatment proved successful in more than one-third of all the clones treated Success is not related to the duration of the heat treatment, since it appears possible to produce healthy clones after 20 days, yet some still carry infection after 30 days

The important factor seems to be the growing-away from the virus rather than the complete inactivation of all the virus present It is therefore necessary to produce very rapid growth under conditions unfavourable to virus multiplication and to isolate the young tip as soon as possible after treatment Tips from control plants not heat-treated were still infected when re-tested

Some of the apparently healthy clones may become re-infected from deep-seated virus at a later date It is also possible that the virus concentration had been reduced to such a level that no symptoms appeared on the indicator plants Since a fairly long period (1½ years) has elapsed, however, since heat treatment, neither of these possibilities seems likely, nevertheless periodic tests will be necessary to confirm freedom from infection

As a clone can be rapidly built up from one individual by vegetative propagation, the heat treatment and tip propagation need be successful in only one individual of each variety and rootstock The clonal descendants from such an individual should remain free from these viruses indefinitely, if contact with infected material is avoided, since there is, as yet, no evidence to indicate their spread in the orchard by other means

A I. CAMPBELL

Research Station,
University of Bristol,
Long Ashton,
Bristol

Use of Goose Cells in Hæmagglutination Tests with Louping ill Virus Antigen

PORTERFIELD[1] recommended the use of goose red blood cells for hæmagglutination and hæmagglutination inhibition (H A I) tests with arborvirus antigens Such tests are susceptible to non-specific inhibition by lipids present in normal animal serum[2,3] These non-specific inhibitors are widely distributed[4], but little is known of their nature or mode of action

Porterfield and Rowe[5] investigated a number of purified phospholipid fractions of human red cells and found that the inhibiting activity against several Group *B* arborvirus antigens was associated with certain choline-containing phospholipids related to lecithin Lecithin itself extracted from hen's eggs was a powerful inhibitor, but they were unwilling to accept the explanation that lecithin itself was the active component, in view of the fact that one fraction without activity in hæmagglutination inhibition was the richest in lecithin by chemical tests, and egg lecithin had only 0 5 per cent of the activity of other fractions

In the course of carrying out hæmagglutination inhibition tests on sheep serum[6] with a louping ill virus (Group *B*) antigen we noted that the goose cells used in the tests became inagglutinable when egg laying commenced in April 1961, and remained so during the egg-laying period, agglutinability returning some time after egg laying had ceased The goose cells from this bird again became inagglutinable on October 5, 1961, when egg laying again occurred At the same time non-specific inhibitor, normally present in goose and gander serum, was no longer detectable in the goose (Table 1) These inagglutin-

Table 1 HÆMAGGLUTINATING CAPACITY OF GOOSE AND GANDER CELLS WITH LOUPING ILL VIRUS ANTIGEN, AND TITRES OF NON-SPECIFIC INHIBITOR (N S I)

Date	Erythrocyte hæmagglutination		Serum N S I titre (reciprocal)		Serum/lecithinase opalescence	
	Goose	Gander	Goose	Gander	Goose	Gander
3 8 61	+	+	160	160		
5 10 61*	–	+	< 10	160	+ +	–
20 10 61	–	+	< 10		+ +	
23 10 61	–	+	< 10		+ +	
26 10 61	–	+	< 10		– +	
31 10 61	–	+	< 10	160	+ +	–
2 11 61	–	+	< 10		+	
8 11 61	–	+	40		–	
9 11 61*	–	+	160		–	
11 11 61	–	+			–	
13 11 61	–	+	320	160	–	–
18 11 61	–	+	160		–	
23 11 61	–	+	320		–	
4 12 61	–	+	320		–	
11 12 61	–	+	320		–	
18 12 61	+	+	320	160	–	–

* Egg-laying period

able goose cells were subjected to various treatments in attempts to reverse the inagglutinability Short-term time-temperature exposures in borate saline to varying pH (5 75–8 7), salt concentration (0 5–2 per cent), trypsin, lecithinase (*Cl welchii* type *A* toxin), and incubation with gander serum failed to render the cells agglutinable Lecithinase added to goose serum induced a marked opalescence in these samples of goose serum devoid of demonstrable non-specific inhibitor, but did not reveal the presence of a lecithin masked inhibitor Gander cells which were always agglutinable could be rendered inagglutinable by exposure to lipid extracted from goose serum or goose egg yolk, and could not be restored to agglutinability by washing with saline Non-specific inhibitor reappeared in the serum simultaneously with the cessation of egg laying, but the red cells remained inagglutinable for a further 39 days

H WILLIAMS
H THORBURN

Bacteriology Laboratory,
Northern Regional Hospital Board,
Inverness

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PSYCHOLOGY

Eyelid Reflex Response to Stretch

AMONG the evidence of consciousness in the human subject, the position of the upper eyelid fulfils a significant role, and varies with the degree of attention[1] It is of interest, therefore, to understand the mechanisms causing eyelid movement and particularly the relationship of local automatic reflexes to this function Consciousness generally also invokes much ocular work both of movement and of visual function, but it has been found that the muscles controlling the position of the eye do not appear to possess simple stretch reflexes[2], nor does proprioceptive information appear to reach a conscious level[3]

Nevertheless, stretch reflexes are possessed particularly by those muscles subserving an anti-gravity function The upper eyelid may be considered to fall into this category

We have examined the response of the upper eyelid to sudden displacements applied extrinsically, and found a reflex contraction in opposition to displacement, which forms part of a more complex response Our method was to record the position of the upper eyelid by means of a transducer device[4], the lever of which was adherent to the upper eyelid Displacements of a given amount were produced by an electromagnet acting on a piece of ferrite attached to the lever, which was otherwise free to move In order to make good mechanical attachment with the eyelid, a curved piece of 'Perspex' was stuck firmly to a large area of the lower margin of the eyelid including the eyelashes By means of such a connexion the force could thus be distributed evenly over most of the eyelid The movement of the contralateral eyelid was simultaneously recorded

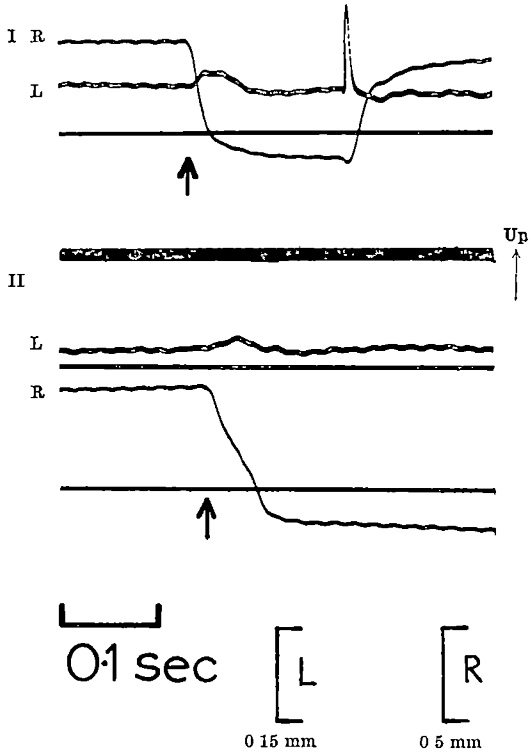

Fig 1 Two records (I and II), each showing simultaneous movement of left and right upper eyelids Downward displacement applied to right eyelid at arrow

We found that the movement of the eyelid to which a downward force had been applied always showed a clear hesitation and delay on the falling slope of the record (Fig 1) The delay in fall thus introduced varied in duration from 70 to 100 msec This check in the downward movement must have been produced by contraction of the levator palpebræ superioris Further evidence for this response was obtained from the other eyelid, which in the human subject shows a high degree of linkage and synchronism[4] The record showed a small contraction to occur during the time of active contraction of the displaced eyelid While a degree of uncertainty exists as to the point of onset of active contraction in the displaced eyelid, it appears that the contraction in the contralateral lid was synchronous with or preceded the displaced lid The relative values of movements were such that a downward sustained displacement of 1 mm evoked a raising of the contralateral lid of 0 1 mm The complete responses of the lids in fact form part of a complex pattern of alternating excitation and inhibition

The stretch response of the upper eyelid may form part of the intrinsic pattern of function of anti-gravity muscles during consciousness It may be considered advantageous during the wakeful state to limit the amount of control referred to the conscious level, by the provision of local controls (stretch reflexes) We may regard the eyelid stretch reflex to be in this category, and to provide some degree of control of position at a low level, while only relatively important decisions are referred to higher brain levels for conscious consideration

D W KENNARD
G L SMYTH

Medical Psychology Laboratory,
University of Cambridge

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Perception of Topographical Information (with Reference to Road Signs)

AN experimental study has compared six layouts of advance road direction signs This communication is concerned with three of these, at present displayed in Great Britain, the Continent and the United States On the first two designs, a road intersection is represented by a diagram on the display, road numbers and place-names are enclosed in rectangular boxes on the British layout, without boxes on the Continental design On the American display the destinations are listed and short arrows at the side indicate direction The distance legibility is greatest for the American type, then the Continental, and the British signs are least legible when signs are compared on back-boards of equal area[1] The order appeared to be determined by the size of lettering The present experiments tested the effect of the difference in the displays on accuracy and speed of interpretation

Signs describing a route with junctions of different complexity were drawn in each of the layouts Subjects sat 9 ft from a screen and off-set 1 4 ft to the right A projection tachistoscope displayed the signs, with black on white contrast, 1/10th to scale for intervals ranging from 0 5 to 2 sec This gave some similitude to the visual angle at which drivers view signs Subjects responded to the signs either by drawing the direction necessary to attain a previously designated destination, or by moving a control stick Throughout the experiment they performed a compensatory tracking task as a substitute for driving, with the tracking display set normal to the subject Performance was measured by (*a*) response-time between the presentation of the sign and the subject's response with the control stick, (*b*) errors of drawn directional movement, (*c*) indirect effects of the sign layouts on an error score on the tracking task In separate experiments, signs in three layouts, selected from all those tested, were compared The sign layouts were presented in a counter-balanced order and a subject followed the route three times

In a subsidiary experiment, signs were briefly exposed to subjects who were required to draw a diagram of the junction represented immediately afterwards, and indicate the direction necessary to reach a destination The diagrams were scored for accuracy

Table 1 SCORES ON RESPONSE MEASURES TO DIRECTION SIGNS IN THREE LAYOUTS

Response measures	Layout British	Layout Continental	Layout American	No of signs per layout	No of subjects per cell
Mean log response time	2 2132	2 1291	2 1365	6	18
Errors of directional movement	43	26	17	9	27
Errors of reproduction	36	39	70	12	12

The tracking task scores showed no difference between layouts Table 1 shows the results of the other measures, significance was tested by analysis of variance

There were more errors of directional movement and a longer response time to the British layout Caution is required in interpreting the latter result as response time was partially determined by interaction effects between the British layout and the others with which it was compared Errors of reproduction were greatest on the American layout, although on all layout errors were more numerous on complex signs On the American layout four-fifths of the attempts at reproducing the complex signs were unsuccessful

These results showed that it was possible to scan and select a direction from a Continental or American sign with almost equal facility, but on the latter layout the numerous errors of reproduction on the complex signs demonstrated that the topographical displays convey an erroneous message about the layout of the crossroads the observer will meet However, before simple junctions, the American list layout may be optimal as it permits larger lettering It was concluded that before simple junctions there are advantages in displaying the Continental or American layouts, before complex junctions, the Continental design with a diagram of the approaching junction is desirable

Part of the problem of designing topographical displays is to emphasize significant details to aid visual resolution, but cognitive processes of interpretation are also important The individual, in route finding, has to locate his own geographical position and to have the intention of proceeding to another known position In doing so, he has the aid of symbolic representations in the form of topographical displays The individual has to match the symbolic representation with the geographical layout, either directly or from memory of the representation, which requires the selective use of environmental cues Bartlett[2] has described the ideational content of direction finding from road signs, placing emphasis on the general anticipatory function of the symbols The present results suggest that not only may the symbols be changed to aid discrimination, but the form of the layout may reduce conceptual work Emphasis on discriminability and the presentation of optimum configuration or 'Gestalten' can present different design problems which are not always easy to reconcile

The work was carried out in collaboration with the Road Research Laboratory I thank Mr J W Whitfield for directing the work

J WALKER

Medical Research Council,
Industrial Psychology Research-Unit,
Psychology Department,
University College,
London, W C 1

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APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Lecturers (2) in Pharmacy—The Registrar, The University, Manchester 13 (August 8)

Biochemist, for research on nucleic acids in normal and abnormal embryonic cells—The Secretary, University College, Gower Street, London, W C 1 (August 10)

Lecturer or Assistant Lecturer in the Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies—The Secretary, The University, Edinburgh (August 10)

Lecturer (preferably with special interests in fluid dynamics or numerical analysis) in Applied Mathematics in St Salvator's College—The Secretary, The University, College Gate, St Andrews, Fife (August 11)

Assistant Lecturer or Research Demonstrator in Physics—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W 8 (August 13)

Lecturer in Computer Science—The Secretary, Birkbeck College (University of London), Malet Street, London, W C 1 (August 13)

Research Curator in Geology—The Professor of Geology, Sedimentary Research Laboratory, The University, Whiteknights Park, Reading (August 13)

Lecturer (Biochemist) in the Department of Medicine at the Western Infirmary—The Secretary of University Court, The University, Glasgow (August 18)

Research Assistants (2) (with an honours degree in zoology), one to work on the aspects of respiratory physiology of vertebrates, and the other to study the ecology and systematics of mammals inhabiting small islands—The Secretary and Registrar, The University, Southampton (August 18)

Administrative Assistant (with appropriate scientific qualifications and at least a working knowledge of French), to be primarily concerned with the work of the British National Committees on Space Research and Co-operation in Geophysics—The Executive Secretary, The Royal Society, Burlington House, Piccadilly, London W 1 (August 20)

Demonstrator in Biology—The Registrar, The University, Keele, Staffs (August 21)

Lecturer or Assistant Lecturer (with a degree in veterinary medicine or physiology) in the Department of Physiology—The Secretary, Royal Veterinary College, Royal College Street, London, N W 1 (August 25)

Senior Lecturer or Lecturer in Mathematics at Newcastle University College, University of New South Wales—The Agent-General for New South Wales, 56/57 Strand, London, W C 2, and Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N S W , Australia (August 30)

Lecturer in Zoology at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, August 31)

Senior Lecturer (with a degree in the social sciences and experience in education) in Sociology of Education—The Secretary, University of London Institute of Education, Malet Street, London, W C 1 (August 31)

Demonstrator in Biology—The Registrar, The University, Keele, Staffordshire (September 1)

Head (honours graduate, preferably under 40, with suitable practical experience) of the Meat Section, to take charge of a division engaged in research related to the manufacturing aspects of meat and other animal products—The Secretary, The British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey (September 1)

Senior Lecturer in Hæmatology in the Department of Pathology, University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, Senate House, University of London, London, W C 1 (September 3)

Research Student in Geography—The Registrar, The University, Hull (September 7)

Senior Lecturer or Lecturer (preferably with an interest in either insect physiology or insects of medical importance) in Zoology at the Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, September 10)

Lecturer (with an honours degree in zoology) in Zoology at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London September 15)

Reader in Cell Structure in the Department of Agriculture, Waite Agricultural Research Institute, University of Adelaide—The Registrar, University of Adelaide, Adelaide, South Australia (September 15)

Lecturers (3) in Civil Engineering at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, September 17)

Senior Lecturer or Lecturer in Inorganic Chemistry tenable at either the Pietermaritzburg or the Durban centre of the University of Natal, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, September 21)

Lecturer (with a degree in biochemistry, organic chemistry, physiology or pharmacology) in Biochemistry—The Secretary and Registrar, The University, Southampton (September 22)

University Demonstrator in Biochemistry—Dr J W Millen, Secretary of the Appointments Committee of the Faculty of Biology "B", Department of Anatomy, Downing Street, Cambridge (October 1)

Chair of Education—The Secretary and Registrar, The University, Southampton (October 27)

Chair of Psychology, Chair of Botany, Chair of Physiological Chemistry, Chair of Geophysics, Dean of the Faculty of Medicine, Senior Lecturer or Lecturer in Chemical Engineering at the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1

Head (honours graduate in mathematics, with experience in the application of statistics to the planning and interpretation of biological experiments and to the general statistical problems arising in agricultural research, particularly with animals) of the Statistics Section—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref 62/5

Hospital Physicist (with a B Sc first- or second-class honours in physics) at the Ahmet Andicen Cancer Hospital, Ankara, to take charge of the Radio Isotope Department—The Director of Recruitment, Department of Technical Co-operation, 3 Sanctuary Buildings, Great Smith Street, London, S W 1, quoting Ref RC 205/151/03

Lecturer (with a degree or diploma in technology in mathematics, physics or electrical engineering, practical experience in the appropriate field, and a special interest in electrical engineering) in Mathematics—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting Ref CST 62/46

Postdoctoral Fellow, for research with Dr H C Clark, on the chemistry or organometallic compounds containing fluorine—Prof. C A McDowell, Department of Chemistry, University of British Columbia, Vancouver, B C , Canada

Senior Scientific Officer and a Scientific Officer (with at least a good honours degree or equivalent) in the Scientific Branch of the Public Health Department, for a wide variety of interesting work on research, development and scientific control—Medical Officer of Health (D 1/c/1759/7), London County Council, County Hall, London, S E 1

Tutorial Research Student in Botany—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey

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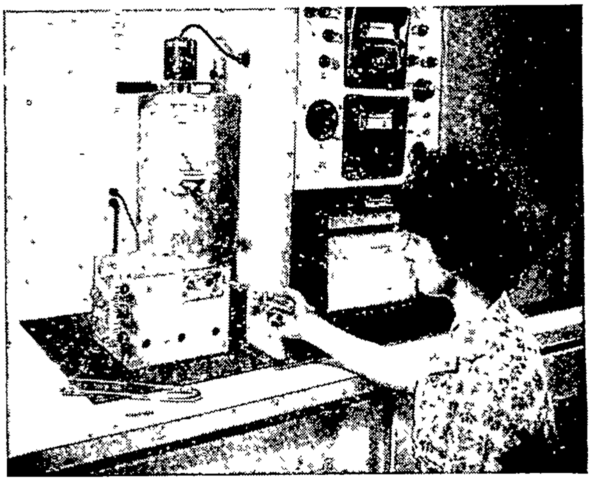

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GOVERNMENT ORGANIZATION OF SCIENCE IN BRITAIN

THE rather rambling debate on science and industry in the House of Commons on July 12 covered many aspects, including the Zuckerman Report on the management and control of research and development, the Trend Committee on the Government organization for the promotion of scientific research in the civil field, the latest report of the Department of Scientific and Industrial Research, the report on industrial research in manufacturing industry issued by the Federation of British Industries, Prof. R. Aron's recent paper on the education of the citizen in industrial society in *Daedalus*, as well as that of Sir Eric Ashby in the same issue, all of which have been discussed recently in *Nature**.

In opening the debate, Mr. G. R. Mitchison sought to obtain more information on the activities of the Minister for Science and of his Parliamentary Secretary or at least some precise definition of the functions of the Ministry. If he scarcely succeeded in this, he drew from Sir Edward Boyle, then Financial Secretary to the Treasury, who concluded for the Government, the announcement that a grant of about £250,000 was being offered to the University of London by the Department of Scientific and Industrial Research to provide a low-power reactor for research and training. This would be a 100-kW *Consort* made by the General Electric Co., Ltd., and would be operated at Silwood Park by the Imperial College of Science and Technology primarily on behalf of the University. However, it would also be available for use by other research organizations in the London area. Early in the debate, Mr. D. Freeth had referred to the establishment by the Department of Scientific and Industrial Research, at the request of the Minister for Science, of the Committee under the chairmanship of Mr. G. B. R. Fielden to consider the present standing of mechanical engineering design and to recommend any changes which are likely to result in improved engineering design of British products, including, in particular, changes in education and training.

Sir Edward Boyle in this speech gave little fresh information about Government policy in general. Commenting on the importance of innovation in industry, he observed that this applied also to commerce, as well as to the Government's own administrative arrangements, though he stressed the value of the flexibility in existing arrangements. Regarding the universities, he merely referred to the research grants of the Department of Scientific and Industrial Research, to the expansion of the colleges of advanced technology, which would cost at least £160 million in five years time, and to the decisions which would have to be taken when the report of the Robbins Committee became available. He also said that the Government intended to do what it could to ensure that more opportunities of administrative work were available to scientists.

Sir Edward also referred to the report of the *ad hoc* Committee of the Royal Society on biological research and agreed with what Mr. Mitchison had remarked as to the exciting vistas in biology which seem to have been opened up. The Minister for Science had received a copy of the report and was seeking advice on it from the Advisory Council on Scientific Policy, the report was also receiving detailed consideration from the Research Councils which supported biological research. He did not, however, comment on the concrete suggestions which Mr. Austen Albu made later in the debate.

Mr. Albu, who directed attention to Prof. R. Aron's paper and deprecated excessive distinction between pure and applied science, had urged that the research associations should be more concerned with basic research and suggested that they needed a stable and more generous grant from the Government. Furthermore, they should be allowed to undertake sponsored research on the condition that the director of the association was satisfied that the proposed research fitted in with his general programme. He thought that the use of development contracts was of increasing importance, and that the Department of Scientific and Industrial Research should receive a block grant on the lines similar to the University Grants Committee and other Research Councils. He also suggested that the Minister for Science should be directly responsible for all major scientific expenditure, including that of the Atomic Energy Authority, the three existing Research Councils, the Nature Conservancy, and a new 'technological development council' which he proposed for identifying subjects for development and research contracts.

Such a council, Mr. Albu envisaged, should be comprised mostly of engineers with a good scientific background, this, he thought, might eliminate the frustration which at present arose under the present arrangements when projects were submitted individually to the Treasury. He also suggested a research council to deal with fundamental research in the universities and another research council for the social sciences. He would make the Minister for Science responsible for research development as well as some sections of the Board of Trade, and suggested also a joint committee of the Ministry for Science and the research departments of the Services and the Ministry of Aviation. In common with other speakers in the debate, he was concerned with the use of scientists as administrators and the training required with administrative or managerial posts. He advocated a permanent staff for the Minister at least of scientists and technologists working half-time or perhaps full-time for three or four years, but not more.

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* See, for example, *Nature*, **193**, 710, **194**, 119, 1105, **195**, 1 (1962)

It is difficult to discuss seriously Mr Albu's proposals without further details, and Mr Albu himself said nothing to suggest what advantages they would offer over the existing set-up if those arrangements were effectively used—if, for example, the link between the Advisory Council on Scientific Policy and the Defence Research Policy Committee were effectively restored In view of the inquiry into the Government organization which is already in progress, it could scarcely have been expected that the Government spokesman would comment on such proposals, however, the debate should at least expedite the inquiry and perhaps induce the Minister to deal with the dilatoriness which has held up for more than a year the promised report on the support of university research by the Department of Scientific and Industrial Research Possibly the inquiry into the research associations could also be included in some constructive statement without excessive delay

Two other features of this debate call for comment First, as in the debate on the National Productivity Year in the House of Lords on the same day, the importance of the quality of industrial management was repeatedly stressed Mr W Stones and Mr J H Osborn suggested that the science of management was one of the most important disciplines to-day, the need for industrial managers to be appropriately qualified if effective use was to be made of either the results of research or Britain's trained manpower was repeatedly emphasized In this connexion, it may be noted that earlier in the day the then Minister of Education, Sir David Eccles, had been asked to establish a central library of publications on business and management education He had replied that he thought it would be better to develop the existing libraries of the British Institute of Management and other bodies which were already well stocked Sir David also referred to the libraries of the colleges of advanced technology, and welcomed the conference on business and management education which the British Institute of Management was planning early next year

While in the House of Commons speakers were concerned with management in respect of the utilization of scientific and technological advance, in the House of Lords the main emphasis was on the handling of British manpower resources and on the human problems of industry However, Lord Marks of Broughton pointed out that Britain could only be fully competitive if there was a concentrated effort at factory-level to exploit the wealth of new and efficient techniques which science and technology were making available Increased productive efficiency and the understanding of human relations must be regarded as a constant challenge which demanded continuous study, education and application Lord Crook, in opening the debate, had stressed the vital importance of the quality of management and the need for a forward-looking approach to all management problems The long-term effects of managerial decisions were too often ignored, and the policy of management in recruitment was of paramount importance

Lord Crook recognized that scientific and technological developments were such that firms must increasingly look to the universities and colleges of technology for recruitment of their future managers, and Lord Baillieu pointed out that apart from technical qualifications, certain qualities of mind and character were essential if the opportunities before us were to be attacked with understanding and responsibility and genuine leadership provided Lord Shackleton urged the need for a more critical approach to problems of management, suggesting in fact much the same approach as the Zuckerman Committee has applied to research and development in Government establishments and which the Trend Committee is now applying to the arrangements for civil research under Government auspices This was welcomed by Earl Waldegrave in replying for the Government, who entirely agreed that the most important factor in the success of the drive for natural efficiency was the solution of the human problems and misunderstandings which still arose The National Productivity Year, which opens in London in November, aims at removing these misunderstandings on both sides of industry

If this debate was a fitting counterpart to the debate in the House of Commons in directing attention to the human factors which must be handled wisely if full advantage is to be taken of scientific and technical advance in industry, a second feature of the debate in the Commons deserves notice Mr Mitchison had pointed out how easily questions of scientific policy involved political decisions, and reminded the House that the allocation of national resources as between biology and nuclear physics, for example, was really a political decision He rather confused the issue both in his reference to the Trend Committee and in that to the Advisory Council of Scientific Policy in this connexion, but the position was correctly stated by the Parliamentary Secretary for Science, Mr Denzil Freeth

Mr Freeth pointed out that the Advisory Council on Scientific Policy were the main advisers of the Minister for Science on general scientific policy They could represent, for example, the form which any space research activity by Britain should take and the size of that effort The decision, however, whether Britain should have a space research programme, the form it should take and its size, was a political decision which the Government alone could take Scientific advisers can only indicate what is required for any such effort to be effective and point out what are the implications of any particular effort

What matters is that the organization for giving scientific advice should ensure that it draws on the best available advisers, that it is representative and takes full account of all the conflicting factors The organization must be such as to command the confidence of the scientists and technologists themselves Above all, it must satisfy them that their time is not being wasted, and that their advice is presented effectively at the level at which political decisions are taken to those who are competent to evaluate the scientific and technical factors along with the other elements that must be taken into account in reaching a decision There are obligations here for the Government as well as the scientist—questions of communication—and it is entirely appropriate that the adequacy of the arrangements should be considered by such a body as the Trend Committee They are not as Mr Mitchison suggested, being asked to take political decisions, but rather to report on the adequacy of the arrangements for ensuring that the scientific and technical aspects of a problem are correctly presented to the Government as a basis for decision

What, of course, could be observed is that some such inquiry might well have been instituted much earlier and also that although the Zuckerman Report has been accepted as laying down a standard of practice, its recommendations appear to be put into practice very leisurely It is to be hoped that further delay will not result from the recent reconstruction of the Government, and since the Minister for Science and his Parliamentary Secretary have retained their offices, they should at least do their utmost to ensure that delay is kept to a minimum Moreover, there is a large field in which it would seem that the final responsibility rests with the Minister for Science and that he alone is to be held responsible for any delay or failure to correct recognized weaknesses The debate in the House of Commons at least affords a pointer to some prodding that he might institute forthwith

NUCLEAR POWER FOR INDUSTRIAL PURPOSES

Nuclear Power Today and Tomorrow

By Kenneth Jay Pp viii + 270 + 16 plates (London Methuen and Co , Ltd , 1961) 25s net

IT is just twenty years since Enrico Fermi's atomic pile went into action in Chicago and in that twenty years unprecedented effort, whether measured in money or in number and quality of scientific investigators, has been spent in the development of large-scale installations for producing from nuclear energy a supply of electrical power on the scale required by modern industry and modern domestic needs A large number of technical books have appeared in recent years dealing both with the general range and with particular aspects of the new subject of nuclear engineering Hitherto, however, there has been lacking a book, accessible to readers with no particular knowledge of nuclear matters or of engineering, which gives an accurate and authoritative account of what has been accomplished in the field as a whole and what may be reasonably anticipated This need is now supplied

The book before us makes clear, in the first place, the scientific foundations of the subject, in particular the reaction with appropriate nuclei of the neutron "This incredibly tiny, electrically uncharged, particle is in fact the key to the new age", as the author says He develops the theme with a skill and happy phraseology that makes the subject simple, explaining such matters as the difference between thermal and fast reactors, the types of nuclear fuel, uranium isotopes and plutonium, that can be used, and the different possible processes involving them Having pointed out that only nuclear fission, and not nuclear fusion, is in question in these industrial applications, he calls the type of nuclei which can be split "fissile fuel", in contrast to coal, which is fossil fuel

The all-important question of cost, especially capital cost, of nuclear power is considered in comparison with that of ordinary electrical power generation The subject is a complicated one, but the various factors which have to be borne in mind are set out with admirable clarity The author then proceeds to give a survey of the nuclear power programmes of the countries most active in the field, namely, the United Kingdom, the United States, the U S S R , Canada, France and Federal Germany The existence at Winfrith in Dorsetshire of an installation, supported by international collaboration, for developing a particular type of gas-cooled reactor will come as news to many

The general functions of moderators, coolants and pressure vessels having been described, the different classes of reactor systems, with their peculiar advantages and difficulties and the circumstances which determine them, are discussed in some detail—the gas-cooled graphite-moderated reactors, hydrogen-moderated systems, fast reactors, the sodium-graphite systems and liquid-fuelled reactors The way in which the essentials are selected bears witness to the width of the author's knowledge it requires much less understanding to give every detail than to set down just what is material The last chapter deals with general questions involved in developing a reactor, such matters as radiation damage to fuel and metals, safety and other outstanding problems being discussed

The diagrams representing in outline the various processes are excellently designed and executed, there are a number of photographic plates showing reactors as a whole and details of construction which bring home the nature of the installations, there is a glossary of technical terms in which the reader will find at once what is meant in the reactor world by for example, breeding An additional recommendation is that the price is very moderate The author is to be congratulated on outstanding success in a very difficult task, having provided an account of the complex subject which is at the same time comprehensive and comprehensible

E N DA C ANDRADE

BIOPHYSICS AND MOLECULAR BIOLOGY

Molecular Biophysics

By Richard B Setlow and Ernest C Pollard (Addison-Wesley Series in the Life Sciences) Pp xiii + 545 (London Pergamon Press, 1962) 84s net

BIOPHYSICS has been defined as the application of the concepts and techniques of physics to biological problems, often with the instinctive approach of a physicist who believes that thought, inspiration and experiment can pick out the essential elements underlying apparently complex phenomena and may often provide satisfyingly simple ways of accounting for them Molecular biology is concerned with the basic properties and interactions of biologically important molecules, studied by whatever approach—biophysical, biochemical, genetical—seems most opportune, and this large and ambitious book was designed by its authors to cover the approach from biophysics

The level of the test is intended to be suitable for advanced undergraduate or first-year graduate students in the United States, it has been written with an eye to the increasing number of biophysics courses now being given, and is in fact based on such a course at Yale University The book contains a large amount of valuable information summarized in one place, though it would perhaps be most useful as a collection of essays to serve as introductory background reading for topics on which one wanted information, rather than a text-book to define an area of study There are fifteen chapters, including (after some introductory discussion) ones dealing with physical methods of determining the sizes and shapes of molecules (36 pp), X-ray analysis (43 pp), intra- and inter-molecular forces (33 pp), absorption spectroscopy (50 pp), enzymes (28 pp), action spectra (39 pp), ionizing radiation (74 pp), microscopes (33 pp), isotopic tracers (31 pp), muscle nerve and eye studies (41 pp), collision and diffusion in cells (33 pp), very well chosen recommendations for further reading are given at the end of each chapter These are all important and interesting topics in biophysics, though their relative importance in present-day molecular biology could perhaps not readily be deduced from the amount of space devoted to each, nor from the degrees of emphasis given in the text A diligent student who mastered the contents of this book might still emerge without having formed, from knowledge of the past history of the subject, a good sense of judgment of how best to set to work on some actual problem

The construction of the book "presupposes some knowledge of mathematics, physics, chemistry and biology beyond the elementary level", and "since most students will not fit into this category" it has been "designed so that students strong in at least two of these fields will have no difficulty in getting through the material" Now it is not easy to see how these three statements can all be true simultaneously and, indeed, they are not The physicist-chemist will not make much sense of the discussion on genetic linkage (incompletely explained) on p 36, nor of that on the recon, the muton, and the cistron, which follows, all too soon, on p 37 The chemist-biologist may, I imagine, be equally at sea in the derivation of the 'random-walk' expression on p 81, in the formulation of the general expression for the amplitude of scattered X-rays on p 129, or in the statement of Poisson's equation $\nabla^2 V = \rho/\varepsilon$ on p 177 These are failings which it is difficult to avoid in a book of practical size In cases of the latter type, of which there are many, it would often, though not always, be possible for a diligent non-physicist to extract some useful formula, table or graph, but these are not separated from the more specialized discussions in any way, nor is any indication given where sections might be omitted without the general understanding of the topic being impaired, and so it is likely to be heavy going in places However, the discussion of the more physical topics is competently done, and most of the information is there The more biological discussions are marred by the assumption of quite considerable prior knowledge, and by an excessive reluctance to say what things are important and basic, so that often the search for the underlying simplicities is obscured There are also some statements (for example, "The nuclear membrane" (presumably of all cells) "is seen" (in thin sections in the electron-microscope) "to be made up of a series of threads running criss-cross, about 10,000 Å long by 500 Å wide") which appear to be in error.

Thus, while the book could not be given an unqualified recommendation as a solitary text for incipient molecular biologists, there is much in it which could be read with advantage by people at a somewhat later stage H E Huxley

EUROPEAN UNIVERSITIES

Universities of Europe

By Anthony Kerr Pp 235 (London Bowes and Bowes (Publishers), Ltd., 1962) 25s net

The Democratic Intellect

Scotland and her Universities in the Nineteenth Century By Dr George Elder Davie (Edinburgh University Publications History, Philosophy, and Economics, No 12) Pp xx + 352 + 8 plates (Edinburgh The University Press, 1961 Distributed by Thomas Nelson and Sons, Ltd , 1961) 50s net

Mr KERR'S attempt to describe briefly university organization, studies and life throughout Europe is certainly bold and it succeeds in answering reasonably for the general reader the question, "What is a university ?" To that extent his book is a useful contribution to the present-day discussion on university expansion in Britain and on the place of universities in the community to-day His concluding chapter directs attention to some of the problems on which decisions must be taken when the Robbins Committee reports and about which it is important that public opinion should be well informed In this chapter, Mr Kerr expresses some personal opinions as to desirable changes, but it is by no means clear as to whom his book is primarily addressed, and in that respect it is not altogether realistic

The first eight chapters deal successively with the northern countries (including Iceland), the Soviet Union, Central Europe, the Benelux Union, France, the Iberian Peninsula, Italy and the Balkans with a separate chapter—the British Isles Some forty pages devoted to the British scene, with its voluminous literature are scarcely in balance with twenty pages for Norway, Sweden, Finland, Denmark and Iceland combined, with twenty on Central Europe and a dozen on Belgium and Holland, on all of which, for British readers, literature is scanty More serious is the almost entire absence of references To append an admirable address of Sir Keith Murray on the 'University Grants Committee' does not compensate for the absence of any select bibliography of authoritative works on the British universities Moreover, universities of all institutions are not to be considered apart from their past, and even the most competent first-hand account of present conditions needs, as background, some reference to previous studies Rashdall and Flexner have not been superseded, and to write of Germany without reference to Flexner, F Lilge's *The Abuse of Learning*, or especially the report of the Commission on University Reform in Germany, seriously weakens the claims of the book to the attention of the very readers to whom it should most appeal Moreover, the paucity of the literature strengthens the claim for some reference to the comparisons between universities presented at the second educational conference of the Association of University Professors and Lecturers of the Allied Countries in Great Britain Likewise, the dozen pages dealing with the Soviet Union should at least have been supported by reference to Mr A G Korol's book

Mr Kerr's painstaking current observations scarcely lift the book above the level of competent journalism, and in this it contrasts sharply with Dr Davie's scholarly but slightly astringent study of Scotland and her universities in the nineteenth century There is no popular appeal in this book and its title is slightly misleading Nevertheless, its exposition of a phase of Scottish history illustrates the nature of some of the interactions between universities and the society in which they function, and points to dangers which arise when academic independence is either infringed or abused It is a book for the student rather than for the general reader, though it has obvious messages for the present-day debate—if they are not perhaps altogether those which Mr J Grimond had in mind when he commended the book recently to Mr H Brooke in the House of Commons

R Brightman

SOCIAL BEHAVIOUR AMONG ANIMALS

Animals as Social Beings

By Prof Adolf Portmann Translated from the German by Oliver Coburn Pp 247 + 32 plates (London Hutchinson and Co (Publishers), Ltd, 1961) 30*s* net

THIS book is a translation of Prof Portmann's *Das Tier als soziales Wesen* and is concerned to present the "basic phenomena of social life" in animals, and the implications for the life of the individual and the group, including, in some cases, human groups It draws largely on the work over the past twenty-five years of those students of animal behaviour known as ethologists The book apparently developed from some lectures delivered to a non-scientific audience with the purpose of describing some of the advances in animal behaviour study It does not claim to be either comprehensive or authoritative (the author's own research has not been in this field), and it is a popularization in the best sense it selects many interesting facts to illustrate chosen themes, often quoting extensively from the original papers of the workers concerned, rendering them in a very readable context

But one's reservations come more to the fore when seeing how these facts are related together in his treatment of the whole field Prof Portmann has read widely in the literature of the subject, but he has a point of view probably untypical of most of the ethologists whose work he quotes, which colours much of the later parts of the book For example, he dislikes the conventional view of natural selection as the major agent of evolution It is of course true that social behaviour raises special evolutionary problems, as Darwin was well aware, but Prof Portmann gives it a marginal role and seems too ready to resort to other, as usual unspecified, agencies, he writes, for example, of "primordial darkness, which can yet be imagined as fraught with creative existence"

Quite apart from this criticism of the general approach, the reader should be warned that the ethological theories of instinct, etc, outlined in parts of the book are in a stage of vigorous metamorphosis, from which they will probably emerge with a very different look

Finally, Prof Portmann has been ill-served by his translator The original must have been extremely difficult to deal with, and perhaps for this reason the English version misses out a certain amount, but there are too many absurdities, for example, the dying act of the male sea-horse (instead of the final act of its courtship), a hierarchy of nerves (instead of a nervous hierarchy), while bees are said to have hindquarters and birds to brush their feathers

J M Cullen

PROGRESS IN PHYSIOLOGY

Annual Review of Physiology

Vol 24 Edited by Victor E Hall, in association with Frederick A Fuhrman and Arthur C Giese Pp v + 604 (Palo Alto, Calif Annual Reviews, Inc, 1962 Published in co-operation with the American Physiological Society) 7 dollars

"THE *Annual Review of Physiology* has selected its contributors because they can write good reviews" The editors of this volume begin their preface on this confident note They have good reason to be pleased with their choice of Prof Wallace O Fenn to write the introductory chapter Under the heading "Born Fifty Years Too Soon" he compares research conditions in physiology as they were a few decades ago and as they are to-day Physiologists and other biologists will enjoy reading and re-reading Prof Fenn's lively reminiscences and penetrating comments, based on his long experience of research in the United States

The editors have restricted the number of topics reviewed in this volume to 16, and this has meant that reviewers have been free to write readable, critical assessments of their subjects, rather than being virtually confined to the recording of card indexes of references Thus, the longest article, on kidney, water and electrolytes (G Giebisch), extends to 64 pages As usual, there are chapters on heart, respiration, digestion, reproduction and peripheral circulation This year's volume also covers growth and differentiation, temperature, cutaneous sensibility, the adenohypophysis and adrenal cortex, and blood volume regulation There are chapters on somatic functions of the nervous system, excitation and synaptic transmission, and on the physiological bases of memory The final two chapters will interest not only physiologists but also biologists in general They deal with the comparative endocrinology of the invertebrates (W G Van der Kloot), and with the effects of latitude on physiological properties of animal populations (F J Vernberg) These two chapters are full of interesting observations and experiments To select just one example, it has been shown that the queen bee secretes an unsaturated 10-carbon acid, 9-oxo-2-decenoic acid, which acts as a 'social' hormone towards the worker bees, preventing them from building new queen cells When this acid is no longer released in the hive, the ovaries of the workers begin to develop, and these bees then start to build cells for new queens

The general high quality of the articles in this volume justifies the editors' claim that they select people who can write good reviews

D C Harrison

EMPIRICISM AND LOGIC IN THE DISCOVERY OF NEW DRUGS*

By Prof. C. A. KEELE

Department of Pharmacology, Middlesex Hospital Medical School, London

THE word 'empiricism' in relation to medicine is defined variously in the *Oxford Dictionary* as "Practice founded upon experiment and observation, ignorant and unscientific practice, quackery". The original Empirics were Alexandrian Greeks of the third century B.C. who held that treatment of disease should be based on experience alone. They were opposed to the Dogmatists who based their treatment on theories, such as the humoral theory of disease. The Dogmatists would have disapproved of an Empiric as "One who in matters of science relies solely on observation and experiment" and one can perhaps see their influence in the debasement of the word to "One who practises physic or surgery without scientific knowledge, a quack, a charlatan". However, I still think that there is a real need for a word to describe "Practice founded upon experiment and observation only" and I make no apology for using the word 'empiricism' in its original sense. For me it is not a smear word, and if anyone wants to say that certain practices are irrational, unscientific or fraudulent, well, why not use these very words and leave 'empiricism' to its earlier meaning of trial, experiment and experience? All scientific observations are empirical and I shall discuss one example to show how even without theoretical principles it is possible to discover a new drug of great practical importance. 'Logic' is defined as "the science or art of reasoning as applied to some particular department of knowledge or investigation". The word 'drug' means "an original, simple medicinal substance, organic or inorganic, used by itself or as an ingredient in medicine".

It is clearly important to make a distinction between the discovery or synthesis of a new chemical compound and its introduction as a drug for therapeutic purposes. To take some outstanding examples. The foxglove was identified and named *Digitalis* by Fuchsius in 1542, but its powerful diuretic properties were not recognized until 1775 and, meanwhile, it was used for various other purposes. Sulphanilamide was first synthesized by a Viennese chemist named Gelmo in 1908 (at a time when Ehrlich's great chemotherapeutic researches were in progress), but its powerful antibacterial properties were not revealed until 1935 when some French investigators showed that the original sulphonamide drug, prontosil rubrum, owed its activity in the body to its conversion to sulphanilamide. Thus, from 1908 until 1935 sulphanilamide was just an organic chemical compound, but in 1935 it became a drug as well. Other examples are the well-known substance DDT, which was first synthesized in 1873 but not used as an insecticide until the Second World War, and penicillin which, though recognized as an antibacterial agent in 1928 by Fleming, was not made available for therapeutic use until the work of Florey, Chain and their colleagues some twelve years later.

Although there may be dramatic moments in the discovery of new drugs, it is impossible to know at once that an observation made in the laboratory, or even in the clinic, will be generally valid in all circumstances. Some apparently important discoveries have just petered out either from failure to confirm early findings or because the new drug has had unsuspected toxic actions. As a pharmacologist, I am sometimes asked what I think about some preliminary report, perhaps in a newspaper, of a new drug for which startling claims have been made. The correct reply is, what I believe that great clinical scientist, Sir Thomas Lewis, used to say when he came across some new observation in the literature, "interesting if true", but I have yet to find anyone who is satisfied with this answer. The establishment of the therapeutic value of a new drug is an empirical process which has to allow for initial enthusiasm, reactive pessimism and final balanced judgment. This all takes time, and until the drug has passed through all the stages of demonstration of pharmacological action, preliminary trial in man, controlled clinical trial and general usage in practice for months or years it is impossible to say precisely what has been discovered, since discovery means finding out the drawbacks as well as the virtues of a drug.

It is commonly thought that from prehistoric times people have everywhere sampled the plants, animals and minerals of their environment for their possible use as food, and that in this process they have stumbled, quite by chance, on pharmacological as well as nutritive actions, and that as a result folklore about the action of crude drugs has accumulated over the centuries. Chauncey Leake[1] has described such empirically obtained and empirically applied knowledge of drugs as 'protopharmacology', but before we smile indulgently at this name we should perhaps remember that the properties of opium, cinchona bark, ipecacuanha, santonin, squills, belladonna, curare, coca, ergot, ephedrine, vegetable purgatives and alcohol were all discovered by protopharmacologists.

However, it may be that some of these drugs were discovered in a different way, and the famous medical historian Henry Sigerist[2] has pointed out that treatment of disease by primitive peoples is a strange mixture of empirico-rational, magical and religious elements. If, for example, disease is attributed to the intrusion of foreign objects or demons into the body, treatment will be based on very different assumptions from those which underlie twentieth-century Western medicine, though starting from primitive premises therapeutic practice appears quite logical. Now the most potent method of driving out spirits was exorcism, the expelling of demons through the magic power of the word, and even other methods of treatment were usually accompanied by incantations. In ancient Egypt (and in medieval England) incantations represented a perfectly legitimate form of therapy not only for disease of magico-religious origin but also for the treatment of conditions such as poisoning by scorpion stings or snake bites, burns of the skin and hæmorrhoids But incantations were transitory affairs, the magician spoke the magic words, some rites were performed, and all was over

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* Substance of a lecture delivered on June 6 at the University of Oxford in the series "Science and Economics in Chemical Technology".

In some cases such a brief ceremony resulted in a cure, but most patients were probably not suddenly improved and felt a strong desire to prolong the influence of the magic force This was done by charging an object with the magic force, making it thus an amulet or talisman Amulets might be simple objects such as a band of fine linen over which the words were spoken or on which they were written, or costly jewellery such as beads of gold, and rings of amethyst In Egypt, as in all ancient civilizations, minerals, metals, plants, every object animate and inanimate, possessed magic virtues Another amulet consisted of evil-smelling herbs, of garlic, and of honey which is sweet for men but horrible to ghosts This was to be applied externally to protect a child against demons, but in other cases herbs, garlic and honey over which a spell had been spoken were consumed by the patient A prescription of drugs thus charged became an internal amulet that exerted its power from inside the body, though it was not the drug in itself that was effective but the words spoken over it made it so The art of medicine consisting in selecting the right drugs, preparing them in the magically correct way and speaking the appropriate words over them One such incantation on drinking a remedy went as follows

"Come remedy ! Come thou who expellest evil things in my stomach and in these my limbs ! The spell is powerful over the remedy Repeat it backwards ! Dost thou remember that Horus and Seth have been conducted to the big palace at Heliopolis when there was negotiated of Seth's testicles with Horus, and he shall get well like one who is on earth "

Sigerist[2] suggests that the majority of Egyptian drugs became part of the materia medica for magic reasons, but that a sound empirical knowledge grew up as well The inherent magical virtue of a drug might coincide with its pharmacological effect The virtue of castor oil taken internally whether magical or empirical was always a cathartic one The day must have come when drugs were prepared and given without incantation This was the moment when magic and medicine separated

Now, although most of the drugs used by ancient and primitive peoples have no significant pharmacological actions in our sense, it is probably a mistake to assume that such remedies were not beneficial Indeed, in the light of recent evidence on the powerful effects of placebos and the considerable influence which an enthusiastic doctor can exert on the effects of drug treatment it seems to me very likely that these 'irrational', 'unscientific' remedies were very effective Primitive people are highly suggestible, and it is not difficult to imagine that an incantation would enormously enhance the virtues of the feeblest of drugs I am sure that some of our primitive ancestors would have made better use of tranquillizers and psychic energizers than does the modern physician participating with Olympian detachment in a controlled clinical trial I have heard rumour of a contemporary medicine man who finds chlorpromazine a tremendous boost to his magical powers This might be the best way of evaluating such drugs !

It is of interest to consider why so few new drugs were discovered from the time of ancient Egypt right up to the nineteenth century The reasons are not far to seek The sources of new drugs were confined to herbs, minerals and animal sources, there was no knowledge of how drugs worked, there was no chemistry to help isolate active principles let alone to synthesize new chemical compounds, there were no fruitful theories of disease, there were difficulties in diagnosis, differences of opinion were settled not by experiment but by argument influenced by an undue respect for authority, and superstition and quackery abounded There were doctrines, if not theories, of disease, and drugs were administered according to the doctrine most generally accepted at any given time The most persistent was the doctrine of the humours which guided therapy from the Hippocratic era until the early years of this century, and was responsible for much purging, vomiting, sweating, blistering and the letting of seas of blood The doctrine appears to have produced no discoveries of new drugs, and its only contribution to human happiness appears to have been its introduction of some delightfully descriptive words like sanguine, choleric, melancholic and phlegmatic into the language

Some people disliked theories about medicine and preferred to rely on experience alone, but Poynter and K D Keele[3] have emphasized that neither theorists nor empirics knew how to answer the question Does a drug work ? This was because they were all subject to the very persuasive logical error that if *B* follows *A* then *B* is caused by *A*—*Post hoc ergo propter hoc* They could not conceive the possibility that if a patient with, say, an acute fever was treated with a drug and recovered, that recovery might have occurred without the drug at all Thus, whatever drug was used was given the credit for the cure Further, they did not seem to be aware of the placebo effect though unconsciously they made full use of it In this climate it is no wonder that in diseases in which the placebo effect could not be exerted their remedies were of little value

After considering the numerous reasons for lack of progress in therapeutics through the centuries, it is most refreshing to turn to the constructive work of a man who in the eighteenth century used the empirical method to make an outstanding and well-documented therapeutic discovery which I shall discuss at some length, because it shows what opportunities had always been there for the taking and what can be done by practice founded on experiment and observation in the absence of general principles.

I refer to the discovery of the actions of digitalis or the foxglove by the famous Birmingham physician, William Withering, who introduced the drug as a treatment for dropsy in 1775 Before Withering's time, digitalis had been variously used as an emetic, a purgative, an expectorant, as a treatment for epilepsy and scrophula (tuberculous ulceration of the skin) and to promote the healing of wounds For one or more of these purposes it had been introduced into the *Pharmacopœia of London* in 1721 but was later omitted

In his book *An Account of the Foxglove*, published in 1785[4], Withering first discusses the ways in which the medicinal properties of animal and vegetables might be found out He realizes that "the more obvious and sensible properties of plants, such as colour, taste and smell, have but little connexion with the diseases they are adapted to cure, so their peculiar qualities have no certain dependence upon their external configuration" He then points out that "we have hitherto made only a very small progress in the chemistry of animal and vegetable substances" and that "Their virtues must therefore be learnt either from observing their effects upon insects and quadrupeds, from analogy, deduced from the already known powers of some of their congenera, or from the empirical usages and experience of the populace.

"The first method has not yet been much attended to, and the second can only be perfected in proportion as we approach towards the discovery of a truly natural system, but the last, as far as it extends, lies within the reach of everyone who is open to information, regardless of the source from whence it springs.

"It was a circumstance of this kind which first fixed my attention on the Foxglove. In the year 1775 my opinion was asked concerning a family receipt for the cure of the dropsy. I was told that it had long been kept a secret by an old woman in Shropshire, who had sometimes made cures after the more regular practitioners had failed. I was informed also that the effects produced were violent vomiting and purging, for the diuretic effects seem to have been overlooked. This medicine was composed of twenty or more different herbs, but it was not very difficult for one conversant in these subjects, to perceive, that the active herb could be no other than the Foxglove."

I have always been intrigued with the apparent ease with which Withering selected the foxglove as the active herb among a mixture of twenty. How did he do it? Was this hindsight when he wrote about it ten years later, or was it indeed a choice based on knowledge or experience of the actions of all the components of the mixture? I think that the latter is probably the case, because Withering was not only a very able physician but also a most distinguished botanist who at this very time was completing his book on *The Botanical Arrangement of All the Vegetables Naturally Growing in Great Britain*. The preface to this book, which was published in 1776, contains much useful information about plants used as foods, and those poisonous to cattle swine, dogs, birds and even insects. As a medical man, he must also have known about the actions and uses of plants in the treatment of disease, so that Withering was extremely well equipped to exploit the clue which chance presented to him. Moreover, he was a natural clinical scientist and pharmacologist, and it is most interesting to watch his reactions to situations comparable with those that arise to-day in the therapeutic evaluation of new drugs.

Withering then undertook a preliminary, or as we should say a pilot, clinical trial, a difficult and crucial stage in the introduction of any new drug, in which ethical problems arise in selecting and treating patients with a new unproved drug that may perhaps be harmful to the patient. Withering solved this problem very simply by making his preliminary observations on the sick poor of Birmingham, of whom he saw between two and three thousand a year free of charge. He gave digitalis to a variety of cases and found it to be a powerful diuretic, though at first he used too large doses, arguing by analogy with squills (long known as a diuretic) that it should act best on the kidney when it excites nausea. He admitted that he had little time to devote to these patients and that two or three of them only, in which the medicine succeeded, did he find mentioned among his papers. Nevertheless, in his book *The Botanical Arrangement* he ventured to assert that the *Digitalis purpurea* "is certainly a very active medicine and merits more attention than modern practice bestows upon it". By 1776, however, he had not yet introduced it "into the more regular mode of prescription", which I take to mean that he had not yet used it on his private patients, but "a circumstance happened which accelerated that event. My truly valuable and respectable friend, Dr. Ash, informed me that Dr. Cawley, then principal of Brazen Nose College, Oxford, had been cured of a Hydrops Pectoris, by an empirical exhibition of the root of the Foxglove, after some of the first physicians of the age had declared they could do no more for him. I was now determined to pursue my former ideas more vigorously than before. . . ."

Withering had first to decide which part of the foxglove plant would produce the most satisfactory and reliable effects. He soon came to the conclusion that the leaf was best, and in his book he gives details as to when the leaves should be gathered and how they should be dried to form a powder of constant activity. He clearly appreciated the need for standardization of digitalis and the fact that his recommended dose of the powdered leaf is still accepted to-day shows how successfully his empirical methods worked.

His *Account of the Foxglove* consists mainly of case reports, largely his own observations, but also communications from other doctors. Withering may not have been a statistician but he certainly knew how necessary it was to avoid a biased presentation of his results. "It would have been an easy task to have given select cases, whose successful treatment would have spoken strongly in favour of the medicine, and perhaps been flattering to my own reputation. But Truth and Science would condemn the procedure. I have therefore mentioned every case in which I have prescribed the Foxglove, proper or improper, successful or otherwise." He is not only honest but hesitant to generalize, perhaps influenced by his Edinburgh teacher of medicine, William Cullen, of whom Withering wrote: "His modesty is such that whatever he advances as new he cautions us thoroughly to examine, and only to embrace with 'a slow consenting academic doubt'." (Fulton[5].)

Now, in any therapeutic trial of a new drug everything possible must be done to minimize the risk to the patient. One way of dealing with this ethical problem is to give the drug only to patients who are desperately ill and most unlikely to recover with available methods of treatment. If a new drug works in such conditions it must be a very effective remedy indeed, and if it should fail or prove toxic, or even cause death, the course of events has merely been hastened, not altered. It was by force of circumstances, rather than choice, that Withering first gave digitalis to cases that "must be considered as the most hopeless and deplorable that exist". He goes on, "for some years I seldom prescribed it but when the failure of every other remedy compelled me to do it; so that upon the whole, the instances I am going to adduce, may truly be considered as cases lost to the common run of practice and only snatched from destruction by the efficacy of the Digitalis; and this in so remarkable a manner that if the properties of that plant had not been discovered, by far the greatest part of these patients must have died". It is against a similar, or even more hopeless, background that many new drugs have been, and are being, tested in patients with inoperable cancer, and so far, with one or two minor exceptions, without the happy issue that Withering frequently achieved with digitalis in the treatment of dropsy.

There is no justification for the introduction of a new drug into medical practice unless it is in some way better than existing drugs or other methods of treatment. A new analgesic should have advantages over morphine or aspirin, a new antibiotic should do something which existing ones do not, and new cortisone-like compounds should be more effective and less harmful than those now in use. Withering made this point clearly in relation to digitalis when he said: "I wish the reader to keep in view that it is not my intention merely to introduce a new diuretic to his acquaintance, but one which, though not infallible, I believe to be much more certain than any other in present use". In many of his case records of the successful use of digitalis he mentions the previous unsuccessful use of squills, until that time regarded as the most effective diuretic drug.

All drugs can produce harmful effects of some kind or other, certainly when an overdose is given and sometimes with doses in the therapeutic range. Withering recognized the effects of digitalis overdosage clearly enough—sickness, vomiting, purging, giddiness, confused vision, objects appearing green or yellow, slow pulse, even as slow as 35 in a minute, cold sweats, convulsions, syncope, death. Some of these effects had long been known, witness its therapeutic use as an emetic and purgative. Withering's contribution lay in his ascribing the beneficial effects of digitalis in dropsy to its diuretic action, and in showing that this action could in favourable cases be brought about by doses too small to produce sickness or any other affliction. Those dropsical patients who failed to show the diuretic response to digitalis were particularly liable to suffer toxic effects. To take account of both favourable and unfavourable cases, Withering recommended that digitalis powder be given in doses of 1–3 grains twice daily and "let it be continued, until it either acts on the kidneys, the stomach, the pulse, or the bowels; let it be stopped upon the first appearance of any one of these effects and I will maintain that the patient will not suffer from its exhibition, nor the practitioner be disappointed in any reasonable expectation".

Withering realized that digitalis was not effective in all cases of dropsy, but in spite of his cryptic comment "That it has a power over the motion of the heart, to a degree unobserved in any other medicine, and that this power may be converted to salutary ends" it was not possible for him to attain to the modern view that digitalis only works in those cases where œdema or dropsy is due to heart failure, that its primary effect is to improve the efficiency of the heart's action, increase the force of ventricular contraction, increase the amount of blood pumped out by the heart and thereby to improve the function of the kidney so that the œdema fluid is excreted as urine. If Withering had indeed expressed such a view it would have been pure speculation, for the very idea of heart failure as a cause of dropsy was not propounded until the early nineteenth century by Corvisart, Napoleon's physician.

I have referred to Withering at some length because I think his *Account of the Foxglove* is a wonderful demonstration of what can be achieved by empirical methods. But I think, too, that it was because there was no rational explanation as to how digitalis worked that the drug was used to such poor effect during the nineteenth century. Even when the association between heart failure and dropsy was appreciated, it was thought that digitalis acted primarily as a diuretic by an action on the kidneys and that the function of the heart improved as a consequence of the removal of the accumulated fluid from the body[6]. It was still used, too, for the treatment of consumption, and even Ferriar[7], who is often regarded as the first to appreciate that digitalis acted primarily on the heart when he said "the power of reducing the pulse is its true characteristic", and Kingslake[8], who claimed that it led to "augmented strength and slowness of pulsation, and also the transmission of a larger quantity of blood through the sanguiniferous system in a given time" were thinking in terms of its beneficial effects in pulmonary tuberculosis and not of its diuretic action in heart failure.

It was not until the early years of this century that Cushing, Mackenzie and Lewis put forward an acceptable rational explanation for the action of digitalis in heart failure. Mackenzie noted that digitalis was most effective in patients who had heart failure resulting from old-standing rheumatic affection of the heart with dilatation and an irregularity of the heart beat which he called "auricular paralysis" and now known as auricular or atrial fibrillation. In these cases it not only slows the rate of the heart "but the size of the heart diminishes, the urine increases and the dropsy disappears"[9]. Lewis[10] wrote that "the chief service of digitalis is to control ventricular rate in cases of this type. By slowing the ventricle it decreases the expenditure of energy". He described digitalis as a "cardiac hypnotic" which conserves the heart's energy and prolongs its rest.

Others, however, were impressed by observations that digitalis was sometimes very effective in heart failure with normal rhythm and in such cases relief can be obtained with very little slowing of the heart rate. Modern experimental work has shown that digitalis works by helping the failing heart muscle to make the most efficient use of the energy released by the chemical reactions within the muscle. In this way it can be of benefit in the treatment of heart failure whatever the rhythm.

Thus digitalis, an outstanding empirical discovery, has only attained its full therapeutic status since its mode of action was understood.

The rational discovery of new drugs was not feasible before the rise of pharmacology as a science during the nineteenth century; this in turn depended on the development of modern chemistry, with its ability to synthesize pure compounds and to extract active principles from natural sources, and also on the growth of physiology and bacteriology. During the nineteenth century the modes of action of the newly isolated alkaloids on the various systems of the body were studied by, among others, François Magendie (1783–1855) and his famous pupil, Claude Bernard (1813–78), who analysed the mechanism by which the arrow poison, curare, caused muscular paralysis. Pasteur's researches on the germ theory of disease paved the way for Lister's demonstration in 1867 of the antiseptic action of carbolic acid, which was the first hint of the possibility of chemotherapy in the modern sense. In the latter half of the nineteenth century the physiological role of the endocrine glands was steadily elucidated, initially by accurate clinical observation, and the way was thus prepared for the subsequent isolation and synthesis of hormones to be used in the treatment of endocrine deficiencies. However, in spite of the introduction of such drugs as nitrous oxide, ether and chloroform as general anæsthetics in the 1840's, of chloral hydrate as the first synthetic hypnotic drug in 1869, of cocaine as a local anæsthetic in 1884, and the synthesis of aspirin in 1899, the nineteenth century was a period rather of preparation than fulfilment. At the turn of the century, fever due to bacterial infections was treated in a semi-logical way by drugs of the aspirin type which merely lowered the temperature without affecting the bacteria; it took thirty-five years and more to find drugs which would lower body temperature by eradicating the infection.

'Chemotherapy' is the term introduced by Paul Ehrlich to describe the logical use of drugs to kill off parasites which cause disease.

Quinine, or rather cinchona bark, is really the origin of chemotherapy. Cinchona bark was discovered at some unknown time by empirical or other means as a remedy for the ague (or malaria) in South America; it was introduced into Europe during the seventeenth century, and in 1820 the active principle, an alkaloid named quinine, was isolated by two French chemists, Pelletier and Caventou. At this time the cause of malaria was not known, but quinine was of established empirical value, though, like digitalis in dropsy, it was not used to the best advantage. A crucial moment in the development of chemotherapy came in 1856 when William Henry Perkin, at the age of eighteen, attempted to synthesize quinine from a chemical compound called toluidine. He failed, but this experiment led directly to another in which he made the first artificial dye, aniline purple or mauve. He thus initiated the synthetic dyestuffs industry, but the full exploitation of this field was taken over by the Germans during the last quarter of the nineteenth century. It was in this environment that Ehrlich grew up and dyes seemed to fascinate him all his life[11]. His M.D. thesis, written in 1876, when he was twenty-four, dealt with the theory and practice of histological staining with particular reference to aniline dyes; he studied the distribution of dyestuffs first in blood and then in living animals and showed that certain dyes stained specifically certain organs or types of cells[12]. With the rapid contemporary developments in bacteriology it was natural that Ehrlich should turn his attention to the staining of bacteria, and after others had shown that some dyes could kill bacteria, he made his first experiment in chemotherapy in 1891. He observed that methylene blue stained malarial parasites very effectively, and Ehrlich and Guttman then successfully used this dye in the treatment of some patients with tertian malaria, though the results were not good enough to warrant the substitution of methylene blue for quinine. It is interesting to consider why this promising development went no further at that time. The reason was that although the malarial parasite had been discovered in the red blood cells of malarial patients by Laveran in 1880, it was not until 1897 that Sir Ronald Ross demonstrated transmission of the parasite by mosquitoes. Thus, in 1891, Ehrlich had no rational method for testing antimalarial substances in animals, and it would have been quite unjustified to subject patients to tests with new and untried drugs. Indeed, it was not until 1924, nine years after Ehrlich's death, that experimental malarial infection of small birds (canaries) was used in Germany for the testing of new antimalarial drugs. This helped in the discovery first of plasmochin and then, in 1933, of atebrin, which under the name 'mepacrine' was of decisive value to the allied armies in the Second World War after the Japanese had overrun Java, the main source of the world's supply of quinine.

As Sir Henry Dale[13] has said, the real opening of Ehrlich's campaign for the synthesis of artificial chemotherapeutic remedies began with the discovery by Laveran and Mesnil in 1902 that mice and rats could be experimentally infected with trypanosomes, the organisms responsible for sleeping sickness. In the following year Ehrlich discovered a benzidine dye that could cure such laboratory infections, and he aptly named it trypan red. Although this dye proved to be unsuitable for the treatment of human trypanosomal infections, it provided "the real point of departure for the new scientific adventure of chemotherapy"[13]. Ehrlich's work was guided by theoretical principles concerning the interaction between drug and parasite. His first principle was "*Corpora non agunt nisi fixata*", meaning that parasites were only attacked by drugs which became fixed to their so-called chemoreceptors. His aim was to find drugs which had a stronger specific affinity for infecting parasites than for the organs of the infected host, or in Ehrlich's terms, a stronger "parasitotropic" than "organotropic" activity. In ordinary language, the drug must kill the infecting parasite without harming the patient. Ehrlich's enthusiasm and hopes for chemotherapy were unbounded and are well summed up in his objective "*Therapia magna sterilans*" or complete recovery with one massive dose of drug.

Ehrlich's outstanding contribution to chemotherapy was the introduction of the organic arsenical drug salvarsan for the treatment of syphilis in 1910. In 1905, the micro-organism responsible for syphilis was identified as a spirochæte and, in 1906, the Wassermann blood serum test for the diagnosis of syphilis was discovered. Actually, salvarsan or '606' (606th compound of the series) was made in 1907, but its importance was at first overlooked by an incompetent assistant, and it was not until a Japanese named Hata joined Ehrlich in 1909 that its remarkable activity in curing experimental syphilis in rabbits was revealed, and in 1910 its successful use in the treatment of human syphilis was announced. One is lost in admiration at the speed with which Ehrlich exploited the opportunities which came his way. His achievements are a wonderful illustration of the blended application of logic, empiricism and opportunism to a problem of practical importance. Sir Henry Dale has written about this discovery: "It would be difficult to exaggerate the effect on medical opinion throughout the world of this first success in producing by an experimental research organized to that end, a specific remedy, acting directly on a disease of such importance as syphilis".

The discovery of drugs for the treatment of bacterial infections—bacterial chemotherapy—which seemed a logical enough development, proved a much harder nut to crack, and it was not until 1932 that the first effective antibacterial sulphonamide—appropriately a dye called prontosil rubrum—was synthesized in Germany by Mietsch and Klarer, and shown to be effective in experimental streptococcal infections in animals by Domagk, the first clinical reports of its successful use appearing in 1935. Why was there such a delay between the discovery of salvarsan for syphilis and prontosil for bacterial infections? One reason seems to be that in testing new compounds, far too much reliance had been placed on their ability to kill bacteria growing in cultures outside the body. Only if a compound could kill bacteria under such conditions in ½ hr was it tested further for its capacity to kill bacteria in an experimentally infected animal. Now, prontosil rubrum had no effect on bacterial cultures growing *in vitro* but was highly active against infections *in vivo*, owing as we have seen to its conversion to sulphanilamide in the body, but even sulphanilamide would not have shown up in a brief *in vitro* test with the culture media then in common use. The famous physiologist, Karl Ludwig, once said, "Die Methode ist alles". Here is a case in point.

But since the sulphonamides were not discovered until the mid-1930's one may well wonder what was happening to penicillin, the activity of which against certain bacteria growing on a culture plate Fleming had first revealed by a brilliant piece of opportunism in 1928. Here is a different story, the difficulties being two-fold. First, when chemists tried to isolate penicillin from the medium on which the mould *Penicillium notatum* had been grown they found that the usual methods of extraction caused it to disappear like a will-o'-the-wisp, and by 1932 they had given up in despair. Fleming's original solutions of penicillin were actually tested on infected human wounds but were not strong enough to have much effect and were far too weak to act on generalized infections. One can only speculate on what would have happened had Fleming tested his solutions on experimental infections in animals, which, following Ehrlich, he should have done. However, the second and equally important reason for the failure to develop penicillin at that time was the prevailing sceptical climate of opinion which regarded bacterial chemotherapy as a pipe-dream. I remember this period very well, as in the early 1930's I was a medical registrar at the Middlesex Hospital, and my duties included the treatment of patients with syphilis. Here, with our neosalvarsan and bismuth, we said, was chemotherapy, but for our patients with pneumonia, septicæmia and infections of the kidney there were no specific drugs at all, and we all felt that bacterial chemotherapy was a hopeless quest. It is true that occasionally we gave injections of drugs such as mercurochrome and gentian violet to patients with septicæmia, who, like Withering's patients, had to be "considered as the most hopeless and deplorable that exist", but with the unhappy difference that the greater part of our patients died. But what did Fleming think about all this a little more than two years after his discovery of penicillin? Early in 1931 he was the main speaker at a discussion at the Royal Society of Medicine on the intravenous use of germicides, and he produced evidence that all the antiseptics which had hitherto been tried in this way were more lethal to the white cells of the blood than to the bacteria they were designed to kill[14]. However, he did find grounds for the faint hope that "some mercury compounds will be elaborated which will be more potent than those cited, and it is possible that by the application of this principle—the use of a chemical not to kill bacteria but to act as an adjuvant to the natural defences—the most beneficial results will be obtained in that dreaded condition, a generalized *Streptococcus pyogenes* infection". Penicillin was not mentioned during the whole meeting.

Such was the atmosphere at that time and I have always felt that the discovery of the sulphonamides, though by itself a great therapeutic advance, achieved even more by fanning a favourable wind of change which, reaching Oxford in 1939, inspired Florey, Chain and their colleagues to produce penicillin as the outstanding antibacterial agent of all time.

I am not going to discuss the numerous further advances in bacterial chemotherapy, but I would like to comment on the reason for the relative failure of what has been called, over-ambitiously, the chemotherapy of cancer. It is both logical and empirically beneficial to use drugs to kill bacteria and other parasites which cause disease. This has been possible because these micro-organisms differ sufficiently from the cells of the body to be susceptible to drugs which do not harm the patient. But the cells of a cancerous growth much more closely resemble the normal cells of the body and drugs which kill off these malignant cells inevitably damage some normal cells as well. A new approach may well be needed here.

In quite other fields an understanding of causal factors in disease has also led to the more rational use of drugs. The antihistamines could only have been developed after it had been established that histamine was a constituent of many tissues and that its liberation in an active form was responsible for allergic reactions such as urticaria and hay fever. The therapeutic administration of hormones in diseases due to reduced secretion from endocrine glands, for example, insulin in diabetes mellitus and thyroid hormones in myxœdema, is entirely rational and empirically effective. The giving of vitamin D for rickets, or vitamin C for scurvy is also rational therapy, but the use of vitamins as 'tonics' is not.

Sometimes a successful therapeutic agent may throw light on the nature of a disease, as happened when Minot and Murphy in 1926 showed, on an erroneous hypothesis, that liver treatment was a pernicious anæmia. In other cases a drug introduced for one purpose may turn out to have a therapeutic effect of quite a different kind. In 1920, Saxl and Heilig were testing an organic mercurial drug for the treatment of syphilis in a patient with heart failure, and the unexpected profound diuresis which followed led to the introduction of the valuable mercurial diuretics. These and other happy accidents recall Francis Bacon's comment "That medicines and cures were first found out, and then after the reasons and causes were discoursed, and not the causes first found out, and by light from them the medicines and cures discovered" (*Celsus—De Medicina—*1*st Cent. A.D.*).

In some diseases we are still too ignorant of causes to know how best to look for therapeutic remedies. For example, the nature of the changes in the brain which provoke disorders of the mind are far too little understood to provide a logical foundation for the use of the many drugs introduced for treatment, and in this case even their empirical value is in doubt.

Over the centuries new drugs have been discovered by lay people, practising doctors, and research workers in university departments or medical research organizations, but latterly the discovery of new drugs has become almost exclusively a business matter. It is therefore interesting to consider how the pharmaceutical industry organizes its search for El Dorado. It realizes that new drugs cannot be designed by logical deductions from valid general principles, that chemical theory alone is not enough. The relation between chemical structure of a compound and its biological activity is the fundamental problem of pharmacology, and most research is conducted on the hypothesis that drugs act by combining with specialized parts of cells called receptors, and that such combination in some way alters cellular activity. However, while a great deal is known about the chemical properties of drugs, very little is known about the nature of receptors, so it is not surprising that interactions between the two should sometimes have unexpected consequences. We have not yet reached the stage optimistically forecast by Thomas Huxley[15] when he addressed the famous International Medical Congress of 1881 and said "sooner or later the pharmacologist will supply the physician with the means of affecting, in any desired sense, the functions of any physiological element of the body". Perhaps 'later' is the operative word. To deal with this situation the research department of a pharmaceutical firm organizes its activities on the basis of provisional hypotheses and empirical tests. Logic provides a direction to research and experience decides whether this is the right direction or no, and in providing information may, by a sort of feedback mechanism, initiate new hypotheses and so lead to fresh experiments.

Pharmacology is the most eclectic of all the medical sciences. Apart from its own theory and practice it seeks inspiration and guidance from chemistry, biochemistry, biophysics, physiology, bacteriology, pathology, immunology and, of course, from medicine itself. The practical application of all this is seen in any industrial research institute where departments, or at least individuals, dealing with these various activities are to be found and where the emphasis is on team work. It is in the nature of things that the chemists who make the potential new drugs should initiate most of the research, but it is the pharmacologist who is the arbiter, usually a very destructive one, since only one compound in several hundred is likely to find any therapeutic application. It might therefore be said that the chemist proposes and the pharmacologist disposes, but the function of the latter is much more constructive than this suggests since the success of the whole project depends on the use of appropriate experimental pharmacological tests, and that it is he who is most likely to make 'lucky strikes'.

Any type of research has to be planned, which means that work must start from some hypothesis, which in our case will take account both of the nature of the disease against which the drug is to be effective and also the chemical constitution and pharmacological actions of drugs previously used for treatment. If the disease is a bacterial infection, experiments will be done to test the new drug's efficacy in killing these particular bacteria when grown *in vitro* and also to find out whether it can protect infected animals. If the aim is to produce a new drug for the treatment of, say, high blood pressure, the problem is more difficult, since the disease cannot be satisfactorily mimicked in experimental animals. In such a case the new drug would be studied for its ability to lower the blood pressure in normal animals and in animals in which the pressure has been experimentally raised, but these are not always reliable criteria. If a drug is to compete with morphine as an analgesic it must be capable of suppressing responses to noxious stimuli—but these examples are enough.

The other side of the picture is the chemical constitution of existing drugs of the different pharmacological or therapeutic groups. Much research is devoted to the making of chemical modifications of existing drugs to try to increase their effectiveness and reduce their toxicity. Thus, we have spates of antihistamines, cough suppressants and tranquillizers. This type of research is profitable to the industry, but excessive multiplication of drugs with similar actions can be most confusing to the doctor.

It is very difficult to plan a major advance in drug therapy, but what a research institute can aim at is 'organized opportunism', the ability to spot and exploit a happy accidental discovery, which may arise from work directed to quite a different end. To avoid missing opportunities, all new chemical compounds are submitted to numerous pharmacological tests of many different kinds—a process called 'screening' a drug. By thus spreading the net it is hoped to pick up the very occasional very big fish, but nothing illustrates better the role of empiricism in modern pharmaceutical research.

Although the discovery of new drugs is a form of applied research, I do want to emphasize very strongly that such research can only flourish if it receives constant nourishment from so-called pure research, conducted in an academic atmosphere where problems are studied for their own sake. This is to my mind the only way in which the process of discovering new drugs can become more rational, so it is very important that fundamental research on the intimate mechanism of drug action, by chemists, pharmacologists, biochemists, microbiologists and others, should be given more support than it now enjoys. I should also like to see freer communication between workers in academic and applied forms of research so that each can better appreciate the other's point of view, and I would recommend that all who work in the microcosmic atmosphere of the laboratory should now and again sniff the macrocosmic air in which patients actually live.

And now for the rest of Huxley's prophecy, made several years before Ehrlich talked of magic bullets: "It will, in short, become possible to introduce into the economy a molecular mechanism which, like a very cunningly contrived torpedo, shall find its way to some particular group of living elements, and cause an explosion among them, leaving the rest untouched." One might perhaps hope that with the incorporation of modern homing devices, and maybe also biological nuclear explosives, Huxley's torpedoes could be given instructions for the logical treatment of all ills; but I for one would welcome an assurance that accidental explosions will never be empirically recorded.

I thank Dr. F. N. L. Poynter, chief librarian of the Wellcome Historical Medical Library, for his help with historical aspects of this article.

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LONGEVITY OF MICRO-ORGANISMS

By Dr P H. A. SNEATH
National Institute for Medical Research, London, N W7

THE maximum time that micro-organisms can survive in a dormant state is of interest from several points of view as a guide to the preservation of cultures, to throw light on the mechanism of dormancy and in relation to the transmission of life through space Lately, the advent of space technology has raised the question of interplanetary contamination by micro-organisms carried by space rockets, and the dangers of this have been discussed elsewhere[1-3] The difficulties in disinfecting space-vehicles[4,5] make it desirable to know how long micro-organisms may survive in them if protected from solar radiation, since these vehicles may remain in orbit for centuries and, should they later fall on a planet, they may be unsuspected sources of microbial contamination if they had not been sterilized before launching

Keilin[6] has summarized the data on longevity of organisms in a dormant state, which he calls 'anabiosis' The most remarkable record is the survival of seeds of the lotus *Nelumbo nucifera* in a peat bog for more than a thousand years Many other plant seeds are known to survive for a hundred years or more Some higher organisms can resist extremes of heat, cold and desiccation, for example, larvæ of the chironomid fly, *Polypedilum vanderplanki*[7] Micro-organisms have long been known to resist even greater extremes of heat, cold, desiccation and chemical disinfection, but much less is known about the length of time they can survive under favourable conditions Most of the information on this is summarized in Engley's extensive review[8] Claims that bacteria have survived in coal since it was deposited are not generally accepted[6] Bacteria have been reported from a mammoth frozen in Siberia[9], but the possibility of later contamination seems considerable, there is uncertainty on how long bacteria survive in permanently frozen soil[10,11] There is information on the survival of many pathogenic bacteria, and those which survive longest are bacterial spores, although some Gram-positive cocci live almost as long It is commonly considered that spores of *Bacillus anthracis* and *Clostridium tetani* are among the most resistant, and there are records of survival of these for thirty or forty years[12,13] One virus survived for 38 years in blood[14] The longest survivals that I have been able to trace are those reported by Wilson and Shipp[15] and by Jacotot and Virat[16] The former authors found viable spores of a thermophilic bacillus in a tin of meat which had been canned 118 years previously The latter authors reported that anthrax spores prepared by Pasteur in 1888 were alive 68 years later

Suitable material for such investigations is not easy to find Most of it is liable to recent contamination, since very little material has been sealed in a container impervious to penetration by micro-organisms The significance of the results therefore depends on showing that the count of viable micro-organisms is higher than can be readily accounted for by recent contamination One promising material for investigation is soil dried on the roots of plants in botanical collections Some of these plants were gathered more than three hundred years ago, and have since then been kept constantly dry The relative humidity in herbaria is generally rather low, and must in any event be low enough to prevent growth of moulds on the specimens There is therefore little likelihood that bacteria found in the soil on these plants can have multiplied since they were collected In a few instances this can be asserted with some confidence since the roots have been painted with strong solutions of mercuric chloride which would exclude any multiplication after the date it was applied. Although powerfully bacteriostatic at extreme dilutions, mercuric chloride is well known to possess very little bactericidal action, and recovery of most of the organisms occurs on culture in media containing an adequate source of sulphydryl groups[17,18] Nevertheless, in material not treated with mercury salts there is a possibility that multiplication of some micro-organisms may slowly occur even under the dry conditions in herbaria, for there are some data on desert soils and on soils studied at low humidities which suggest that this can occur[19] For various reasons mentioned here, this does not seem to be a likely explanation of the findings reported here.

Soil samples Through the kindness of the Keeper of the Herbarium, Royal Botanical Gardens, Kew, Surrey, and the Keeper of the Herbarium, British Museum (Natural History), I was able to obtain samples of soil from the roots of a number of plants, mostly grasses, which had been gathered and dried between 1640 and the present day Granules of soil were detached from the roots with sterile forceps and placed in small sterile tubes Small fragments of mounting paper and of florets and leaves served as control specimens The soil was crushed to a fine powder with a sterile glass rod, and by using a small platinum scoop which held a known weight of soil and which could be sterilized by flaming, known amounts of soil were transferred to the culture media described here Where necessary, dilutions were made in the culture media or in sterile distilled water Replicate viable counts gave reasonably consistent results

The following media were used for viable counts

Nutrient agar (for 'total' counts) Meat extract agar[20] to which had been added 0 1 per cent (w/v) of Difco yeast extract and 0 1 per cent of thioglycollic acid (to neutralize mercury ions) was used as a pour plate medium The soil inoculum was added to 20 ml of the melted medium (cooled to 56°), and this was poured on to a solid basal layer of 20 ml of the same medium in a Petri dish After solidification, a top layer of 5 ml of uninoculated medium was poured. The plates were incubated aerobically at 25° for five days, and the colonies counted

'Thermoacidurans' agar (selective medium for *Bacillus coagulans*) Difco 'Thermoacidurans' agar was used, composed as follows Bacto yeast extract, 5 gm , 'Proteose' peptone, 5 gm . glucose, 5 gm ; dipotassium hydrogen phosphate, 4 gm , agar, 20 gm , distilled water, 1 litre The medium was

autoclaved and cooled to 56° and than adjusted to *p*H 5 0 The agar, in 20-ml amounts, was inoculated with soil and pour plates were made A top layer of 5 ml was added The plates were incubated aerobically at 50° for two days

'*Oxoid' reinforced clostridial medium* (Oxo, Ltd, Queen Street Place, London, E C 4) This was used for pour plates and incubated anaerobically at 30° for five days Some strains of *Clostridium* were isolated, but with the oldest samples the colonies proved to be strains of *Bacillus*

'*Oxoid' Littman ox gall agar* (for fungi) was used with the addition of 30 µgm /ml of streptomycin Plates of this medium were inoculated with soil suspensions, spread with a sterile glass spreader, and incubated aerobically at 25° for five days

'*Oxoid' wort agar* (for fungi) was used with the addition of 'Novobiocin', 100 µgm /ml This antibiotic was recommended as selective for fungi by Butler and Hine[21] Surface inocula were made as above, and the plates were incubated for five days at 25°

Benedict agar plus 50 *µgm /ml 'Pimaricin'* (for streptomycetes) This medium is recommended for the isolation of streptomycetes[22] Surface inocula were made as above, and the plates were incubated at 25° for twelve days Some bacteria and some fungi grew in addition to the streptomycetes, and the colonies of the latter were therefore confirmed by examination with the low power of a microscope

Identification of bacteria Almost all the bacteria grown from the older soil samples were species of *Bacillus*, and they were identified by the usual tests[23-25]

The results of the examination of these soils are shown in Figs 1 and 2 It can be seen from them that fungi and streptomycetes do not survive for many years in dry soil samples This is in keeping with what little is known about their survival in soils, though some fungi may survive longer[26] The only viable organisms which are found in the older samples are sporing bacilli, almost all being aerobes of the genus *Bacillus* In Table 1 is shown the identity of these It is seen that the majority of those from the older soils are strains of the *Bacillus subtilis–pumilus–licheniformis* group Most of them were typical The longevity parallels the heat resistance of the spores[27]

Table 1 SPECIES OF *Bacillus* ISOLATED FROM SOIL SAMPLES OF DIFFERENT AGES

Species	No of isolates from soils of ages (yr) 0–50	50–100	100–200	200–320
B subtilis	29	12	9	4
B licheniformis	5	2	2	1
B pumilus	1	0	1	0
B coagulans	6	4	0	0
B firmus	1	0	0	0
B megaterium	3	0	0	0
B cereus	3	0	0	0
B circulans	4	4	0	0
B polymyxa	1	0	0	0

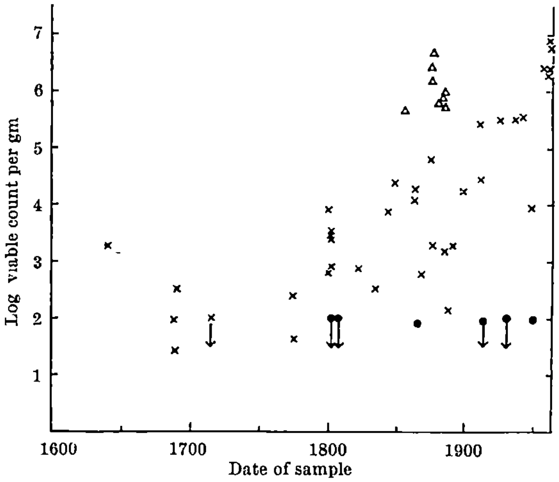

Fig 1 Viable counts of soils on aerobic nutrient agar The triangles indicate counts on sealed samples The circles indicate counts on materials used as controls, for example, leaves, florets, paper The arrows indicate that the counts were less than the value shown (no growth from 10 mgm of material)

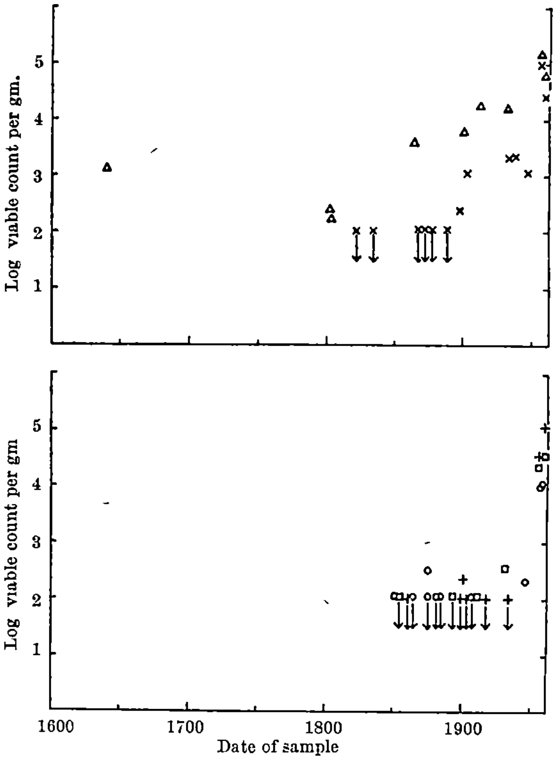

Fig 2 Viable counts of soils on selective media Upper part bacteria lower part fungi and streptomycetes × 'Thermoacidurans' agar, △, reinforced clostridial medium (anaerobic), □ Littman ox gall streptomycin medium, +, Wort agar with 'Novobiocin', ○, Benedict medium with 'Pimaricin'

For the meaning of the arrows, see legend to Fig 1

Fig 1 shows that the death-rate of bacteria in soil follows a line which may be somewhat curved There is a rapid fall in total viable count in the first few years owing to the death of many non-sporing bacteria Thereafter, there is a slower rate of death of more resistant forms such as fungal and bacterial spores After about 50 years the survivors are almost all spores of species of *Bacillus*, and the death-rate of these is slow There is a suggestion of a still slower component, representing the spores of the *Bacillus subtilis* group, which are viable in the oldest samples One would expect such an appearance on drying soil containing a very mixed population of microorganisms, and the overall curve is not unlike the decay curve of a mixture of radioisotopes of different half-lives

It is not easy to fit a curve to the very scattered points in Fig 1 We may, however, estimate that for soils more than 50 years old the slope is such that nine-tenths of the survivors die every 50 years, possibly with a more long-lived component (nine-tenths dying approximately every 100 years) If the last figure is correct, one can estimate that a ton of dry soil would still contain a few viable spores even after 1,000 years It will be seen from the figures that material such as the mounting paper, small fragments of leaves and florets, etc , had very low viable counts, which gives some confidence that the counts on the soil are not due to contamination since the date of collection The narrow spectrum of surviving forms in old soils (contrasting with the wide range of bacteria and moulds which can be isolated from freshly dried soil or from dust) also supports this There was no great difference between the viable counts from soils which had been treated with mercuric chloride and those which had not

A few samples of soil from Rothamsted Experimental Station were made available through the kindness of the director, Mr F C Bawden These had been collected from agricultural land in the latter half of the nineteenth century, and had been dried, powdered and stored in sealed jars These samples gave heavy counts of aerobic sporing bacilli, ranging from 3×10^{5} to 5×10^{6} per gm , most being of the *Bacillus cereus* and *Bacillus subtilis* groups Though they antedate the discovery of penicillin, many of these strains produced large amounts of penicillinase The high counts, as high as those in many recently collected samples, may have been due to better survival in sealed containers, or possibly to multiplication to very high levels during the early treatment of the soils (of which details are now not available) These results have not been included in estimating the death-rates, but are shown in Fig 1 as the triangular symbols These samples had very few moulds or streptomycetes, but some *Clostridium* colonies were found in anaerobic plates

A number of other materials have been briefly examined by the techniques given above Through kind permission of Dr P S Nutman, eighty soil cultures, mostly of soil bacteria, which had been set up in 1925 by Sir Gerard Thornton at Rothamsted, were examined These had been set up by adding to dry, sterile soil in plugged vials enough of a culture of the organism in liquid medium to moisten it thoroughly, the cultures were allowed to dry out and were preserved at room temperature and humidity in the dark In 1939 Sir Gerard Thornton had found 55 of the cultures still viable on culturing about 100 mgm of the soil In 1960 twenty of these were still viable As expected, most of the sporing bacilli were still alive, and most of the non-sporing bacteria were dead These results are similar to those of Jensen[28], though he obtained growth of *Rhizobium* after 45 years Viable counts on the known spore-forming organisms are of interest as indicators of the likely death-rate of different species in natural soil It was found that the viable counts per gram of three strains of *Bacillus cereus* var *mycoides* were approximately 5×10^{7}, 2×10^{8} and 9×10^{7}, for one strain of *Bacillus megaterium* it was approximately 4×10^{8}, and for seven strains of the *Bacillus subtilis* group the counts were approximately $1\ 5 \times 10^{7}$, 1×10^{8}, 4×10^{5}, 4×10^{6}, 4×10^{9}, 3×10^{9} and 6×10^{8} Very probably the original spore counts were about 5×10^{9} per gm when the soil tubes were first inoculated These figures do not suggest that *Bacillus subtilis* spores have a much slower death-rate than spores of the other species the data are, however, by no means conclusive

Through the kindness of Dr E O Callan, of Macdonald College, Quebec, some coprolites from Mexico were examined These were human fæces from the Meso de Guaje culture, and date from about 1500 B C They have been preserved in caves by very dry conditions The outer surface was thoroughly flamed and the coprolites broken open with aseptic precautions, and the fibrous material of the interior was cut out and cultured This material was sterile when about 100 mgm quantities were cultured in nutrient broth or in pour plates of nutrient agar aerobically and anaerobically No bacteriophages active on *Escherichia coli K*-12 were isolated In such fæcal material one would expect there to have been coliform bacilli, fæcal streptococci, spores of *Clostridium welchii*, and possibly spores of other *Clostridium* species and of *Bacillus* species The fæcal material was excellently preserved, and microscopically the structure of the plant residues was easily seen, and a few objects morphologically resembling bacteria were seen among the plant residues This material was examined by Miss Joan Webb of this Institute by gas phase chromatography, and was found to have the characteristic fatty acid pattern of fæcal lipids[29]

Dr Karl Schlabow, of the Schleswig-Holstein Museum, kindly sent some intestine from a peat burial of about A D 1000 (ref 30) The bowel was opened aseptically and found to contain white, waxy material, apparently fat derived from fæces, and it also showed the fatty acid pattern of fæcal lipids This was also sterile when about 50 mgm was cultured in pour plates as above However, the material had a low *p*H (about 5 4), as would be expected of material embedded in peat, and this would have caused accelerated death of the bacterial flora There was probably considerable multiplication of anaerobic bacteria after death and before the preservation of tissue by the penetration of acid materials from the peat so one would expect that *Clostridium welchii* spores and probably spores of *Cl sporogenes* and other putrefactive clostridia had been present originally Microscopically no objects resembling bacteria or their spores were seen

The results reported here seem unlikely to be accounted for by recent contamination of soil samples by dust or handling If they are accepted as substantially accurate, it is clear that viable micro-organisms can survive for centuries in soil under conditions of normal temperature and humidity. We cannot, therefore, expect contaminated space-vehicles to become sterile even after several centuries, and this implies that they may remain contaminated for periods longer than accurate records of their orbits and other particulars are likely to be preserved. It is therefore doubly important to avoid the launching of unsterilized space-vehicles, they may initially strike a planet and contaminate it, or they may become forgotten but still contaminated solar satellites It is also clear that even intense radioactive contamination of our planet is unlikely to lead to the extinction of all forms of life, since in protected places in glaciers or caves micro-organisms would survive until the radioactivity had largely decayed However, whether this is a cause for rejoicing is another matter The few data on the oldest material are not of much significance, they have been exposed to conditions which were not ideal for survival Under other conditions, for example the extreme cold of the antarctic ice-cap survival may be better Prof R H McBee (personal communication) has found more than 10^{3} viable coliform bacilli per gm in pony fæces from Scott's Antarctic Expedition of 1911, which had been continuously frozen for nearly fifty years

How long could life survive under the best conditions ? By this is meant conditions such that labile but essential metabolites are preserved, or provided in the environment at recovery, so that the organism has no difficulty in re-establishing its metabolism (probably a main cause of death in anabiosis) When we consider the survival of organisms over very long periods, we have to take into account the spontaneous chemical breakdown in those organic molecules which form the essential organizational structure of the cell, in particular, proteins and nucleic acids It seems likely that proteins break down more rapidly than nucleic acids Different amino-acids have different degrees of stability, alanine, for example, being very stable and tryptophan very unstable, and this is borne out by the results of examination of fossilized protein materials of various ages[31] These considerations are not entirely academic, since, as Lederberg points out[2], we should not discard out of hand all possibility of Avogadro's panspermia hypothesis that life has spread through the universe, and the Moon may have collected biological material in the past[1,32]

Becquerel[33] calculated that, at temperatures around absolute zero, micro organisms would survive for millions or billions of years This is obtained by extrapolation of survival curves obtained at much higher temperatures, but there seems to be no reason to doubt the general conclusion At such low temperatures not only would essential and labile metabolites be preserved, but the structural breakdown of proteins and nucleic acids would also be exceedingly slow The effect of the very high vacuum in space on micro-organisms is still uncertain, though recent work[34] suggests that it may not have a seriously lethal effect, and it would not apply to protected material

One of the uncertain factors in estimating the possible survival of life over very long periods of time is the influence of accumulated ionizing radiation While the chemical breakdown discussed here would not be important at temperatures near absolute zero, it is likely that ionizing radiations would produce damage to cells, either directly or through the production of ions and free radicals which, though they might be unreactive at these low temperatures, would presumably remain in the cell and become reactive when the cell reached an environment permitting recovery This would then, in theory, set a limit to the survival time even at absolute zero

We still know little about the effect of irradiation at low temperatures or *in vacuo*, but we may for present purposes assume a logarithmic death-rate such that 90 per cent of bacterial spores (which are among the more resistant of organisms) will be killed by an accumulated radiation dose of about 10^5 roentgens[35] If this dose is increased twenty-fold, we expect the survival to become effectively zero since, assuming the usual logarithmic relation between dose and survival, the survivors would be 1 in 10^{20} We may consider spores which are protected by a few centimetres of material from direct cosmic rays, electrons, ultra-violet radiation, etc In deep interplanetary space the radiation will probably be about 10 roentgens per year, most of it due to secondary X-rays formed by bombardment of the surface material In interstellar space the radiation may be much less, so that the radioactivity of the protective material itself becomes the main factor If the material is similar to granite, the dose will be about 0 1 roentgen a year, and in iron meteorites it is likely to be still smaller, perhaps 0 001 roentgen a year To achieve 10^5 roentgens will require in these cases respectively 10^4, 10^6 and 10^8 years In potassium-containing rocks, however, spores would receive radiation from the radioactive isotope of potassium (potassium-40) all around them, and this may be quite appreciable The range of the β-particles will be about 2 mm , and in orthoclase (potassium feldspar, $K_2O\ Al_2O_3\ 6\ SiO_2$) the dose-rate will be about 0 5 roentgen a year, due to the potassium-40

There remains one further limiting factor, and that is the irradiation produced by the decay of the potassium-40, which no organism can escape since potassium is universally distributed in living creatures A bacterial spore weighing 10^{-12} gm contains about 1,250 atoms of potassium-40, of which about 500 decay in 10^9 years, and in this period would yield a dose within the spore of about 750 roentgens This dose would kill very few of the spores, but little is known of the damage in cells due to the recoil energy of decay of potassium-40 If this behaves like decay of phosphorus-32 (ref 36), then a high proportion of spores might be killed over a period of 10^9 years For shorter periods this would not apply, and we may conclude that life could probably be preserved for periods of more than a million years, if suitably protected and maintained at temperatures close to absolute zero This may be relevant to the present-day interest in the possibility of life-forms in meteorites[37]

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CLOUD PHYSICS

PROF B J MASON delivered his inaugural lecture on "The Physics of Clouds, Rain and Lightning" at the Imperial College of Science and Technology, London, on November 14 The lecture marked the occasion of Prof Mason's appointment to the first chair of cloud physics in the College*

Of the many problems which confront the cloud physicist some which have attracted particular attention are, first, the detailed processes which bring about the condensation of cloud droplets from water vapour, secondly, the mechanism which causes the tiny water droplets to freeze, and thirdly, the manner in which the small water droplets or ice crystals grow to a size sufficient to permit them to fall out of the cloud and reach the ground as precipitation

Broadly speaking, clouds form when air containing water vapour rises to levels at which the pressure is lower The resultant quasi-adiabatic expansion is accompanied by cooling until the temperature falls below that at which the air becomes saturated with water vapour The excess vapour then condenses, but it can only do so on small particles which act as nuclei for the small droplets The atmosphere contains many such particles One c c of London air may contain 10^5 particles the radii of which range from 5×10^{-7} cm to more than 10μ Sea-salt particles make good condensation nuclei They are formed over the oceans in the foam of breaking waves when air bubbles burst to produce small droplets of sea-water The latter evaporate, leaving behind the salt nuclei 0 1μ–10μ in diameter, or 10^{-15} gm –10^{-9} gm in mass The way in which these small salt particles form has been studied by high-speed photography of the bursting air bubbles

The formation of a cloud involves the growth of a population of droplets on condensation nuclei of various sizes Condensation occurs first on the larger hygroscopic nuclei, if the available water vapour is not used up, supersaturation of the air increases and allows condensation to take place on smaller nuclei Thus, the rate at which the cloud grows is dependent on the rate of cooling of the air and the nature, numbers and sizes of the condensation nuclei present Differential equations have been set up and an electronic computer used to calculate the evolution of a population of cloud droplets Recent results have shown a resemblance between a theoretically produced drop size distribution and those which are actually observed

A typical cloud without rain formed over land contains a few hundred droplets per c c of air, but over the ocean there may only be a tenth of this number Typical droplet radii are 10μ

There are two mechanisms which bring about the growth of cloud droplets to the size of drizzle or raindrops The most recently established process explains the release of rain from clouds which are everywhere warmer than 0° C In the early days of cloud physics it was thought that rain could not fall from clouds unless their tops penetrated above the 0° C isotherm Later it was noticed that rain fell, particularly in the tropics, from clouds which were observed to be well above 0° C It was shown that cloud droplets exist in various sizes, because they form on condensation nuclei of different sizes, and grow under different conditions of temperature and supersaturation in different parts of the cloud A droplet which is larger than its neighbours will fall through them, and in doing so it may collide and coalesce with others lying in its way

A study of the coalescence process has been made by L M Hocking, of University College, London His theoretical computations inferred that cloud droplets of less than 19μ radius were unable to capture droplets of smaller size, so that their existing size must therefore be reached by condensation probably on large salt nuclei These theoretical calculations have been verified experimentally by laboratory results which agree with Hocking's values that droplets of radius less than 20μ do not take part in the coalescence process The problem of coalescence is being investigated further by a technique by means of which water is forced through a hypodermic needle which is attached to the diaphragm of an ordinary headphone The application of an audio-frequency signal of the right frequency causes the needle to vibrate and throw off a stream of drops The size and separation of the drops can be controlled Thus, the behaviour of streams of drops of different sizes may be examined through a microscope Collision is usually followed by coalescence, but sometimes the droplets rebound Behaviour of the droplets under an electric field is also being examined The introduction of an electric field assists coalescence

The second mechanism of precipitation release arises from the fact that cloud droplets exist in the supercooled state As the temperature in the ascending air cools below 0° C some of the droplets freeze In order to freeze at these temperatures they must become infected with special minute particles called ice nuclei It is not until the temperature reaches $-40°$ C or less that supercooled water droplets of a few μ freeze spontaneously Thus, as the temperature falls lower and lower below 0° C, more and more ice nuclei become active and more and more ice crystals appear among the supercooled water droplets In such a mixture of ice crystals and water droplets the saturation vapour pressure over the water droplets exceeds that over the ice crystals so that there is a vapour pressure gradient from the water droplets towards the ice crystals The ice crystals therefore grow much more rapidly than the droplets and soon reach a size sufficient to give them an appreciable falling speed In falling they may join together and form snowflakes which settle through the air in a tumbling motion until they reach warmer levels and melt into raindrops

Much of the work of Prof Mason and his group has been concerned with the basic physical processes of the ice-crystal mechanism of precipitation release Not the least of the questions involved is that of the freezing process itself Observations have been made of the freezing of a large number of drops of distilled water of diameters 10μ–1 cm at the interface of two immiscible liquids to avoid the nucleating effects caused by contact with solid surfaces Fig 1 shows the freezing temperature distribution about the most frequent value ($-24°$ C) of 1,100 such droplets of 1-mm diameter The curve shows that nucleation is

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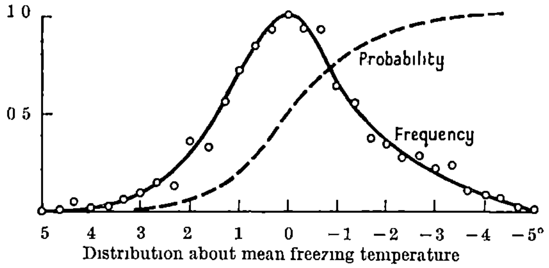

Fig 1 Distribution of freezing temperatures of 1,100 water drops of 1 mm diameter about the most frequent value of −24° C

a statistical process Mason and other workers have shown that there is a relationship characterizing the nucleation of water-drops containing small foreign particles This may be interpreted in terms of the fact that the numbers of particles which become active as ice nuclei in the atmosphere increase roughly logarithmically with decreasing temperature In experiments in which great care was taken to remove particles from the water it was possible to supercool large numbers of drops of diameter 1μ to −41° C and drops of diameter 1 mm to −33° C Such experiments suggest that groups of water molecules undergo random fluctuations of position and velocity, and become locked by chance into an ice-like arrangement to serve as nuclei to initiate freezing

The ice nuclei which initiate freezing of supercooled cloud droplets may be regarded as the fundamental particles of cloud physics Since 1 m^3 of air in the atmosphere may contain only 10 active ice nuclei among a population of 10^{11} other particles they are difficult to detect and identify One technique which has been used is to fill a cloud chamber with saturated air, chill it below 0° C, and then subject it to rapid expansion During the sudden cooling the supercooled droplets which contain active ice nuclei will freeze and grow into ice crystals These may then be counted for successively lower temperatures caused by successively greater expansions

Measurements made from aircraft over land and sea show a variable ice-nucleus population Down to about −30° C their concentration increases almost logarithmically with decreasing temperature, while below −35° C spontaneous nucleation causes a rapid increase in the number of crystals present The fact that the concentration of the nuclei appears to fall at times below the minimum value needed to release precipitation efficiently may be used as an argument for the belief that some additional precipitation may be produced by seeding clouds with artificial nuclei such as silver iodide

The nature and origin of ice nuclei are somewhat controversial Prof Mason believes their origin is the Earth's surface Dr E G Bowen, on the other hand, has suggested that meteoric showers may be an important source In an attempt to test these rival theories, laboratory experiments were conducted on various types of soil and mineral-dust particles, and also meteoric material, in order to test their ice-nucleating ability Of all these substances, the most abundant of the active particles was kaolinite, a minor constituent of the Earth's crust, which initiates ice formation at −9° C Dusts produced by grinding and vaporizing meteorites were not effective above −17° C Additional evidence from electron microscope and electron diffraction techniques in Japan has shown that 85 per cent of the identified particles were mineral, of which 80–90 per cent were clays, 50–60 per cent being kaolin

A search for substances which might be more effective than silver iodide for cloud-seeding purposes has not been successful Within the range −4° C to −11° C silver iodide, being more potent and more easily dispersed than its rivals, remains the best

Another problem in cloud physics which has attracted Prof Mason is that posed by the extraordinary variety of shape exhibited by natural snow crystals There are three main forms the hexagonal prismatic column, the thin hexagonal plate, and the branching star-shaped form The crystal form is related to both the temperature at which they form and the supersaturation of air Artificial crystals have been grown under controlled conditions in order to investigate the factors which influence the particular form they take Broadly speaking, the temperature governs the basic habits of the crystal, but supersaturation affects the speed of growth and the development of various secondary features Evidence is now accumulating which suggests explanations of the basic processes involved in crystal shape formation

The final topic on which Prof Mason touched was the generation of electricity by thunderstorms The thunderstorm may be regarded as an electrostatic generator in which the positive charge is concentrated in one region of the cloud and the negative charge in another C T R Wilson showed thirty years ago that the negative charge is centred in the lower part of the cloud where the temperature is about −5° C, while the positive charge is situated several km higher at temperatures usually below −20° C

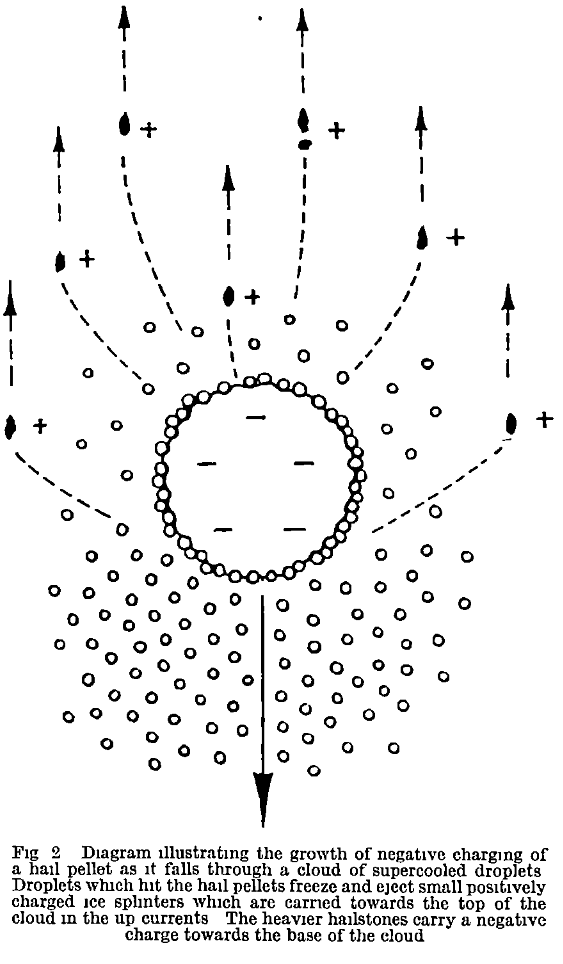

Fig 2 Diagram illustrating the growth of negative charging of a hail pellet as it falls through a cloud of supercooled droplets Droplets which hit the hail pellets freeze and eject small positively charged ice splinters which are carried towards the top of the cloud in the up currents The heavier hailstones carry a negative charge towards the base of the cloud

It has been calculated that the average rate of charge generation in a typical thunderstorm between the appearance of precipitation that is detectable by radar and the first lightning flash is about 1 coulomb/km.^{3}/min. Existing theories appear incapable of producing charge at such a rate. Prof. Mason's theory is that the formation of hail pellets in a cloud by the impaction and freezing of supercooled droplets leads to the formation of negatively charged hailstones which fall towards the base of the cloud. Meanwhile, positively charged ice splinters, formed by the bursting of the ice shell and the ejection of small ice splinters when drops of 0·03-mm.–1-mm. radius freeze, are carried by the up-currents towards the top of the cloud (Fig. 2). The electrification of artificial hailstones by the impaction, freezing and splintering of supercooled droplets has been examined in the laboratory by means of specially designed apparatus.

In a typical experiment at an air temperature of -15° C., droplets of 80μ diameter impacting at 10 m./sec. froze to produce, on average, twelve splinters and a charge of 4×10^{-6} E.S.U. per drop. These laboratory measurements lead to the conclusion that the charging of hailstones by the impaction, freezing and splintering of supercooled droplets is indeed capable of producing electric charge and electric fields at the rates required by observation, and that this is, in effect, the main mechanisms of thunderstorm electrification. It is further suggested that the large-scale generation of electricity in thunderstorms and the origin of lightning depend essentially on a thermo-electric property of ice, a preferential migration of protons under the influence of a temperature gradient which exists across the ice particles. This temperature gradient occurs because the outside of a supercooled water droplet freezes first and at a rate determined by the dissipation of the latent heat of fusion to the surroundings. The outside ice shell afterwards cools towards the lower air temperature, while the inner surface is at 0° C. Now protons tend to migrate down the temperature gradient, thus producing a positive space charge in the outer layers of the ice. When the ice droplet bursts by expansion of the freezing centre the splinters ejected from the outer layers will carry away a positive charge leaving the remainder negatively charged, as is observed.

Prof. Mason concluded his lecture by remarking that the subject of cloud physics was linked on the atomic and grand scales. Thus, the atmosphere sets the problem; this may be analysed in terms of basic physical processes which may be studied in the laboratory; finally, the pieces are put together back in their atmospheric setting. The past decade has seen considerable progress in our understanding of cloud- and rain-forming processes. All these problems will be investigated on a steadily expanding scale in the years ahead.

A. H. GORDON

A NEW RADIOHELIOGRAPH FOR AUSTRALIA

By DR. E. G. BOWEN

Chief of the Division of Radiophysics, Commonwealth Scientific
and Industrial Research Organization, Sydney

THE Ford Foundation has announced the grant of 550,000 dollars to the Radiophysics Division of the Australian Commonwealth Scientific and Industrial Research Organization for the construction of a radioheliograph for photographing the Sun in its natural radio emission (Fig. 1). The instrument represents the latest prospective addition to C.S.I.R.O.'s radio astronomical equipment and the announcement follows hard on the heels of the completion of the 210-ft. steerable radio telescope at Parkes in New South Wales. It is likely that the radioheliograph will be situated near the 210-ft. paraboloid; indeed, the two instruments will share facilities and will together form the nucleus of the Australian National Radio Astronomy Observatory which was inaugurated last year.

The function of the proposed instrument is to obtain high-resolution radio pictures of the Sun, at wave-lengths near 3·5 m., at regular intervals of time varying between seconds and minutes depending on the phenomena being recorded. The angular size of each complete picture will be 2°, and the angular resolution within this field will be equivalent to a 60-line television picture. It is designed to record both the evolution of solar bursts, such as those which accompany solar flares, and the general structure of the thermal corona.

During the post-war years, C.S.I.R.O. radio astronomers, under the leadership of Dr. J. L. Pawsey, have been actively engaged in research on solar radio emission. In particular, observations in the metre-wave spectrum have been made by J. P. Wild and his colleagues with radio spectrographs and interferometers; these have not only contributed significantly towards an understanding of the basic processes occurring in the Sun's corona at the time of flares, and of the solar–terrestrial relationships, but have also posed many new questions associated with the coronal plasma. But such studies have lacked comprehensive positional data, and the new project, designed to fill this gap, represents the next logical step in the Division's solar programme.

The instrument, which will operate at a wave-length near 3·5 m., is designed to scan the Sun rapidly with a pencil beam of reception of angular size 3·5 min. of arc between points of half-power. This will be accomplished with the use of 100 paraboloid aerials, each 42 ft. in diameter, arranged around the perimeter of an ellipse of minor axis (east–west) of 3 km. and major axis (north–south) of 3·6 km. These dimensions are so chosen as to make the resolving power of the instrument equivalent to that of a uniform circular aperture of diameter 3 km. when viewed from the direction of the Sun at noon at equinox.

Signals from the aerial elements will be taken along open-wire feeders to a central station at the southern focus of the ellipse, where they will be combined in a multiplicity of phase relationships to form the final picture on a cathode-ray tube. The sensitivity of the equipment will be enhanced by the use

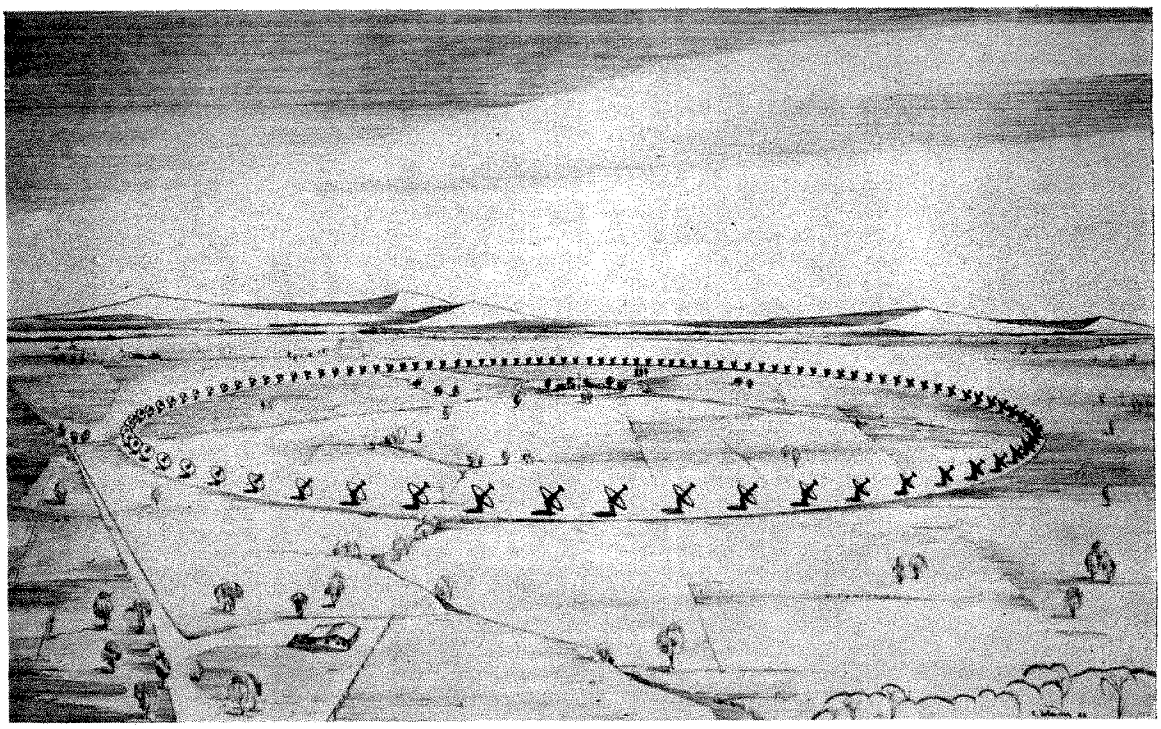

Fig. 1. Artist's impression of the proposed radioheliograph. The aerial array consists of a hundred 42-ft. paraboloids around the perimeter of an ellipse with major and minor axes of length 3·6 and 3·0 km. respectively

of 50 separate detectors, thus allowing the instrument to look in 50 different directions, along a north–south line, at the same time. The picture will be formed by sweeping these 50 beams across the face of the Sun in the east–west direction. A special post-detector computer will be incorporated to perform the process of aerial correction[1] required to make the thin annular aerial array equivalent to a full aperture.

The design and scientific direction of the project are being undertaken by Mr. J. P. Wild, who is in charge of the Division's programme of solar research. The generous grant of the Ford Foundation will enable constructional plans to proceed immediately, and it is hoped that the instrument will be ready for operation in about three years time, near the start of the next solar cycle.

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[1] Wild, J. P., *Proc. Roy. Soc.*, A, **262**, 84 (1961).

NEWS and VIEWS

Chemistry at York : Dr. R. O. C. Norman

DR. R. O. C. NORMAN, whose appointment, as from 1965, as professor of chemistry in the new University at York, has recently been announced, is just thirty. He was educated at St. Paul's School, London, and Balliol College, Oxford, where he was a Brackenbury scholar. At Oxford he was the Gibbs scholar in chemistry of his year and gained first-class honours in 1955. In 1956 he was elected to a junior research fellowship at Merton College, where two years later he was appointed as tutorial fellow in organic chemistry and has, since 1960, been acting as dean. Dr. Norman's research work for his D.Phil. degree was carried out under the supervision of Dr. W. A. Waters and dealt with aspects of homolytic substitution of polycyclic aromatic compounds. Since 1958 Dr. Norman has been directing the activities of an independent research group in the Dyson Perrins Laboratory which has substantially contributed accurate quantitative data concerning heterolytic substitution of benzene derivatives, making use of a sensitive technique of vapour phase chromatography which was developed in conjunction with his Merton colleague, Dr. C. S. G. Phillips. From this work Dr. Norman and his students have been able to put forward new quantitative theories concerning rates of aromatic substitution and the puzzling problem of the *ortho/para* ratio: for this Dr. Norman was this year awarded the Meldola Medal.

The importance of quantitative aspects of organic chemistry is year by year becoming more evident, and since it requires co-ordination both with the latest of analytical techniques and with basic physico-chemical theories, it could be an excellent basis for the development of a new school of chemistry in Britain. The University of York has clearly chosen a man with youthful vigour and research enthusiasm, supported by experience of the tutorial approach to undergraduates which gives them the close personal contacts and stimulations that have proved to be so valuable in the older universities.

Chemistry in the University of East Anglia : Dr. A. R. Katritzky

DR. ALAN ROY KATRITZKY, who has recently been appointed to the chair of chemistry in the new University of East Anglia, was born in London, where he received his early schooling, and then, after national service in the Royal Air Force, entered the University of Oxford in 1948 as a member of St Catherine's Society to read chemistry He took his B A degree with first-class honours, and then the B Sc and D Phil degrees after research with the late Dr S G P Plant and Sir Robert Robinson, respectively He was appointed an Imperial Chemical Industries Fellow and later a lecturer and tutor at Pembroke College In 1958 he was appointed a University demonstrator at Cambridge, and a year later an Official Fellow and director of studies in chemistry at Churchill College Dr Katritzky's research has been mainly in heterocyclic chemistry, where he has been engaged in particular in the application of physical methods, especially infra-red, ultra-violet and nuclear magnetic resonance spectroscopy, to the study of the structure, tautomerism and reaction mechanisms of various heterocyclic systems He is also well known for his text-book, *Heterocyclic Chemistry* (with Mrs J M Lagowski), which appeared in 1960 and contains a very considerable mass of material in a comparatively slender volume At the age of thirty-three he has about a hundred published research papers and in addition to the above text-book has other books, written or edited, in the press He is the senior editor of the English translation of *Russian Chemical Reviews*

Metallurgy at the Manchester College of Science and Technology

Prof K M. Entwistle

Dr Kenneth Mercer Entwistle has been appointed to a newly created chair in metallurgy by the Councils of the University of Manchester and the Manchester College of Science and Technology Dr Entwistle, who is thirty-seven years of age, was educated at Urmston Grammar School, Manchester, and at the University of Manchester, where he graduated with honours in electrical engineering in January 1945 He was awarded the degree of M Sc in December 1945 and after postgraduate research in the Department of Metallurgy was appointed assistant lecturer in metallurgy in the University of Manchester in October 1947 He was awarded the degree of Ph D in July 1948 He became lecturer in metallurgy in October 1949, senior lecturer in metallurgy in October 1954, and reader in metallurgy in October 1960 He has been a member of the United Kingdom Group at the World Metallurgical Congress, Detroit (1951), and was British Council visiting lecturer at the Bergakademie, Clausthal-Zellerfeld, West Germany, for two weeks in 1954 He is a past-president of the Manchester Metallurgical Society Dr Entwistle will take up his new appointment on September 1

Biochemistry at Queensland

Prof E C Webb

At the age of forty-one, Dr E C Webb has been appointed the first professor of biochemistry in the University of Queensland, Brisbane, Australia, and will take up his duties there in August Dr Webb was born in Dorset and educated at Poole Grammar School and Clare College, Cambridge He went to Clare College in 1939 to read science and was placed in the First Class in both parts of the Natural Sciences Tripos, with biochemistry as the subject for Part II From 1942 until 1944 he engaged in extra-mural research at Cambridge for the Ministry of Supply, which related to the mechanism of action of the so-called nerve poisons This was carried out in collaboration with Dr Malcolm Dixon, with whom he has remained in close partnership ever since From 1944 until 1946 Dr Webb held a Beit Memorial Fellowship for Medical Research and then was appointed successively demonstrator, and lecturer, in biochemistry in the University of Cambridge His research work has been particularly concerned with the isolation of enzymes and the study of their kinetics and specificity of action He was co-author with Dr Malcolm Dixon of the monumental book on *Enzymes* published in 1958 Dr Webb has been a member of the Commission on Enzyme Nomenclature set up by the International Union of Biochemistry, and is secretary of the Standing Committee which keeps the subject of enzyme nomenclature under review He was formerly a member of the Cambridgeshire County Council, and for twelve years has been a member of the Board of Governors of the United Cambridge Hospitals He has also served as a member of the Governing Bodies of the Cambridge Technical College, of the Perse School for Girls and of the Perse School for Boys Dr Webb goes to Brisbane with a firmly established scientific reputation, wide experience of university teaching and good experience of administrative matters

Unit Cost of Nuclear Power Production

In a written statement circulated with the official report on July 16, the Parliamentary Secretary to the Ministry of Power, Mr J Peyton, gave the following estimated total costs of generation at nuclear power stations, 1962–66 Berkeley, 1 05*d* per unit, Bradwell, 0 95*d* , Hinckley Point, 0 85*d* ; Trawsfynydd, 0 81*d* , Dungeness, 0 66*d* , Sizewell, 0 66*d* , Oldbury, 0 66*d* The average cost of generation at conventional stations in 1960–61 was 0 88*d* per unit, and varied from an estimated 0 55–0 7*d* for plant commissioned in 1962 to about 0 5–0 65*d* for plant commissioned in 1960

British Manpower in Countries now Independent

In a written answer in the House of Commons on July 26, the Secretary for Technical Co-operation, Mr D Vosper, gave the number of pensionable British expatriate officers now serving in Ghana as 30 compared with 580 at the time when that country gained independence For Sierra Leone the corresponding figures are 115 and 250, and for Nigeria Federation, 555 and 870, North, 640 and 900, East, 90 and 105, West, 110 and 135 Mr Vosper had no firm figures of contract officers

British Assistance to Latin America

In replying for the Government to a debate on the international situation in the House of Lords on July 25, the Minister of State for Foreign Affairs, the Earl of Dundee, said that while Lord Lindgren had criticized, perhaps justly, certain features of the execution of American aid in Latin America, he thought that President Kennedy's plan of massive aid to South American countries towards economic progress with liberty, "The Alliance for Progress", was one of the greatest and most imaginative projects of this century Moreover, Britain would wish to help as much as her balance of payments allowed In recent years, Britain had greatly expanded her cultural and information efforts in Latin America, and lately a plan had been started for technical assistance now costing £70,000–£80,000 a year What the South Americans perhaps needed most was the expert technical knowledge to use their resources Together with the University Grants Committee, Britain had also been considering ways and means of expanding the scope of Latin American studies in British universities, and it was hoped to announce a practical first step towards this end in the autumn

Medical Research Council Vision Research Unit

THE Medical Research Council have announced that, on the assumption of financial responsibility for the major part of the work of their Ophthalmological Research Unit by the University of London from August 1, the Unit's Visual Research Division will be reconstituted as a separate Council Unit under the direction of Dr H J A Dartnall, at present head of the Council's Visual Research Division It will be known as the Vision Research Unit and will continue to be housed in the Institute of Ophthalmology, Judd Street, London, W C 1

Supply of Science Teachers

A REPORT entitled *The Supply of Science Teachers in England and Wales* by the Joint Committee for Science and Education (Pp 19 London Joint Committee for Science and Education, 15 Half Moon Street, 1962 1*s*) states that, immediately, there is need for an increase of between 28,000 and 34,000 over the present number of graduate science teachers in schools, technical colleges, training colleges and universities, which stands to-day at about 20,600 In addition, the report states that an increase of 12,000–18,000 non-graduate specialists in science teaching will be needed in schools The shortage of science teachers is seen to be one part of the general teacher shortage, but many reasons are given for regarding it as a separate and specially urgent problem The present and future supply of mathematics teachers is an equally grave problem The whole education system, and not only science teaching, will suffer without an adequate supply of mathematics teachers But, in the Committee's view, the supply of mathematics and of science teachers presents two distinct problems The sooner this is accepted, "the sooner will policies be forthcoming which are adequate to the solution of both problems" There is a general awareness of the increasingly urgent need for more scientists and technologists in industry and the public services This report emphasizes the need to consider not only the training of these specialists but also the training of non-specialists "We need not only more specialists We need a common culture which includes more science To achieve this we must foresee science being taught to all children throughout their school lives "

Science Laboratories in Grammar Schools

Provision and Maintenance of Laboratories in Grammar Schools (1961) is the title of a report compiled from 762 answers to a questionnaire sent to grammar schools in England and Wales (Pp 16 London John Murray Published for the Science Masters' Association, 1962 2*s* net) The object of the survey, the third of its kind, was to find out whether laboratory facilities in schools were satisfactory and, if not, in what respects they were unsatisfactory. The report compares existing facilities, first, with the standards recommended by the Ministry of Education and, secondly, with the standards recommended by the Industrial Fund for the Advancement of Scientific Education in Schools Compared with 1959, there has been an increase from 45 to 55 per cent in the number of local education authority schools which have, according to Ministry of Education standards, adequate laboratories Local education authority schools, however, still lag behind direct grant and independent schools More local education authority schools now employ one or more laboratory technicians, but only 8 per cent of these schools employ a sufficient number of technicians In many cases the local authority makes no provision for such assistance, and the report stresses the importance of adequate technical assistance in the teaching of science Laboratory finance is expressed as the amount spent per annum per pupil taking science—the minimum sum regarded as adequate being 12*s* 6*d* Local education authority schools for boys spend on the average 13*s* 6*d* , compared with 12*s* 3*d* in 1959 The report points out that 50 per cent of these schools spend less than 12*s* 6*d* per annum per child, which is not adequate Girls' schools, particularly, have inadequate facilities for teaching science, as well as suffering from an acute shortage of science teachers Mixed schools, in general, lie between boys' and girls' schools, while the laboratory facilities in comprehensive and bilateral schools vary considerably

Bulletin of the Royal Society International Scientific Information Services

THE Royal Society has established a special library for the publications of the International Council of Scientific Unions, the International Scientific Unions, Commissions, Special Committees and Permanent Services, as well as of certain other publications such as those of the United Nations Educational, Scientific and Cultural Organization in the Arid Zone, Humid Tropics and Marine Sciences With this library as a basis, it is planned to provide an information service to facilitate use in the United Kingdom of the international contacts of the Royal Society The first issue of the *Bulletin of the Royal Society International Scientific Information Services*, which it is proposed will be published regularly, has become available (1, No 1, July 1962 Pp 80 London The Royal Society, 1962) It contains complete lists of the holdings of the Society, arranged under the International Council and under the different International Unions or other bodies responsible for publication Future issues will keep the catalogue up to date, and in his preface Sir Patrick Linstead expresses the hope that the *Bulletin* will keep scientists in touch with international developments It is also proposed to include occasional articles on international scientific affairs and notes on exchanges, visits, grants and similar matters The first issue is well produced and admirably designed for its task of making these scientific publications more widely known

The British Journal for the History of Science

SHORTLY after the British Society for the History of Science was formed in 1947 it produced a *Bulletin* which, owing to the stringency of the times, was virtually a broad-sheet Later, by arrangement, the *Bulletin* was published as a supplement to the *Annals of Science*, with Dr N H de V Heathcote as a connecting link between both journals, being editor of one and on the editorial board of the other Yet even this arrangement had its difficulties, as papers read to the Society could not be published *in extenso* Now, however, a major step forward has been taken with the issue of *The British Journal for the History of Science*, 1, Part 1, No 1, June 1962 Pp 95 (Published twice annually Ravensmead, Keston, Kent The British Society for the History of Science, 1962

15*s* per part) Under the editorship of Dr H D Anthony, it offers five papers, all read to the Society Dr J S Wilkie, on "Some Reasons for the Rediscovery and Appreciation of Mendel's Work in the First Years of the Present Century", Dr C H Talbot, on "Simon Bredon, Fellow of Merton, Mathematician, Astronomer and Physician", E A Marland, on "British and American Contributions to Electrical Communications", Thomas Martin, on "The Origins of the Royal Institution", Leonard Clarke, on "Greek Astronomy and its Debt to the Babylonians" There is a précis of four other lectures, together with a useful bibliographical note on papers in other journals and a book review As a journal it should be widely used in universities and technical colleges

Natural Resources in Scotland

A REVIEW of the volume entitled *Natural Resources in Scotland*, which recorded the proceedings of the symposium organized by the Committee on Natural Resources of the Scottish Council during October 31–November 2, 1960, has already appeared in *Nature* (193, 514, 1962) The Committee has now issued its report as a cyclostyled foolscap-sized document, adopted by the Scottish Council (Development and Industry) It is based on the evidence presented to the symposium, which was studied and carefully sifted by seven working parties The lack of accurate factual information is again emphasized, and the report calls for detailed surveys as a pre-requisite for planning The keynote of the thirteen-point plan is the need for integration—notably of farming and forestry—and for the multiple use of land with the over-riding objective of maintaining and increasing the productive capacity of the land Copies of the report are available from the Council, price 21*s* (Pp v+194 Edinburgh The Scottish Council (Development and Industry), 1962)

Strontium-90 in Human Bone

No 4 of the *Medical Research Council Monitoring Report Series* contains initial analyses of strontium-90 in human bone for the first half of 1961 The analyses were carried out by the Atomic Research Establishment and by research workers in Glasgow The results indicate that, for most age groups, the average amounts of strontium-90 in bone during the first half of 1961 were approximately the same as in the second half of 1960, but for the important age group of 0–5 years the analyses made at the Atomic Energy Research Establishment show a decrease in the average level from 2 65 to 2 07 strontium units (micro-microcuries of strontium-90 per gram of calcium) The highest single value recorded in the United Kingdom during the first half of 1961 was 6 5 strontium units The period covered by this report precedes the resumption of nuclear weapon testing in the autumn of 1961

Expedition to Iceland

A CHELSEA College expedition to Iceland was first suggested in 1956 by David Bellamy, then a student in the Department of Botany and Zoology Bellamy's suggestion was not followed up, however, until the summer of 1960, when W A Stevens initiated the planning of the College's first scientific expedition Seven students took part in the expedition, which lasted two months and was centred on the Baegista Valley in the north of Iceland a report of the expedition has now been published (Chelsea College Union *Biological and Geological Societies' Expedition to Iceland, Summer 1961—Final Report* Pp 52 London Chelsea College Iceland Expedition, 1962 3*s* 6*d*) The purpose was to make detailed botanical surveys and geological maps of the area visited, to correlate botanical and geological studies so far as possible, to make comprehensive collections and records of the plants, rocks and minerals found, to examine the more interesting, instructive or natural sights of Iceland, including the volcanic thermal and glacial phenomena, to make ornithological observations where possible, and to learn as much as possible about the Icelandic countryside and nation The illustrated report gives full details of the expedition and could be of great value to other students planning to make surveys in various countries overseas

Chemotherapy of Cancer

DIFFERENT people may hold different views on the political side of the United Nations, but no reasonable person can deny a great belief in, and his full support of, the admirable work of the United Nations' welfare, medical, scientific and cultural organizations One of these bodies is the World Health Organization with its headquarters in Geneva It is very gratifying to find that its efficient staff have turned their attention recently also to the chemotherapy of cancer (which includes leukæmias), and with the help of a panel of experts have issued their first report on this subject (*Technical Report Series* No 232 *Chemotherapy of Cancer* First Report of an Expert Committee Pp 52 Geneva World Health Organization, London H M S O, 1962 2 Swiss francs, 3*s* 6*d* , 0 60 dollar) It is true that so far as this report represents a critical assessment of the present situation in this field by enumerating the general principles of cancer chemotherapy and making a review of available and tested agents, other publications have appeared from time to time written by individuals or co-operative groups However, as the report is also looking forward and touches on topics such as combined treatments and on the intricate problem of chemical trials of new drugs, the Expert Committee, and particularly its secretaries, Drs A V Chaklin, W Davis and Prof L F Larionov, should be congratulated on a fine piece of work This will be most useful to the investigator, scientific or medical, in cancer chemotherapy and also to anybody interested in any form of medical science as applied to the treatment of pathological states

Society for Applied Bacteriology

AT the annual meeting of the Society for Applied Bacteriology, held in Sutton Bonington on July 17, the following were elected officers of the Society *Honorary President,* Dr T Gibson, *Honorary Secretary,* Dr Ella M Barnes, *Honorary Treasurer,* Mr G Ellis Jones, *Honorary Editors,* Dr S E Jacobs and Dr D J Jayne-Williams, *Honorary Publications Manager,* Mr A H Walters, *Honorary Advertising Manager,* Mr E J Mann, *Members of the Committee,* Dr A Hurst, Dr N D Harris, Dr J G Murray, Dr J R Norris, Dr A M Paton, Dr Joan Taylor, Dr Agnes A Nichols, Dr Patricia M Scholes, Dr A Seaman, Mr G Sykes (*ex-officio*)

Stability of Vitamin Preparations

A COLLABORATIVE investigation into the stability of seven vitamins (ascorbic acid, cyanocobalamin, folic acid, nicotinic acid, nicotinamide, riboflavin and thiamine hydrochloride) during two years storage of different preparations has been initiated in India (Government of India Ministry of Health Report of the Central Drugs Laboratory, April 1958 to March 1959 By Dr H K Banerjee Pp ii+56 Delhi Manager of Publications, 1961 Rs 11 50 nP , 18*s*) The collaborators are the Central Drugs Laboratory, Calcutta, the Haffkine Institute, Bombay, and the Defence Research Laboratory (Stores), Kanpur Results from the different centres will be co-ordinated and statistically analysed in the Central Drugs Laboratory For experimental investigations, the pure vitamins were obtained from Hoffmann-LaRoche, Basle (except for cyanocobalamin, which was obtained from Glaxo Laboratories, Ltd) The physical and chemical characteristics of the products were tested in the Biochemistry Department of the Central Drugs Laboratory and, finally, ten pharmacopœial preparations were chosen for collaborative investigations on the stability of the preparations when stored under prevailing conditions of temperature and humidity in Calcutta, Bombay and Kanpur The preparations are (1) ascorbic acid tablets, coated (25 mg/tablets), (2) ascorbic acid tablets, uncoated (25 mg/tablets), (3) nicotinamide tablets (50 mg/tablets), (4) nicotinic acid tablets (50 mg/tablets), (5) riboflavin tablets (1 mg/tablets), (6) vitamin B_1 tablets (5 mg/tablets), (7) folic acid tablets (5 mg/tablets), (8) vitamin B_1 injection (25 mg/ml) (9) nicotinamide injection (50 mg/ml), (10) cyanocobalamin injection (50 µg/ml). The preparations were distributed to the collaborators towards the end of March 1959 for the assay of the initial content of the vitamin in each of the preparations at the three centres Thereafter, the vitamin content was determined at intervals of three months for a period of twenty-four months

Antibody to Enzymes—a Three-Component System

A MEETING on "Antibody to Enzymes—a Three-Component System" is to be held in New York during September 5–7, and will deal with the following topics *Section* 1, aspects of two-component immunology, *Section* 2, reaction mechanisms of the interaction of enzymes, antibody and substrate, *Section* 3, the effect of antibody on various enzymes, *Section* 4, interaction of enzyme, co-enzyme and substrate with antibody (*a*) reaction mechanism, (*b*) multiple forms of enzymes, *Section* 5, use of antibodies in the discrimination of multiple molecular forms of enzymes, *Section* 6, antibodies as tools in studies of biosynthesis of enzymes The meeting is being held under the auspices of the New York Academy of Sciences and will draw its speakers from Australia, Britain, Canada, France and the United States Further information can be obtained from Dr B Cinader, University of Toronto, Department of Medical Biophysics, 500 Sherbourne Street, Toronto 5

The Tenth International Botanical Congress

THE first circular giving general information about the forthcoming Tenth International Botanical Congress, to be held in Edinburgh from August 3–12, 1964, has now been issued to some 9,000 botanists and botanical institutions throughout the world The majority of recipients are those who have attended previous Congresses, but it is believed that there may be quite a number of botanists who would wish to have a copy of this circular and who have not yet received one These may be obtained on request from the Secretary (Executive Committee), Tenth International Botanical Congress, 5 Hope Park Square, Edinburgh, 8 Enclosed within the circular is a preliminary registration form which is to be returned before September 30, 1962, by those interested in the Congress Subsequent Congress circulars and registration forms will be distributed only to those who complete one of these preliminary registration forms

Physiology and Psychology of Arid Lands

IN *Nature* of July 21, p 232, it was announced that a symposium on "Environmental Physiology and Psychology in Arid Conditions", being organized by Unesco. was to be held in Naini Tal, India, during August 27–September 1 It has now been announced by the Department of Natural Sciences of Unesco that for various reasons the date and venue for this symposium have been changed it will be held at the Central Drug Research Institute, Lucknow, India, during December 7–12

Conference on Palæoclimates

A CONFERENCE on "Palæoclimates", sponsored under the Nato Advanced Institute programme, will be held in the Physics Department, King's College, Newcastle upon Tyne, during January 7–11, 1963 The subjects to be covered range from palæobotanical and palæontological inferences on climate to the information gained from the rocks themselves, evaporites, glacial deposits, limestones, etc Supplementary information from geophysics will also be introduced The aim of the meeting is to stimulate research, consequently the accent will be on the problems of palæoclimates rather than on results Although it is intended to limit the number of participants, it is still possible to extend invitations to research workers and postgraduate students with an active interest in the subject Some financial assistance may be available for students Further information can be obtained from the Administrative Assistant, Department of Physics, King's College, Newcastle upon Tyne, 1

The Field Survey Association Annual Prize, 1962

THE Field Survey Association is offering a prize of £50 for the best paper on a surveying subject, consistent with their aims and objects, submitted before December 31, 1962, provided that this paper is considered to be of sufficient merit for the award of the prize The prize is open to any person under the age of thirty-five on December 31, 1962, who may be either within or outside the membership of the Association, but those outside must be eligible for membership The rules of the prize, the history and objects of the Association and the conditions of membership can be obtained from the honorary secretary of the Award Committee, Commander R Bill, Vickers House, Broadway, S W 1

The Beilby Medal and Prize, 1963

AWARDS from the Sir George Beilby Memorial Fund are made by the Administrators of the Fund, representing the Royal Institute of Chemistry, the Society of Chemical Industry and the Institute of Metals Sir George Beilby had been president of each of these three bodies, and they jointly sponsored the appeal for subscriptions whereby the Fund was raised as a memorial to him after his death in 1925 The Beilby Medal and Prize, consisting of a gold medal and a substantial sum of money, is specified as being 'For Advancement in Science and Practice' Such an award is offered at intervals of two years, but more than one may be made on the same occasion if there are several candidates of sufficiently outstanding merit The awards are made to British scientists in recognition of independent original work of exceptional merit, carried out continuously over a period of years and involving the development and application of scientific principles in any field related to the special interests of Sir George Beilby, namely, chemical engineering, fuel technology or metallurgy, in their modern interpretations The awards are intended as an encouragement to men and women (preferably under the age of forty) who have carried out work of practical significance in any of these fields Consideration is to be given to the making of an award from the Fund in 1963 Outstanding work of the nature indicated may be brought to the notice of the Administrators, either by persons who desire to recommend the candidate or by the candidate himself, not later than December 31, by letter addressed to the Convener of the Administrators, Sir George Beilby Memorial Fund, the Royal Institute of Chemistry, 30 Russell Square, London, W C 1

The Night Sky in September

FULL moon occurs on Sept 14d 04h 12m U T and new moon on Sept 28d 19h 40m The following conjunctions with the Moon take place Sept 1d 05h, Mercury 6° S, Sept 3d 02h, Venus 8° S, Sept 11d 03h, Saturn 1° S, Sept 13d 04h, Jupiter 1° N, Sept 19d 12h, Aldebaran 1° S, Sept 22d. 13h, Mars 2° N In addition to these conjunctions with the Moon, Mars is in conjunction with Pollux on Sept 30d 13h, Mars being 6° S Mercury is too close to the Sun for observation Venus is an evening star, visible low in the west after sunset Mars rises at 23h 30m, 23h 10m and 22h 55m on Sept 1, 15 and 30, respectively It is at a distance of 148 million miles on September 15, and its stellar magnitude is +1 2 Jupiter sets at 5h 15m, 4h 10m and 3h 05m at the beginning, middle and end of the month, respectively It is in Aquarius, its stellar magnitude is −2 4 and its distance from the Earth in the middle of the month is 373 million miles Saturn is in Capricornus, setting at 2h 10m, 1 h 05m and 0h 05m on Sept 1, 15 and 30, respectively Its stellar magnitude is +0 6 and its distance from the Earth in the middle of the month is 858 million miles Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich Sept 17d 23h 01 5m, +11° 445 (*R*); Sept 21d 00h 18 7m, 64 Ori (*R*) *R* refers to reappearance The autumnal equinox occurs on Sept 23d 12h

University News: Edinburgh

THE following appointments to lectureships have been announced J Hogg (biophysics), Dr G S G Beveridge (chemical technology), A W Read (chemistry); R P Kirby (geography), C J Shaddock and Captain E T W Smyth (mathematics), Dr B L Ginsborg (electropharmacology), Miss Sybil M Lloyd (physiology)

Sheffield

DR B A BILBY, at present reader in theoretical metallurgy, has been appointed to the chair of theoretical metallurgy, as from October 1 Dr Bilby was the Sorby Research Fellow of the Royal Society in the University from 1951 until 1957

Southampton

DR K A MUNDAY, senior lecturer and head of the Department of Physiology and Biochemistry in the University, has been appointed to the newly established chair of physiology and biochemistry, with effect from October 1

Yale

A NEW Department of Biology has been established through the fusion of the former Departments of Botany and Zoology Prof D F Poulson, professor of biology, has been appointed chairman and director of biological laboratories Prof G E Hutchinson, Sterling professor of zoology, will serve as director of graduate studies Six major scientific fields are represented in the new Department genetics and cytology, developmental biology, physiology and biochemistry, comparative physiology and behaviour, ecology, and evolution and systematics The Department is at present housed in the Osborn Memorial Laboratories and occupies five floors in one wing of the Josiah Willard Gibbs Research Laboratories New facilities for research in experimental biology, to be housed in a central building of the new Kline Science Center, are in an advanced stage of planning

Announcements

PROF K C DUNHAM, professor of geology in the University of Durham, has been elected president of the Institution of Mining and Metallurgy for the session 1963–64 Prof Dunham was elected to membership of the Institution in 1941 He has served continuously as a Member of Council since 1950, holding office as a vice-president for the three sessions 1957–60

THE Society of Environmental Engineers will be holding the first meeting in its winter programme at the Imperial College of Science and Technology, Exhibition Road, London, S W 7, on September 12 Mr Russell Lowe, vice-president of the Barry Wright Corporation of Glendale, California, will speak on structural damping allied to the isolation of electronic equipment Further information can be obtained from the Society of Environmental Engineers, Suite 7, 167 Victoria Street, London, S W 1

THE Basic Science Section of the British Ceramic Society is holding a meeting on "Mechanical Properties of Non-Metallic Crystals and Polycrystals" at Hastings during October 16–17 Further information can be obtained from Dr J P Roberts (Honorary Section Secretary), Houldsworth School of Applied Science, the University, Leeds 2

A CONFERENCE on "Fœtal and Infant Liver Function and Structure", sponsored by the New York Academy of Science, is to be held during November 7–10 Further information can be obtained from Dr J A Sterling, 1351 W Tabor Road, Philadelphia 41, Pa

ERRATUM Mr Dhruba K Sen regrets that in his article entitled "Role of Carbonyl Chloride in the Production of Uræmia", which appeared on p 889 of the March 3 issue of *Nature*, paragraph 11, line 13 should read " *p*-dimethyl aminobenzaldehyde " instead of "*p*-diethyl aminobenzaldehyde"

IRRIGATION IN GREAT BRITAIN

THE water supply problem created by the spread of irrigation in Great Britain has already attracted the attention of the Central Advisory Water Committee of the Ministry of Housing and Local Government A preliminary study was undertaken by the Sub-Committee on the Growing Demand for Water and forms the subject of the committee's second report issued in 1960 It was, however, a very brief document of two pages, the first detailed and objective study of irrigation in Great Britain in which practically all aspects have been covered has been prepared and recently published as a report of the Natural Resources (Technical) Committee of the Office of the Minister for Science[1] It is a notable document which casts considerable light on a significant agricultural trend about which few quantitative data have hitherto existed

The report, prepared by a strong sub-section of the Committee on Agriculture, examines the possibilities and consequences of irrigation in Great Britain It brings together a considerable range of available information on crop responses, costs and returns, and assesses the likely value of irrigation to the farmer and to the community Tentative estimates are also made of the potential demands for water for irrigation.

It is quite clear from the report that irrigation of certain crops in parts of Britain can lead to a major increase in productivity With the continued loss of agricultural land to housing, industrial and amenity uses, and the need to produce at home the major portion of the food for the inhabitants of these islands, the extension of irrigation in the right areas could become a significant factor in promoting economic recovery coupled with an increasing standard of living The main problem is, of course, the need to provide the water in the right place at the right time

The area where irrigation can be utilized to increase output, reduce unit costs and improve efficiency of production is very largely that part of England receiving less than 35 in, together with that part of Scotland receiving less than 30 in, of rain a year The frequency of the need is greatest in the south and east, being about nine years in ten in the Thames Estuary and diminishing north and westwards towards the higher rainfall areas In most years up to 4 in of rain would be needed to make good the shortage At present some 130,000 acres of land are irrigated, and this is increasing by about 15,000 acres per annum If expansion continued at this rate some half a million acres might be subject to irrigation by 1980 The application of 4 in of water to this area would, according to the Natural Resources Report, require 50,000 million gallons Rough estimates of the eventual practical limits suggest an area of 1½ million acres with a peak annual demand of well over 100,000 million gallons This represents a possible peak daily demand for water ranging from about 2,000 million gallons a day in April and September to 3,000 million gallons a day from mid-June to early August This is in excess of the total amount of water at present being supplied throughout the country by all statutory water undertakers

The water so far used in irrigation has been obtained from a variety of sources of supply, and increasing competition with other users has raised many problems The irrigation demand varies considerably and reaches its maximum when supplies are often at their lowest It is clear that substantial expansion in irrigation will only be possible by increased conservation, mainly by storage of excess winter flow The report considers the ways and means whereby this can be most effectively undertaken and suggests in the first instance the extension of farm conservation schemes by means of storage reservoirs More than 250 of these have already appeared in the past year or two Eventually, however, the problem can only be solved by the transfer of surplus water from the wetter highland zone of Britain to the drier lowland zone

At present the water is largely applied by overhead spray methods To be effective the farmer needs to know when to spray and how much to apply The report details the technique which has evolved so far Some interesting figures are also given for the likely return in both production and value Maincrop potatoes receiving about 4 in of water may give an increased yield of 3 tons per acre, valued at about £50, cauliflowers, receiving 2 in, an increase of 3 tons, valued at up to £150, soft fruits, receiving 2–4 in of water, an increase of yield of from 20 to 50 per cent valued at between £50 and £100 per acre Grass may give an increased return of about £20 per acre from 5 in of water, but here the cost of the water becomes critical

The actual costs of the irrigation vary according to the availability of water and equipment For the now almost standard overhead spray method capital costs come out at about £25 per acre Where water is 'free' running costs plus depreciation and interest may average out at 45*s* per acre-in of water for an application of 3 in The detailed economics of irrigation have yet to be worked out, however But it is quite clear that the method pays dividends almost everywhere with vegetables, fruit, potatoes and peas, but that it might be marginal with grass, sugar-beet, field beans and cereals

The Committee comes, without difficulty, to the conclusion that the judicious use of irrigation in agriculture and horticulture should be encouraged It recommends that active steps should be taken to improve conservation of water, especially by local storage in small units close to the land to be irrigated In the larger sphere conservation works should be properly planned in relation to each other and in conjunction with the proper development of the water resources of a particular river basin Demands for water for irrigation should, it is thought, be considered together with demands for water for industrial purposes Further research is also considered desirable on the fundamental, technical and operational aspects of irrigation and for this purpose a research station or experimental husbandry farm is proposed The need is also expressed for longer-term quantitative rainfall forecasts to avoid wasteful application of water

A few years ago any suggestion of irrigation in Great Britain would have produced a derisory reaction in most individuals This new Report, however, demonstrates quite clearly that the scientific application of water over much of lowland Britain would constitute a minor agricultural revolution, increasing output and strengthening our economic position immeasurably To bring this about, however, requires a national approach to the problem of water conservation in Great Britain Encouraging progress is, however, now being made in this direction[2,3]

W G V BALCHIN

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[1] *Irrigation in Great Britain, a Report by the Natural Resources (Technical) Committee, Office of the Minister for Science, London* (H M S O. 1962) 5s 6d

[2] *Water Conservation, England and Wales* (London H M S O , 1962) 1s 3d

[3] *Nature*, **193**, 1244 (1962)

DIAMOND PHYSICS

THE 1962 Diamond Conference, sponsored by the De Beers Group, was held at Cambridge during June 25–26, the delegates were accommodated in Gonville and Caius College, and the conference sessions took place in the Hopkinson Memorial Theatre The sixty or so members were drawn from universities, research establishments, and the research laboratories of the diamond industry; in addition to England and South Africa, the United States and several European countries were represented The attendance might have been greater had the First International Congress on Diamonds in Industry, with its scientific and technological sessions, not taken place in Paris only four weeks previously

The Diamond Research Laboratory in Johannesburg was the originator of this conference, and much of the work in this field is done by members of that body, either in their own departments or during secondment to other laboratories While the conference is not in itself a novelty (it has been held on twelve occasions previously), it has always been regarded up to now as a wholly private occasion, and for this reason no reports have been issued This has in no way implied a prohibition on publication (most of the papers presented have been published in some form in this or other scientific or technical periodicals), but formal Proceedings have not been issued, and direct reporting by the Press has not been allowed Although this meeting is still private in the sense that attendance is only by invitation, the papers are to be published as from the conference and reports of its activities will be permitted

As at all the previous Diamond Conferences, the range of the subjects covered was extensive, from the history of the early attempts at diamond synthesis to the latest investigations of present-day synthetics, and from geology to nuclear physics In fact, although the history of research into the nature and properties of diamond has a respectable antiquity, there is still a vast field for further exploration into a material that is unusual from a number of points of view

The conference was divided into four sessions, the morning of June 25 was devoted to the nuclear physics and electronics aspects of diamond, while the afternoon covered the geological aspect and the growth history of diamonds Tuesday morning had as its principal subject the mechanical strength of diamond, and the afternoon was given up to a miscellany of papers The conference ended with a dinner in the hall of the College, not the least important part of conferences such as these is the opportunity they give for meeting and talking informally to other workers in the same field

As has now come to be expected on these occasions Prof S Tolansky (Royal Holloway College) produced a number of remarkable slides of the surfaces of diamonds, both natural and synthetic, produced by the multiple beam interference method which he has developed to a high degree of excellence Another paper illustrated by extremely interesting pictures was that prepared by Dr T. Evans and C. Phaal (University of Reading) and presented by Dr Evans The subject was "Transmission Electron Microscopy in Diamond", and the description of the techniques used for the preparation of the samples was as interesting as the results obtained

The three papers presented by Dr D R Grantham (Selection Trust), Dr. P Stevenson (Royal Holloway College) and Dr M. Seal (Engelhard Industries Inc) on coated diamonds and growth history of diamond crystals produced some controversial matter, it is clear that this is a field in which a considerable amount of investigation still remains to be carried out Even the meaning of the commonest of all the surface features of natural diamonds—the trigons that appear so numerously on the octahedral faces—still remains in doubt, and the two schools of thought that relate them respectively to growth and to etching seem to be still far from reaching agreement, it is interesting to note that these features seem to be rare on synthetic diamonds, although very common on the natural stones

Dr E M Wilks (University of Oxford) produced some interesting facts on the effects of neutron irradiation on the mechanical strength of diamond, including a checkered cracking effect produced during polishing the diamond (by methods similar to those used in gem polishing), these, to judge by the illustrations shown, may well be analogous to the 'fire marks' known to be produced in polishing synthetic sapphire, which are due to local strain

The cleavage of diamond and the propagation of fractures were dealt with by Dr J H Brunton (University of Cambridge) and Dr J E Field (University of Cambridge) respectively Dr Field's ultra-high-speed photography and the calculations resulting from it have produced interesting information about the speed and method of the propagation of cracks started by small explosive charges and mechanical impact at different points in sample pieces, and Dr. Brunton's paper made it clear that the differences of energy involved are not in themselves sufficient to account for the marked preferential cleavage of diamond along octahedral planes

In a second paper, read during the last session of the conference, Dr Seal reported on electron microscope investigations made on the 'Hannay Diamonds' from the British Museum (Natural History), and although his results were not conclusive he appears to have added some further evidence for the genuineness of Hannay's success in diamond synthesis, Dr. H J Milledge announced that University College, London, was undertaking further work on these diamonds using X-ray diffraction methods, and the results of these investigations will be awaited with interest

As is natural with a conference of this type occurring annually, much of it was taken up by what can only be called interim reports, giving the outline of the advances made during the past twelve months, rather than final and conclusive statements on completed programmes, this does not detract from its interest, but rather adds interest to the prospects for the future occasions J F P H Greene

INDUSTRIAL CHEMISTRY IN BRITAIN

The ninety-eighth annual report on Alkali, etc , Works by the Chief Inspector for England and Wales covering the year 1961* records 10,583 visits and inspections during the year, including 264 special visits by the Chief and Deputy-chief Inspectors, compared with 9,861 in 1960 Of these, 429 were to, or in connexion with, works not registered under the Act, 21 were concerned with control of radioactive emissions and 102 were to, or in connexion with, colliery spoilbanks During the inspections, 6,992 quantitative analyses were made of gases evolved from the processes in operation, compared with 3,724 in 1960 Most of the increase is due to a special investigation concerning salt glazing of earthenware, where 3,065 tests were made, compared with 1,060 in 1960 In addition, 541 special samples, compared with 486 in 1960, were taken and submitted for analysis to the Government Chemist

As a result of the special investigation just mentioned, it was concluded that for the present the practice of salt-glazing must be accepted There is no available suitable alternative to common salt and scrubbing the gases would involve a heavy financial burden on the industry Pending a solution of the acid or mist removal problem, or the adoption of other glazing techniques, the provisional 'best practical means' is a chimney not less than 120 ft high for salt addition up to 1 5 cwt , research work by the British Ceramic Research Association should continue In connexion with sulphuric acid works, the report directs attention to a tendency for over-reliance on recording instruments, which especially on acid works need routine check by conventional analytical methods, particularly following a shut-down, temporary by-passing, etc , when for one reason or another the instrument does not always respond The report includes interesting reviews of developments in the fertilizer industry during the past 80 years and of the many developments in chlorine works since chlorine came into commercial use, also the experience of the Inspectorate of the past three-and-a-half years in the iron and steel industry is also briefly reviewed

The Chief Inspector for Scotland reports that progress in dealing with industrial air pollution to bring it within the requirements of the Clean Air Act has out-paced reduction of pollution from its main evil—the domestic fire Besides 552 visits of inspection under the Act, 178 miscellaneous visits were paid and 79 visits on work not directly related to the Act A study was made of existing industrial chimneys and the heights of 150 chimneys were plotted against the fuel-burning rates of the furnaces When industrial chimneys were built to create draught, even quite small installations were provided with chimneys higher than present-day standards In consequence, smoke was taken well clear of any downward movement of air due to wind turbulence created by surrounding property—a point liable to be overlooked to-day

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* Ministry of Housing and Local Government, Department of Health for Scotland Ninety-eighth Annual Report on Alkali, etc , Works by the Chief Inspectors, 1961 Pp 73+4 plates (London H M Stationery Office, 1962) 5*s* 6*d* net

ARID ZONE RESEARCH

In the budgetary year 1956–57 the United Nations Educational, Scientific and Cultural Organization decided to convert its research programme on the problems of arid lands into a "Major Project" for a period of five years This meant that larger allocations of funds and personnel were channelled into this field of work Though some polar areas may in fact be deficient in moisture, the arid regions which were calculated to cover 23 per cent of the Earth's land surface lie mainly in two broad but interrupted belts in northern and southern tropical and subtropical latitudes in lands which are, with the major exception of South Africa, members of Unesco One major result of the research programme has been the publication of a series of quarto volumes under the general title *Arid Zone Research*, of which the one under review* is Vol 18

By virtue of its status as a unique international organization Unesco has been able to call on the best brains throughout the world in the preparation of these research volumes As authoritative statements of the present state of knowledge and research in such fields as climatology, hydrology, ecology, salinity, land use and many others, they stand unrivalled Whereas the whole series should automatically be on the shelves of every serious library, certainly in every university departmental library covering the many fields concerned, the truth is that their circulation and use remain lamentably small and restricted To take but one example in the research series, *The History of Land Use in Arid Regions*, with its world coverage, has a wealth of material not previously available in the languages of Western Europe and certainly not gathered together in one volume, yet indispensable if any planning for the future is to have a firm scientific basis

As a major project the arid zone programme comes to an end this year and this lengthy report of the main symposium, if it should prove to be the last volume in the series, would form a fitting climax The symposium was attended officially by 250 scientists from 33 countries, but the list of participants does not include the names of a number of world figures who certainly were present at the meetings.

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* The Problems of the Arid Zone Proceedings of the Paris Symposium (*Arid Zone Research*, Vol 18) Pp xii+481 (Paris Unesco, London H M Stationery Office, 1962) 47 25 NF , 69*s* , 15 30 dollars

Apart from the inaugural and presidential addresses, 27 papers are printed, most were presented by their authors and followed by a discussion which is given in summary form The papers are arranged in four groups the state of scientific knowledge, nomadism, alternative uses of scarce water supplies and, finally, four papers on public awareness of the educational problem The thirteen papers in the general section deal with water, ground-water, climatology, microclimatology, soils, salinification, plant physiology and ecology, human ecology, insects, winds and solar energy and saline water conversion It is interesting to find nomadism objectively studied by the Food and Agriculture Organization and viewed as a proper use of semi-arid lands under adequate control—thus extending on a world-wide basis the famous Cain and Abel experiment of Israel

It would be invidious to select papers from this rich collection for individual comment , but three conclusions would seem to emerge The first is that the research needed is usually in the borderland between present-day accepted fields of knowledge, in fact, the interrelation of the many factors The second is the delicate balance, easily upset, which exists in arid lands between the biota, including man, and the environment The third is the need for an open mind, willing constantly to re-think and reassess the problems and their solution

L Dudley Stamp

DAIRY RESEARCH IN SCOTLAND

THE Hannah Dairy Research Institute at Ayr now has a staff of about ninety, of whom some thirty are qualified in agriculture or science The recent triennial report* shows that the entire process of dairying is being covered, from the production of food on the farm to the chemistry, biochemistry and bacteriology of milk and milk products The programme of research is agreed in consultation with the Agricultural Research Council (London) and the Department of Agriculture for Scotland

The previous report described a unique range of new equipment for respiration experiments with sheep and cattle, and a splendid new climatic laboratory It is evident that these have been put to immediate use and that the work in these fields is wisely receiving emphasis Thus the careful reader will find on p 33 further support for the conclusion that while rations differing considerably in composition may be equally valuable for satisfying the maintenance requirements of ruminants, their nutritive value for production may differ widely Much vital supporting information is now being assembled, on such questions as the effect of environment, movement and lactation on the energy requirements of sheep and cattle The eventual effect of this work may well be felt wherever feeding standards are used

Capital for the climatic laboratory was provided by the Colonial Office in order to obtain basic knowledge of value in the improvement of cattle in hot countries A broad and vigorous approach is being made to the study of factors that influence temperature regulation, heat tolerance and acclimatization Much of this may be of value also in temperate countries

This Institute is clearly making a major contribution to research on many aspects of Britain's greatest and most vital agricultural enterprise. It may be symptomatic of the times, but it will nevertheless be surprising in some other countries that less than 1 per cent of the money required annually should now come from voluntary contributions

Sufficient background is provided to enable the general reader to appreciate the reason for doing each piece of work While a great deal of factual information is included, ample references are given to enable the specialist to trace detailed reports As always, this report is impeccably edited

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* Hannah Dairy Research Institute Report for the three years ended 31st March, 1962

UPTAKE OF GLUCOSE, AMINO-ACIDS AND VITAMINS BY HUMAN AMNION CELL CULTURES INFECTED WITH A TICK-BORNE ENCEPHALITIS VIRUS

By Dr P HALONEN, L HJERP, M. PYHTILA and P. WECKSTROM

Department of Virology, University of Helsinki, and Microbiological Laboratory, Laaketehdas Orion Oy, Helsinki

CHEMICALLY defined media designed primarily for cell cultures are extensively used for the propagation of viruses in such cultures To obtain the maximal yield of viruses requiring a lengthy incubation and maturation time in cell cultures, change of the medium must usually be omitted Accordingly, the best medium for such virus propagation may not necessarily be of the same composition as that for optimal cell growth, when frequent change of the medium is possible To examine the basic aspects of this problem it appeared important to know whether a critical loss of metabolites from a chemically defined medium could be obtained during prolonged incubation of uninfected and virus-infected cell cultures This communication describes the changes in the concentrations of glucose, amino-acids and vitamins of the B-group in Eagle's minimum essential medium[1] in uninfected human amnion cell cultures and in cultures infected with a tick-borne encephalitis virus

Strain *A* 52 of the tick-borne encephalitis virus was selected because in the continuous line of human

Table 1 AMINO-ACID CONCENTRATIONS (mM) IN EAGLE'S MINIMUM ESSENTIAL MEDIUM IN TICK-BORNE ENCEPHALITIS VIRUS-INFECTED HUMAN AMNION CELL CULTURES AND CONTROLS

l-amino-acids	Time (hr.) after addition of final medium 0		6		12		22		34		46		57		70	
	v	*c*	*v*	*c*	*v*	*c*	*v*	*c*	*v*	*c*	*v*	*c*	*v*	*c*	*v*	*c*
Arginine	0·60	0·60	0·27	0·31	0·01	0·04	0·03	0·02	0·02	0	0·04	0·03	0	0·03	0·02	0
Cystine	0·10	0·10	0·02	0	0·01	0	0·02	0·01	0·01	0·01	0·03	0·01	0·03	0·02	0	0·01
Glutamine	2·00	2·00	3·40	2·32	2·69	2·01	2·26	2·44	1·48	1·57	0·60	0·88	0·12	0·40	0·11	0·09
Histidine	0·20	0·20	0·16	0·14	0·17	0·10	0·14	0·15	0·14	0·13	0·12	0·13	0·09	0·15	0·10	0·12
Isoleucine	0·40	0·40	0·31	0·33	0·30	0·34	0·22	0·26	0·17	0·22	0·16	0·19	0·10	0·12	0·08	0·07
Leucine	0·40	0·40	0·33	0·36	0·27	0·33	0·23	0·28	0·18	0·20	0·13	0·15	0·08	0·11	0·07	0·06
Methionine	0·10	0·10	0·07	0·09	0·06	0·08	0·04	0·06	0·05	0·06	0·05	0·06	0·03	0·04	0·03	0·04
Phenylalanine	0·20	0·20	0·20	0·22	0·17	0·18	0·14	0·16	0·13	0·14	0·16	0·16	0·11	0·13	0·11	0·11
Threonine	0·40	0·40	0·36	0·37	0·36	0·33	0·31	0·34	0·29	0·30	0·26	0·29	0·22	0·22	0·28	0·30
Tryptophan	0·05	0·05	0·04	0·04	0·02	0·01	0·03	0·03	0·02	0·01	0	—	—	0	0·01	0
Tyrosine	0·20	0·20	0·18	0·19	0·17	0·17	0·16	0·17	0·15	0·16	0·19	0·16	0·16	0·15	0·10	0·13
Valine	0·40	0·40	0·35	0·44	0·35	0·35	0·27	0·31	0·22	0·25	0·19	0·24	0·13	0·17	0·12	0·13

v, virus-infected, *c*, control

amnion cells used[2] it multiplies well for 10–14 days without any apparent cytopathogenic effect. The cells were grown in Roux bottles in 0·5 per cent lactalbumin hydrolysate medium with 20 per cent calf serum. At the time of virus inoculation the bottles were washed three times with Hanks's solution and incubated for 2 hr. at 37° C in Hanks's solution in order to starve the cells in a medium devoid of amino-acids and vitamins. The cells, approximately 25 million per bottle, were infected with 1:100 diluted virus grown in mouse brain, that is, approximately 7 × 10^6 mouse infectious doses per bottle. After incubation for 1 hr. at 37° C the unadsorbed virus was washed twice with Hanks's solution. 60 ml. of the final medium containing Eagle's minimum essential medium and 0·1 per cent bovine albumin ('Bovine Plasma Albumin, Fraction V', Armour Pharmaceutical Co., Ltd., Eastbourne, England) was added to each bottle. The *p*H of the medium was kept at 7·0–7·2 with sodium bicarbonate. Periodically, samples were taken from the medium of 3–5 virus-infected bottles, pooled and centrifuged at 2,000 r.p.m. for 15 min. The supernatants were stored at −25° C and −70° C. Before chemical and microbiological assays the virus was inactivated for 1 hr. at 56° C. Identical samples were taken from the medium of the control bottles.

On each pooled sample virus titration was carried out in mice. Glucose, amino-acids and vitamins were determined as described elsewhere[3].

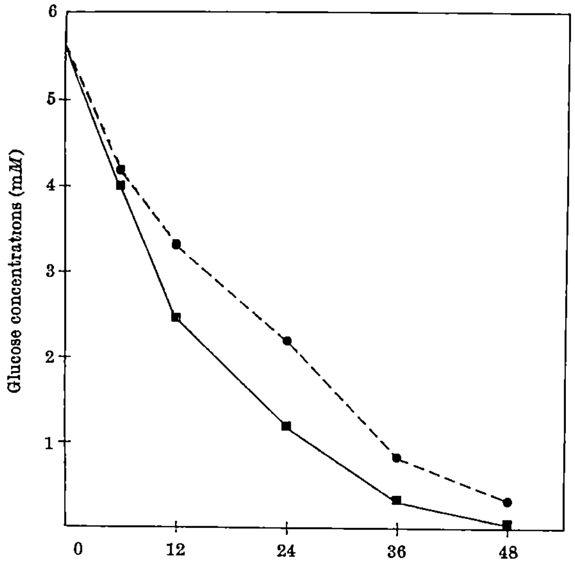

Fig. 1. Glucose concentrations (mM) in Eagle's minimum essential medium in tick-borne encephalitis virus-infected human amnion cell cultures and controls. ■, virus; ●, control

In a series of experiments the infectivity titre of the virus was about 5·0 log units/ml. at 24 hr. and 6·0 log units at 48 hr. After three days the titres decreased gradually but slowly. The titrations indicated that the maximal multiplication of this thermolabile virus occurred within 48 hr., but a considerable release of new infectious virus particles continued for at least 10 days.

Glucose concentrations were slightly lower in the virus-infected than in the control samples (Fig. 1). In 36–48 hr. they were less than 0·5 mM in both samples.

No significant change was observed in the concentrations of folic acid, inositol, pantothenate, riboflavin and thiamin in the medium of either the virus-infected cell cultures or the controls. In both samples, however, the concentration of nicotinamide decreased. After 8 days it was 20 per cent of the original biological activity in both samples and in 12 days below the detectable level in the samples taken from virus-infected cell cultures. The pyridoxal concentrations likewise fell, their values after 5 days being about 50 per cent of the original biological activity, but thereafter no further decrease was observed.

The amino-acid concentrations in a representative experiment are shown in Table 1. Three amino-acids (arginine, cystine and tryptophan) disappeared completely during some stage of the observation period of 70 hr. from the samples of both virus-infected and control cell cultures. Cystine disappeared from the control samples in less than 6 hr. In both samples glutamine concentrations were higher during the first day than in the original medium, but decreased rapidly during the next two days. The concentrations of isoleucine, leucine, methionine and valine also decreased significantly, the concentrations of histidine, phenylalanine, threonine and tyrosine to a lesser extent. In this connexion it is interesting to note that of the 5 amino-acids which are essential for human cell cultures in addition to the eight essential in feeding experiments in man[1,4], arginine and cystine disappeared from the medium in 12–34 hr. and glutamine almost completely in 70 hr., but in histidine and tyrosine a decrease of only about 50 per cent was observed in 70 hr.

It appears that, as regards the uptake of the metabolites in a chemically defined cell culture medium, only minor differences exist between human amnion cell cultures infected with a tick-borne encephalitis virus and uninfected controls. On the other hand, the disappearance of glucose and some amino-acids from the medium at an early stage may have influenced the multiplication of the virus after two days' incubation. Similar experiments are in progress with other viruses, and an investigation is being made of the effect on virus yields of the addition of various metabolites[5].

A preliminary report of some of the results presented in this article was given at the thirteenth Scandinavian Congress for Pathology and Microbiology in Turku[6].

We thank Mrs. T. Nummela for technical assistance.

This work was supported by grants from the Sigrid Jusélius Foundation and the Finnish Medical Research Council.

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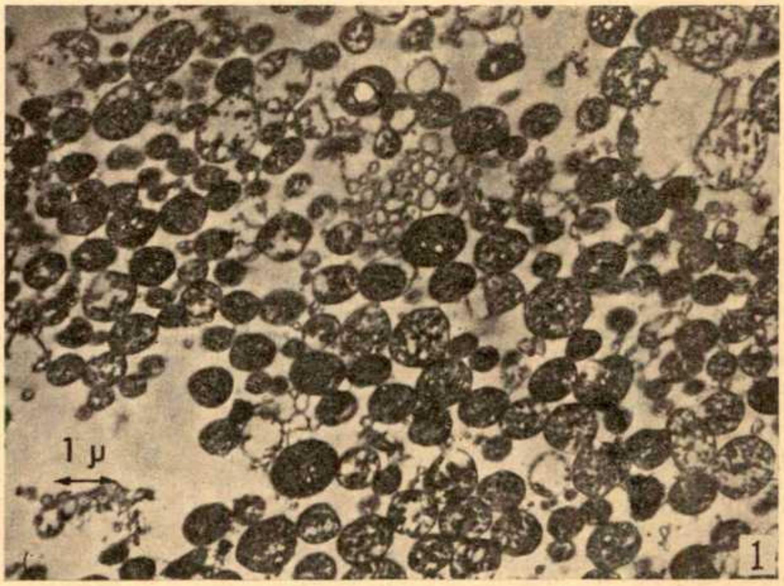
STRUCTURE AND ENZYME ACTIVITY

By P. F. BAKER, DR. D. H. NORTHCOTE and SIR RUDOLPH PETERS, F.R.S.

Department of Biochemistry, University of Cambridge

AT the present moment there is an awakening of interest in the relation of structure to the function of various organelles of the cell, as the background to a more complete understanding of the behaviour of the living cell[5]. In this connexion, it seemed interesting to examine the action of long-chain fatty acids on mitochondrial structure. The effect of long-chain fatty acids (C_{10}–C_{14}) on the oxidation of pyruvate (pyruvate oxidase system) in brain brei (pigeon) has been known for many years, since it was found that a concentration of about 1·0 mM at 37° stopped the action of the pyruvate oxidase system[6]. For 1·6 mM of C_{12} acid the decrease in respiration was 79 per cent and for C_{10} 59 per cent. It appeared that the acids were displacing some essential component of the pyruvate oxidase system by a surface action. This action seemed to be analogous to an effect of these acids at a similar concentration on the green chromoprotein, ovoverdin, from the egg of the lobster; addition of the acid changes the colour from green to red, which is the colour of the component astaxanthin. It was known in 1938 that the pyruvate oxidase system was a complex of enzymes, and it is to be noted that the succinate system in brain tissue was found to be more stable[6].

Since these experiments were reported, it has been found that pyruvate oxidation in brain tissue of the pigeon does involve the enzymes of the citric acid cycle[1,7], which enzymes are organized inside the mitochondria[3,8]. In view of this more recent information, we have investigated the effect of dodecanoic acid (lauric, C_{12}) on the respiration and the structure of mitochondria prepared from the kidneys of guinea pigs. It was con-



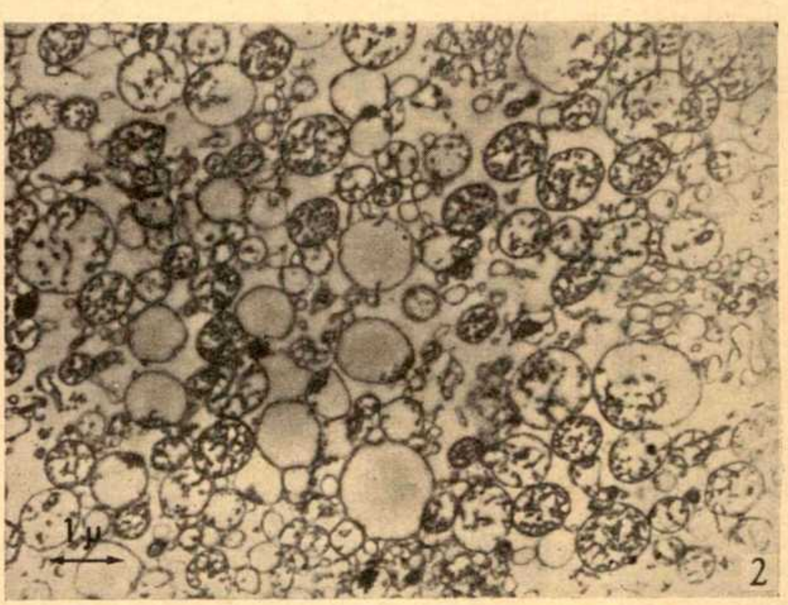


Fig. 1. Section of a normal preparation of mitochondria from kidney of guinea pig. The material was fixed in osmium tetroxide

Fig. 2. The same preparation as shown in Fig. 1 after treatment with sodium laurate for 2 min at 37° C

Table 1. EFFECT OF DODECANOIC ACID (C_{12}) ON THE METABOLISM OF KIDNEY MITOCHONDRIA

The reactions of the citric acid cycle were blocked with fluorocitrate at the aconitase stage, so that the citrate formed is a measure of the activity of the reactions from pyruvate to citrate.

Experimental. The kidneys from one guinea pig were homogenized in a hand homogenizer in 1 per cent potassium chloride solution at 0° C. After removal of nuclei, etc., by slow centrifugation for 15 min the supernatant was centrifuged at 7,000 r.p.m. (approx.) for 20 min. The pellet of mitochondria was resuspended in 11 ml. of 1 per cent potassium chloride solution and 11 ml. imidazole buffer (0·05 M) at *p*H 7·0. Each 50-ml. Erlenmeyer flask contained 2·0 ml. of the mitochondrial suspension, magnesium 4·0 μmoles (0·1 ml.), adenosine triphosphate 1·2 μmoles (0·2 ml.), sodium fumarate 20 μmoles (0·2 ml.), sodium pyruvate 10 μmoles (0·1 ml.), fluorocitrate (syn) 20 μg 0·05 ml., sodium dodecanoate neutralized to *p*H 7·0, 1·0 mg (or 0·5 mg) in 0·1 ml. and 1 per cent potassium chloride to a total volume of 3 ml. The duplicate flasks were gassed with 95 per cent oxygen, 5 per cent carbon dioxide and incubated for 30 min at 37° C, after which 1·0 ml. 25 per cent trichloroacetic acid was added. The duplicates were pooled, filtered and made up to 20 ml. for estimation of the citric acid present.

Citrate formed in 30 min at 37° C per flask in μmoles.

Control	1·85
C_{12} acid 0·5 mg	0·664
C_{12} acid 1·0 mg	0·078

firmed that, as in brain tissue, laurate (1·6 mM) stopped the respiration of pyruvate (Table 1).

Two experiments were made for examination of the structure of the mitochondria by the electron microscope, and in each case the mitochondria from a single preparation were divided into two samples, one of which was treated with laurate and the other sample used as a control.

In the first experiment the mitochondria were exposed to laurate (1·0 mM) (neutralized to *p*H 7·0) at 37° for 10 min. After this the suspension was cooled to 0° C and centrifuged to form a pellet. This pellet was fixed with 1·0 per cent osmium tetroxide dissolved in the medium used for the mitochondrial preparation for 30 min at 18° C. After dehydration the fixed mitochondria were embedded in a mixture of butyl methacrylate, 85 per cent; methyl acrylate, 15 per cent; and thin sections cut with a glass knife and examined with the electron microscope. In this first experiment a complete breakdown of the mitochondrial structure was shown. A second experiment was therefore performed in which the exposure to the fatty acid was less drastic; the mitochondria were divided into three batches which were exposed for 2 and 5 min respectively at room temperature, and at 37° C for 2 min. After this they were embedded and cut as before.

As would be expected, the untreated mitochondria prepared in 1·0 per cent potassium chloride were rounded, though they showed good biochemical behaviour so far as pyruvate oxidation was concerned. The mitochondria exposed to laurate at room temperature showed some changes; several of the mitochondria had lost their internal structure. But in those exposed to lauric acid at 37° C practically no cristæ were visible (Figs. 1 and 2). Only the limiting membranes of the mitochondria remained and even these were sometimes ballooned.

It is clear that the loss of the biochemical activity in mitochondria exposed to long-chain fatty acids is correlated with a virtual disruption of the internal structure; this may result from the penetration of an excess of undissociated fatty acid molecules. This can be inferred from the fact that in an interface between a lipoid and aqueous phase[4] long-chain fatty acids are less dissociated than they are in a bulk solution of the same *p*H. Our observations are clearly related to those of Emmelot[2]. He considers that adsorption of long-chain fatty acids may explain some details of the behaviour of mitochondria isolated from strains of hepatoma; for example, the decreased dinitrophenyl-activated adenosine triphosphatase of the hepatoma particles as compared with those of liver mitochondria, and also the pattern of swelling, which is different in the tumour preparations. It was pointed out in the earlier paper[6] in 1938 that traces of fatty acids would influence experimental results in isolated enzyme systems.

In conclusion, it has been found that treatment of mitochondria at 37° C for 2 min with the C_{12} fatty acid (dodecanoate) removed the internal structure at the same time that it eliminated the oxidation of pyruvate by the enzymes of the citric acid cycle. The fact stresses the importance of structure in some organized actions of enzyme systems.

We thank Mrs. P. Legge for technical assistance, and also the Shell Co. and the Wellcome Trustees for grants, and Prof. F. G. Young for facilities privided.

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EFFECT OF MAGNETIC FIELDS ON TUMOUR IMMUNE RESPONSES IN MICE

By DR. LEO GROSS

Waldemar Medical Research Foundation, Port Washington, New York

THE biological effects of magnetic fields have been investigated in several recent studies. Barnothy *et al.*[1] have shown that a leucocytosis is produced after exposure to static magnetic fields, and Audus[2] has shown a magnetotropism in plants. We[3] have tested for an effect of magnetic fields on tumour growth in mice. Gross and Smith[4] have reported a delay in wound healing produced by magnetic fields.

We have sought a means of assaying the biological effects of magnetic fields. An experimental situation wherein 50 per cent of the experimental animals succumbed to the treatment as commonly used in pharmacological dose-level tests was felt to be a sensitive point at which to test. My colleagues and I have reported a means of protecting mice against transplantable tumours by a series of immunizing injections[5] of X-ray-attenuated tumour cells. The extent of protection was determined by challenging with a measured number of viable tumour cells, and an LD_{50}-level was established.

These mice received weekly intraperitoneal injections of 5 million Ehrlich's ascites carcinoma cells that had been previously attenuated by X-irradiation with 4,000 r A series of six such injections resulted in 50 per cent of the mice (LD_{50}) being able to withstand a challenge of 10 million live untreated cells injected intraperitoneally one week after the course of immunization Animals so protected and challenged were then placed in magnetic fields, and a marked effect was detected

Twenty-four such protected and recently challenged *C3H* mice were placed within a relatively uniform magnetic field of 4,000 gauss produced by 'Alnico' magnets with 2-in diam pole faces and a 2-in gap The variation across the field of the 24 magnets did not exceed 200 gauss for the volume within which the mice were confined

Animals under test were housed for the duration of the experiment in individual cubical containers constructed of non-magnetic stainless steel wire mesh approximately 2 in on a side Some of the controls and the mice exposed to magnetic fields were so housed, additional control animals were housed in the usual manner in our mouse colony with six animals per pen All animals received food and water *ad libitum* These groups were equalized with respect to age, weight, and sex

The mice were observed after the challenge dose, for tumour growth and survival

Highly inbred mice of *C3H* strain were used in this experiment (Table 1)

Table 1

	Magnetically treated	Untreated Individually housed	Untreated Normally housed
No of animals	24	24	24
No surviving challenge	3	12	13
Average life-span for animals succumbing to challenge (days)	15 9	16	16
Mortality-level (*LD*) (%)	87 5	50	45 8

For a second test, some *C3H* animals which had been treated with purified protein derivative of mycobacteria and with X-ray attenuated Ehrlich's ascites were used The effect of the purified protein derivative thus used as an adjuvant was to raise the protection-level as determined by challenge and survival to an LD_{50} at approximately a 30 million cell dose This higher dose was injected into the animals in this second test (Table 2)

Table 2

	Magnetically treated	Untreated individually housed
No of animals	23	17
No surviving challenge	2	4
Average life span for animals succumbing to challenge (days)	15	17
Mortality-level (*LD*) (%)	91 3	76 5

Analysis of the results shows an LD_{50}-level in the control animals, and a higher mortality in the magnetically treated animals, equivalent to an $LD_{87\ 5}$ In the second trial, the immunization-level of the mice was higher but the dose was not accurately estimated to produce an LD_{50} in the controls Nevertheless the same relationship of fatalities in magnetically treated mice to controls was obtained, namely, 91 3 to 76 5 per cent The *P* values of these tests are significant with a probability level of $P < 1$ per cent

Tests conducted with mice that did not receive a prior course of immunizing injections before challenge showed no perceptible difference between magnetically treated mice and their controls

This effect of the magnetic field is marked Mice kept within the magnetic field succumb in larger numbers to an LD_{50} challenge dose of a transplantable tumour than do similarly treated controls

It is fair to assume that the protective mechanisms of the mice arrayed against Ehrlich's ascites tumour is in some way impaired by the action of the magnetic field At the critical LD_{50}-level or close to this level, this impairment in magnetic fields is revealed with more sensitivity We have postulated, in view of the delay in wound healing in magnetic fields[4] and in light of developmental anomalies on fruit flies observed by Mulay *et al* [6] in magnetic fields, that the production of antibody titres would be reduced in the presence of magnetic fields We are measuring such antibody titres in mice, and results to be published later show a diminution of antibody titre-levels in mice resident in magnetic fields

We thank the Raytheon Manufacturing Co for providing the magnets used in these experiments This work was supported in part by U S Public Health Service, National Institutes of Health, grant No. *C*4561*C*2

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GLUCOSE METABOLISM IN RAT BRAIN *IN VIVO*

By Dr R VRBA

Medical Research Council, Neuropsychiatric Research Unit, Carshalton, Surrey

THE brain is unusual among the larger organs in its dependence on glucose[1,3] It is generally believed that processes of glycolysis, of the tricarboxylic acid cycle and of the direct oxidative pathway through pentose phosphate are the main metabolic pathways of glucose in the brain[1-3]

During the past ten years evidence has accumulated that glucose can be converted into amino-acids in the brain Labelled α-amino-acids were isolated from brains of neonatal and young mice[4] after injection of glucose-U-^{14}C and from the brains of adult rats after injection of glucose-1-^{14}C and glucose-6-^{14}C (ref 7). Similarly, rapid formation of labelled amino-acids was found *in vitro* after incubation of brain cortex slices with glucose labelled with carbon-14 (ref 5) and after introduction of glucose-U-^{14}C into 'artificial blood' used for perfusion of the brain of a cat with isolated circulation[6]

In this article the main results are presented of experiments in which some pathways of glucose metabolism in brain were followed from 1 min up to 5 hr after introduction of glucose-U-^{14}C into intact circulation These experiments form part of a more extensive investigation which has been published in full elsewhere[9]

Glucose-U-^{14}C was injected into the tail vein of rats which were decapitated at intervals, of 1, 2, 5, 10 and 30 min after injection 0 5 ml of blood was collected immediately and deproteinized with acid The brain was quickly dissected and placed in ice-cold 0 25 *M* sucrose, homogenized at 0° C in a mechanical homogenizer[8], deproteinized by addition of concentrated perchloric acid to a final concentration of 5 per cent of the acid (w/v) and fractionated into various chemical fractions, as outlined in Table 1 It may be seen from Table 1 that already 2 min after injection of radioactive glucose, 41 per cent of the total radioactivity found in the brain was there in the form of α-amino-acids, and this figure increased to 75 per cent after 30 min

Table 1 DISTRIBUTION OF RADIOACTIVITY IN RAT BRAIN AFTER INTRAVENOUS INJECTION OF GLUCOSE-U-^{14}C

Fraction	Sub-fraction	Time between injection of glucose-U-^{14}C and decapitation of the rat (min) 0†	1	2	5	10	30
Acid-soluble	*a* Non-amino-acid fraction	95 84	66 67	58 01	42 63	41 76	22 35
	b Amino-acids	3 51	26 51	40 97	55 99	55 36	74 78
Lipid	*a* Acetone extract	*	*	0 54	0 85	1 96	1 60
	b Ethanol-ethyl ether extract	*	*	*	*	*	*
	c Chloroform methanol extract	*	*	*	*	*	*
	d Acid chloroform methanol extract	*	*	*	*	*	*
Nucleic acid	*a* Hot HClO$_4$ extract	*	*	*	*	*	0 29
	b Lipid impurities	*	*	*	*	*	*
Protein	*a* Carbohydrate impurities	*	*	*	*	*	0 15
	b Amino-acids	*	*	*	0 17	0 33	0 65
	c Humines	*	*	*	*	*	*
	Total	100	100	100	100	100	100

Results are expressed as percentages of total radioactivity in 1 gm fresh weight of brain Only counts exceeding the double of the background are considered of significance Values replaced with asterisks can be considered as practically negligible because they are below limits of the reliable resolution power of the method The results were corrected for background and self-absorption Each active sample was plated in duplicate and at least 6,000 counts were taken in a Nuclear Chicago automatic Geiger counter For further details of methods see ref 9

Female litter mates (120 gm each) were injected into the tail vein with 0 2 ml of glucose-U-^{14}C (15 μc) in presence of 2 38 mgm. carrier glucose The glucose was provided by the Radiochemical Centre, Amersham, England

† Control = animal was not injected but glucose-U-^{14}C added to the solution in which the dissected brain was homogenized All other procedures were identical with those to which the brains of animals injected *in vivo* were subjected This serves as a check for the separation methods

Among the acid-insoluble substances, the acetone-soluble lipids were those most labelled It was possible to demonstrate their radioactivity within 2 min of injecting glucose-U-^{14}C into the circulation It is unlikely that the counted radioactivity is due to adsorption, because it is evident that the number of counts and percentage of total brain activity in this fraction were not dependent on the absolute amount of radioactivity in brain, but on the time which had elapsed from the moment of injection of glucose-U-^{14}C into circulation The labelling of brain proteins was relatively small during the first 30 min , although 5 min after injection of glucose-U-^{14}C it was possible to demonstrate the presence of carbon-14 in brain proteins

Table 2 A COMPARISON BETWEEN BLOOD AND BRAIN ACID-SOLUBLE FRACTIONS AFTER INTRAVENOUS INJECTION OF GLUCOSE-U-^{14}C

Fraction	1 Non-amino-acid fraction		2 Acid-soluble α-amino-acids	
Remarks	Cold HClO$_4$ (5 per per cent w/v) extract neutralized, and evaporated after elimination of KClO$_4$ and redissolved in a small amount of water Absorbed on 'Zeo-Karb-225 *H*' form cation exchange resin and eluted with water		After elution with water (see 1) the 'Zeo-Karb-225' column was eluted with 1 *N* NH$_4$OH	
Time after injection of glucose-U-^{14}C (min.) Tissue	Blood	Brain	Blood	Brain
0* (control)	(–)	*96 47%* (11,344)	(–)	*3 53%* (415)
1	*99 74%* (6,224)	*71 55%* (840)	*0 26%* (16)	*28 45%* (334)
2	*99 67%* (42,648)	*58 61%* (9,853)	*0 33%* (139)	*41 39%* (6,958)
5	*99 62%* (34,972)	*43 23%* (10,925)	*0 38%* (132)	*56 77%* (14,348)
10	*99 45%* (15,012)	*43 00%* (5,100)	*0 55%* (83)	*57 00%* (6,761)
30	*99 05%* (19,632)	*23 01%* (8,205)	*0 95%* (189)	*76 99%* (27,454)

Results expressed as percentages of total acid-soluble radioactivity obtained from 1 ml blood or 1 gm fresh weight of brain Figures in parenthesis indicate counts/min /ml. blood or 1 gm brain respectively For all other conditions see Table 1

Only 30 min after intravenous injection of glucose-U-^{14}C was it possible to show unequivocally the presence of carbon-14 in the 'nucleic acid' fraction The relatively high rate of conversion into amino-acids points to the importance of this process in the function of the brain This is corroborated by the fact that conversion of glucose into amino-acids proceeds in brain at a rate of several times that in liver, kidney, heart, lungs, spleen, skeletal muscle or blood[9] The results shown in Table 2 show unequivocally that the radioactive α-amino-acids were formed from glucose in brain and not transported to the brain by circulation. Even 30 min after injection of glucose-U-^{14}C the α-amino-acids in blood contain less than 1 per cent of the total acid-soluble radioactivity of blood The specific activity of α-amino-acids in the blood is only a small fraction of the specific activity of α-amino-acids in brain The amino-acids of brain were further separated on 'Dowex' anion exchange resin into monocarboxylic and dicarboxylic α-amino-acids using Gaitonde's method[10]. The dicarboxylic α-amino-acids were then separated into glutamic acid and aspartic acid by chromatography on alumina according to Darling's method[11] These separations provided information on the distribution of carbon-14 between various α-amino-acids Data in Table 3 show that the dicarboxylic α-amino-acids have (several times) higher specific radioactivity than the monocarboxylic α-amino-acids Both glutamic and aspartic acids are highly labelled It may be further

Table 3 RELATIVE SPECIFIC ACTIVITIES OF ACID-SOLUBLE α-AMINO-ACIDS IN BRAIN AFTER INTRAVENOUS INJECTION OF GLUCOSE-U-^{14}C

Time after injection of glucose-U-^{14}C (min)	0 (control)	1	2	5	10	30
Monocarboxylic* α-amino acids	*0 001* (15)	*0 217* (273)	*0 404* (6,868)	*0 538* (13,823)	*0 264* (3,227)	*0 410* (15,066)
Dicarboxylic* α-amino-acids	*0 113* (1,322)	*0 584* (736)	*2 153* (36,563)	*2 163* (55,436)	*2 147* (26,217)	*2 591* (95,156)

* α-amino-acids were isolated from the acid-soluble fraction of brain using 'Zeo-Karb-225 *H*' form cation exchange resin (cf Roberts *et al*[4]) Thereafter they were passed through a 'Dowex'-carbonate column according to Gaitonde[10] (anion exchange resin '*AG*'1–10, 200–400 mesh, Bio Rad Laboratories, Richmond, California) and separated into monocarboxylic and dicarboxylic Before this separation glutamine was hydrolysed to glutamic acid

S A Specific activity = counts/min /mgm α-amino nitrogen

$$\text{Relative specific activity} = \frac{\text{specific activity}}{\text{counts/min /gm fresh weight of brain}}$$

Relative specific activity in italic figures, specific activity in brackets

Table 4 ACCUMULATION OF RADIOACTIVITY IN RAT BRAIN DURING FIRST 30 MIN AFTER INJECTION OF GLUCOSE-U-^{14}C*

Time after injection of glucose-U-^{14}C (min)	$\frac{\text{Specific activity of brain}}{\text{Specific activity of blood}}$
1	*0 202*
2	*0 400*
5	*0 730*
10	*0 809*
30	*1 852*

* Specific activity of brain = counts/min /gm fresh weight of brain, specific activity of blood = counts/min /ml. of blood acid-soluble fraction.

seen that the relative specific activity of α-amino-acids reached an almost constant level in brain in less than 5 min after injection of glucose-U-^{14}C and an equilibrium of specific activity of α-amino-acids with total radioactivity in brain is reached and maintained. On the other hand, the data of Table 4 clearly indicate that during the first 30 min there occurs a constant enrichment of brain with carbon-14 derived from glucose-U-^{14}C injected into the circulation As shown in Fig 1, this is not in contradiction with the state of relative equilibrium mentioned above About half an hour after the injection of glucose-U-^{14}C into the circulation, the process of progressive elimination of carbon-14 from brain prevails (Table 5, row *f*) On the other hand, the carbon-14 still present in the brain is progressively more and more converted into acid-insoluble substances, mainly lipids and proteins (Table 5) Thus the main features of glucose metabolism in the rat brain *in vivo* can be characterized as follows

(1) Part of the glucose–carbon, which enters the brain from the circulation, is retained there for at least half an hour A fraction of this remains in the brain even after 5 hr and probably much longer.

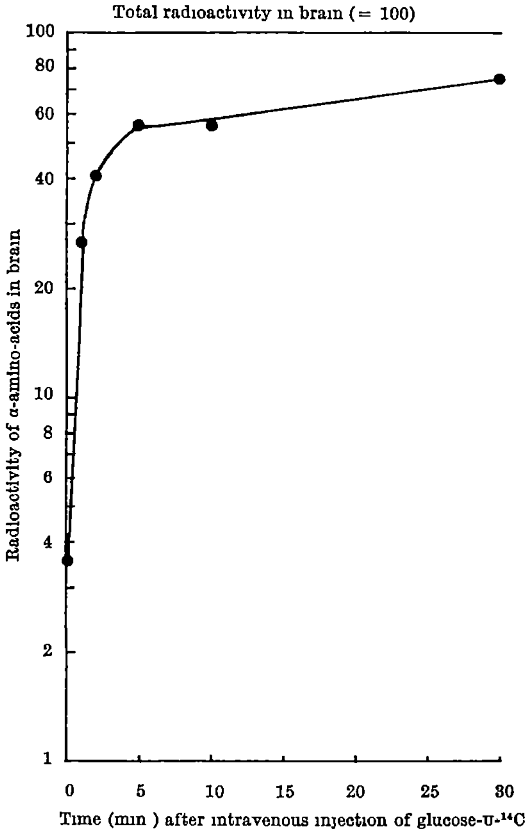

Fig 1

Table 5 DISTRIBUTION OF RADIOACTIVITY IN BRAIN DURING 5 HR AFTER SUBCUTANEOUS INJECTION OF GLUCOSE-U-^{14}C

Time after injection of glucose-U-^{14}C (hr)	0 5	1 0	2 0	4 0	5 0
a 'Acid-soluble' fraction	98 3	93 0	79 9	49 6	30 7
b 'Lipid' fraction	0 8	3 2	8 6	29 4	36 8
c 'Nucleic acid' fraction	0 5	1 0	2 3	5 6	8 2
d 'Protein' fraction	0 3	2 8	9 2	15 4	24 3
e Total (per cent)	100 0	100 0	100 0	100 0	100 0
f Total (counts/min / gm fresh brain)	6,126	3,001	2,152	840	1,070

Female litter-mates, 120 gm each, were injected subcutaneously over the left flank with 7 μc glucose-U-^{14}C in 0 2 ml water and 2 mgm carrier glucose Results expressed as percentage of total activity in brain

(2) At 2 min. after injection of glucose into the circulation, about 40 per cent of the glucose–carbon retained in the brain is in the form of α-amino-acids, mainly glutamic and aspartic acids In less than 5 min equilibrium of conversion of glucose into α-amino-acids is reached At this time more than 50 per cent of glucose-derived carbon present in the brain can be accounted for as α-amino-acid carbon.

(3) During the next 5 hr more than 80 per cent of the glucose-derived carbon is eliminated from the brain by a metabolic process involving oxidation of α-amino-acids, since the highly labelled α-amino-acids formed in the brain do not appear in any significant quantity in blood The glucose-derived carbon not eliminated from the brain during this period is converted into lipid, protein and other carbon substances.

It has been established by the classical work of Himwich and others[1], that the increase of carbon dioxide and lactic acid in brain venous blood can be accounted for quantitatively by the glucose consumed in the brain My experiments show that glucose–carbon introduced into the brain via the circulation is partially retained in the brain for a considerable period of time in the form of various substances The logical conclusion is that carbon dioxide eliminated from the brain is at least in part derived from oxidation of amino-acids, proteins, lipids and other 'non-glucose' substrates Only in this way could an equivalent amount of carbon dioxide be liberated by the brain in order to replace the glucose–carbon retained in the brain

It should be noted that there is independent evidence that a part of the carbon dioxide eliminated from the brain is derived from oxidation of non-glucose substances, particularly from α-amino-acids, lipids, proteins and nucleic acids[3,6,12,13]

I thank Dr Derek Richter for his support in this work

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HYPOCHROMISM IN THE ULTRA-VIOLET ABSORPTION OF NUCLEIC ACIDS AND RELATED STRUCTURES

By DR. H. C. BOLTON
Department of Physics
AND
PROF. JOSEPH J. WEISS
Department of Chemistry
King's College, University of Durham, Newcastle upon Tyne

IT has been known for some time that there is a considerable increase in the ultra-violet absorption of nucleic acids (RNA and DNA) on degradation to the corresponding nucleotides, that is, the arrangement of nucleotides in nucleic acids and polynucleotides causes what has become known as a hypochromic effect.

Earlier statements that this effect is due to the interaction of the π-electrons of the bases do not provide an explanation. In a more recent paper, Tinoco[1] attempted to apply exciton theory to account for this hypochromism. However, as has already been shown by Rhodes[2], this treatment cannot account for the effect.

Rhodes[2] attempted to give a theory for the hypochromic effect on the basis of what he terms 'first order exciton theory'' taking into account dispersion forces, but there is no clear statement in his paper about quantitative results. Moreover, it appears that Rhodes's considerations are based on the assumption that hypochromism is solely due to the coil–helix transformation. His considerations leave unexplained the fact that hypochromism is not confined to the strand–helix transformation, as in fact it has been shown[3] that even dinucleotides can exhibit some hypochromic effect (~10 per cent), which in some simple polynucleotides was found to reach a limiting value up to 35 per cent at chain lengths of about 10 nucleotide units.

Previous work on the basis of the exciton theory has dealt mainly with the structure of the absorption spectra of solids, that is, the shift and broadening of the absorption bands[4]. However, in going from mononucleotides to nucleic acids, there is no marked change in the position and shape of the absorption band but a very pronounced decrease in the extinction coefficient at maximum absorption, that is, hypochromism. The explanation of this effect must therefore be sought on different lines.

It had been suggested by one of us[5] that the hypochromic effect is basically a 'medium effect' and is related to some interaction between the nucleotide units within the 'molecular crystal' of a polynucleotide.

In general, the behaviour of a molecule with a dominant absorption band under the influence of a light wave may be considered as a classical electron oscillator in the field of the light wave and the average field of the other oscillators. The classical theory of an isolated oscillator was given by Rosenfeld[6] and the equivalent quantum mechanical theory by Heitler[7].

The reduced light absorption arises from the fact that in a medium consisting of similar units (for example, in a crystal) there is a reduced dissipation of energy as the energy lost by an oscillating dipole is regenerated to a greater or lesser extent by the coherent action of the radiation coming from the other dipoles. It should also be clear that, in these situations, the simple spectroscopic sum rule does not apply as its derivation relates only to the strength of the absorbed (or scattered) radiation expressed in terms of the incident amplitude at the position of the absorbing molecule and does not allow for a modification of this incident amplitude by the presence of other scatterers or absorbers.

To obtain a quantitative estimate of the light absorption of a polymer crystal of N monomer units, one can start with a model where the polarizability tensors of each of the N units are arranged in some pattern in the macro-molecule. The light absorption in the polymer is related to the polarizability tensor of the monomer through the theory of the internal field in the N-mer.

We include absorption by treating the polarizability as a complex quantity; similarly the refractive index can be written as

$$n = n' - ik \qquad (1)$$

where k is the absorption coefficient. Decreased light absorption, that is, hypochromism, would then mean that the absorption coefficient per monomer unit of an N-mer is not a constant but decreases with increasing number of N up to a certain limit. This, at first sight, may seem surprising, but this is simply due to the fact that any selected unit has its near neighbours *correlated* according to the structure of the polymer and not randomly arranged. The problem is essentially similar to that first discussed by Ewald[8].

One can now see immediately how the absorption maximum of an N-mer will behave compared to that of N separate monomers. The corrections with the field acting on a particular molecule arising from local structure will be the fields of neighbouring dipole oscillators in the polymer. At the maximum absorption these absorbing dipoles will have a polarizability which has a large if not dominant imaginary component. There will thus be a quadrature component of the local field in addition to the usual field, and the resulting component, which is exciting the dipole moment, is reduced. It is the introduction of the local correlation which produces decreased light absorption (hypochromic effect).

It must be emphasized that we are assuming polarizable units and consequently localized dipoles. Since a polymer is itself a single molecule or even a one-dimensional crystal, excitation and absorption are in a sense a collective property of the polymer and not of the separate units.

Calculation for Dimers and N-*mers*. The method of calculation which has been adopted can be illustrated by the following particularly simple model, namely, the case of a dimer with isotropic units. The field acting on any particular absorbing unit will be the radiation field and the field of all the other molecules

The random arrangement of molecules implies that there are two transverse and one longitudinal positions and therefore, for example, the average polarizability of the monomer unit (within a dimer) is given by $\alpha\,\xi$, where

$$\xi\,(X) = \frac{1 - X}{1 - X - 2X^2} \tag{2}$$

and $X = (\alpha/d^3)$ is a dimensionless factor, α is the polarizability of the monomer and d the distance between the dipole oscillators

The equations needed to link polarizability and absorption coefficient have been given in Seitz[9] At the centre of the absorption band where $\nu = \nu_m$, the polarizability α of a single unit is given by

$$\alpha = f\,e^2/(4\,\pi^2\,m\,i\,\nu_m\,\nu_d) \tag{3}$$

where f is the oscillator strength, ν_d is a damping frequency, e and m are the charge and mass respectively of the electron We introduce the non-dimensional parameter

$$y = f\,e^2/(d^3\,4\,\pi^2\,m\,\nu_m\,\nu_d) \tag{4}$$

where d is the shortest distance between the absorbing units in the polymer.

The expression for the maximum absorption coefficient k_m is given by[9]

$$k_m = C_N(y)\,N_0\,y\,d^3\,(4\,\pi/3)\,(n' + 2)^2/6n' \tag{5}$$

where N_0 is the concentration of monomer units and n' the refractive index of the solution. The influence of the correlation on the light absorption of an N-polymer is expressed by the factor $C_N(y)$ which is the real part of the correction factor $C_N(y) = Re\,\xi\,(-iy)$

It is an easily observable fact that in going from a mononucleotide to a polynucleotide the position and width of the absorption bands, which are at about 2600 Å, do not change very much, therefore the maximum absorption coefficient of an N-mer in solution per monomer unit can be expressed to a good approximation by

$$k_m/N_0 \propto C_N(y) \tag{6}$$

Correlation factors for several N-mers are given here the subscript is the number of units in the molecule and the superscript indicates whether the transverse (T) or longitudinal (L) moments or both have been taken into account

For isotropic polarizabilities ($L + 2T$) one obtains for the dimer ($N = 2$) (from equation (2)),

$$C_2^{L+2T} = \frac{1 + 3\,y^2}{1 + 5\,y^2 + 4\,y^4} \tag{7}$$

There are only algebraic complexities to extend the argument to longer chain polymers For an infinitely long chain we need the summation $\sum\limits_{S=1}^{\infty} 1/s^3$ which is the Riemann zeta function of order 3, it was given by a Ferranti–Pegasus computer programme to a sufficient accuracy as 1.2021 along with other series needed in other calculations

Thus one obtains for an infinitely long chain ($N = \infty$)

$$C_\infty^{L+2T} = \frac{1 + 17.3\,y^2}{1 + 28.9\,y^2 + 133\,y^4} \tag{8}$$

For N-mers taking into account only transverse polarizability

$$C_2^T = 1/(1 + y^2) \tag{9}$$

$$C_\infty^T = 1/(1 + 5.78\,y^2) \tag{10}$$

Taking into account only the longitudinal polarizability

$$C_\infty^L = 1/(1 + 23.1\,y^2) \tag{11}$$

In the last three cases, the right-hand side of equation (5) should be multiplied by a factor of 1/3 because of the averaging

Double Helix in Nucleic Acids The model chosen is a helix of base pairs of linear oscillators arranged in planes, the two dipoles making an angle 10.4° to each other[2] The line of the oscillator is the line of long axis of the base Each base pair is rotated with regard to its neighbours by an angle 36° For simplicity, the only interactions considered are those between dipoles in the base pair and the four neighbouring dipoles in the two adjacent base pair planes. For the helical dimer one gets for $N = 6$, that is, the interaction of one base with its five nearest neighbours, the following equation

$$C_6^{\text{helix}} = \frac{1 + 14.3\,y^2 + 45.9\,y^4}{1 + 20.2\,y^2 + 102\,y^4} \tag{12}$$

and to a first approximation it may be assumed that $C_6^{\text{helix}} = C_\infty^{\text{helix}}$ The distance d is taken here to be both that between dipoles and between the planes of the base pairs

The foregoing results show that the transverse polarizability, that is, in the plane of the bases, makes a contribution towards hypochromism but the longitudinal polarizability (along the helix) has a relatively much stronger effect, as can be seen by a comparison of equations (10) and (12) with equation (11) In a single strand with a rigid structure the longitudinal polarizability could be very important This will, however, not be the case when the single strand assumes, to a greater or lesser extent, the configuration of a 'random coil', as then the hypochromism due to longitudinal polarizability may be negligibly small If, however, two strands are combined to form a double helix which behaves like a 'rigid rod', correlation due to the longitudinal polarizability will be restored and the full hypochromic effect due to it will manifest itself

From these considerations the magnitude of the observed hypochromic effect in nucleic acids, that is, an increase in the maximum absorption coefficient in the ratio of about 1.14 in going from the double helix to the single strands and a similar increase in going from a single strand to the mononucleotides, can be calculated on the basis of the Watson–Crick model[10] using equations (10) – (12), with values of the parameters $y^{(T)} = 3 \times 10^{-1}$ and $y^{(L)} = 2 \times 10^{-1}$ for the transverse and longitudinal polarizability respectively. These numerical values correspond to mean oscillator strengths of $f^{(T)} = 0.21$ and $f^{(L)} = 0.14$ as derived from equation (4) for an absorption frequency $\nu_m = 10^{15}$ sec^{-1} and a damping frequency $\nu_d = 10^{14}$ sec^{-1} and $d = 3.5$ Å

In this way, these calculations give some insight into the light absorption of nucleic acids connected with the 'random coil'–helix transformation It is not only the regular arrangement of the bases in the helical configuration but particularly the fact that the formation of the double helix leads to a more or less rigid, well-ordered, rod-like structure which is responsible for the hypochromism due to correlation effects in the nucleic acid molecular crystal

We thank Prof R E Peierls for his advice Our thanks are also due to the Rockefeller Foundation and the Medical Research Council for financial support of this work

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REGENERATION OF PROTHROMBIN ACTIVITY BY PLASMA ALBUMIN AND GLOBULIN FRACTIONS

By Dr. B C. DAS*
Case Institute of Technology, Cleveland 6, Ohio

THE agents responsible for blood coagulation must be able to circulate freely in the blood and yet be able to form a clot at the site of injury to the body. Two agents of primary importance in the process of blood coagulation circulate in the blood as the proteins prothrombin and fibrinogen Prothrombin is a precursor to thrombin, it is converted to thrombin when blood leaves the circulation and comes into contact with some foreign surface The thrombin can then convert the soluble protein fibrinogen into insoluble fibrin, which forms the network of fibres known as a blood clot While the biochemical conversion of fibrinogen to fibrin by the action of thrombin has been described in some detail the conversion of prothrombin to thrombin is not so well understood[1] The latter process may be investigated experimentally by partial activation of prothrombin, which reduces its capacity to form thrombin, and subsequent regeneration of prothrombin-like activity so that thrombin can be formed The work described here reports the capacity, hitherto unrecognized, of plasma albumin and globulin fractions to regenerate prothrombin-like activity from a partially active prothrombin derivative

Ware and Seegers[2] were the first to observe that a substance exhibiting prothrombin-like activity could be generated from a partially active prothrombin derivative The reaction was afterwards observed by others[3-8] The partial activation of prothrombin and subsequent regeneration of a substance exhibiting prothrombin-like activity, referred to as prothrombin-*R*, provides an experimental model for testing the effects of enzymes and other plasma protein fractions which may be serving as clotting factors in the process of converting prothrombin to thrombin

To examine systematically the influence of the plasma albumin and globulin fractions on the formation of prothrombin-*R*, a series of experiments was conducted using Cohn plasma fractions and plasma fractions purified by DEAE-cellulose anion exchange chromatography Purified prothrombin was prepared from bovine plasma[9], its specific activity was approximately 30,000 U /mgm tyrosine The prothrombin was diluted with Michaelis veronal acetate buffer[10] at *p*H 5 to an activity-level of 1,000 units/ml (ref 11) To obtain the partially active prothrombin derivative, rat liver light mitochondria (lysosomes)[12,13] were added to the prothrombin at the rate of 10 μgm protein/1,000 units prothrombin activity[11] When the activity-level was reduced by half, the reaction was stopped by cooling to − 2° C, centrifuged at 14,000*g* for 10 min to remove the mitochondria, and the *p*H increased to 7 9

DEAE-cellulose anion exchange chromatography[14] (buffer 0 005 *M* triphosphate, *p*H 8 6) was used to obtain α-, β- and γ-globulins and albumin from Cohn fractions III, IV and V (Pentex, Inc, Kankakee, Illinois). The homogeneity of the Cohn fractions and the chromatographed fractions was tested by immunoelectrophoresis[15] Fig 1 presents photographs of the immunoelectrophoretic slides for commercial Cohn fractions III (rabbit origin) and IV (rabbit and human origin), and the chromatographed albumin, β- and γ-globulin fractions (rabbit origin) The fractions which were not chromatographed showed multiple precipitation lines, indicating that they were composed of several immunoelectrophoretically distinguishable protein components The chromatographed fractions showed single precipitation lines, and may be regarded as homogeneous according to the immunoelectrophoresis procedure

Single plasma fractions, both Cohn and chromatographed, were mixed in varying concentrations with the partially active prothrombin derivative and were tested for prothrombin activity[11] immediately after mixing (0 min) and at successive intervals up to 70 min Table 1 presents the experimental data at 0, 30, 60 and 70 min for optimal concentrations of

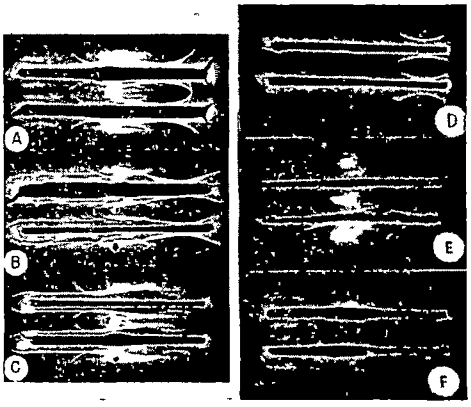

Fig 1 Photographs of immunoelectrophoretic slides (original size 76 mm × 26 mm) *A*, Cohn rabbit fraction IV, *B*, Cohn human fraction IV, *C*, Cohn human fraction III, *D*, DEAE-cellulose chromatographed rabbit albumin, *E*, DEAE cellulose chromatographed rabbit β-globulin, *F*, DEAE-cellulose chromatographed rabbit γ-globulin

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* On leave from the Indian Statistical Institute, Calcutta

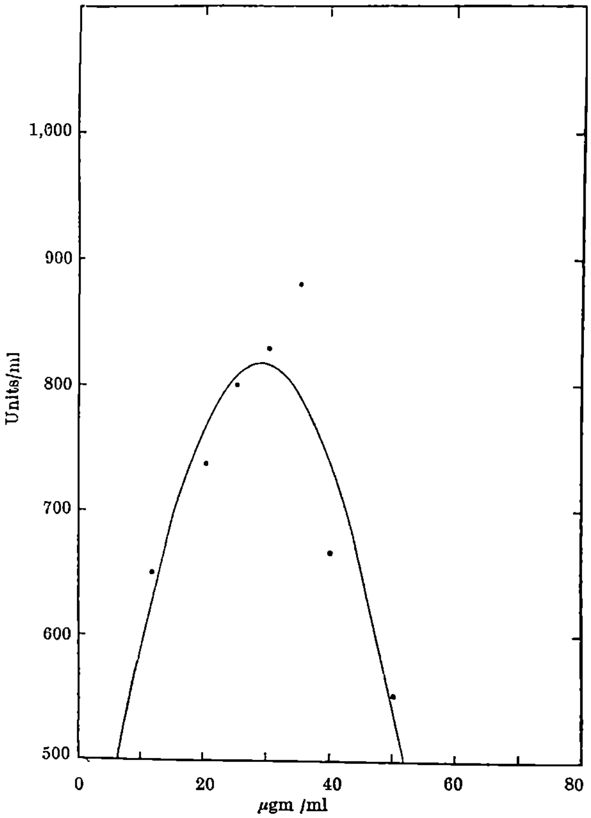

Fig 2 Regenerated prothrombin activity at 60 min in relation to the concentration of Cohn fraction II The ordinate gives prothrombin activity in units/ml, and abscissa gives protein concentration of the plasma fraction in μgm /ml The parabolic curve gives the expected yield of prothrombin-*R* and the points give the observed yield of prothrombin-*R*

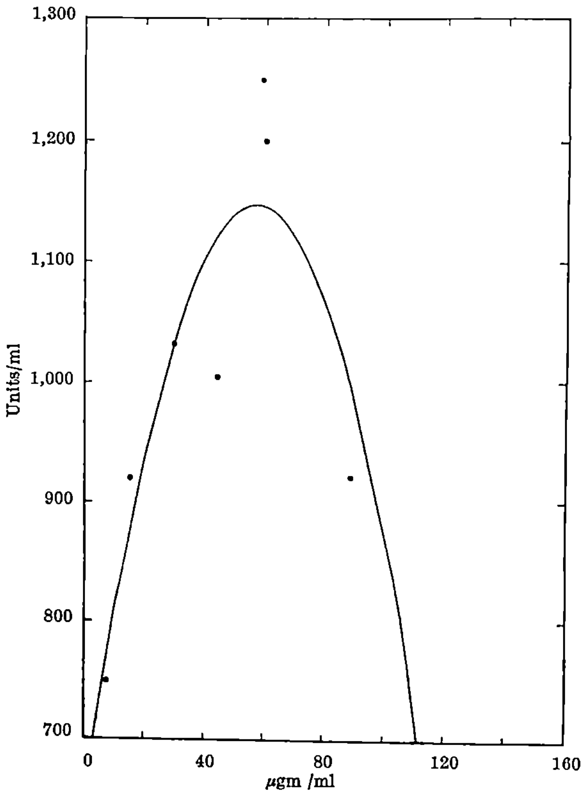

Fig 3 Regenerated prothrombin activity at 60 min in relation to the concentration of DEAE-cellulose chromatographed β-globulin (See Fig 2 for further explanation)

the fractions and for selected lower and higher concentrations The prothrombin activity of fractions at 60 min was found to be a parabolic function of the protein concentration of the plasma fraction, and Table 1 presents the parabolic equations fitted to all the experimental data by the non-reduction method of least squares[16]. Figs 2 and 3 present the expected amount of prothrombin activity as a curve, and the observed data as points, for Cohn fraction II and for purified β-globulin The prothrombin activity at 60 min can be expressed as a percentage of the activity of the prothrombin derivative prior to mixing it with the plasma fraction, these percentages are also given in Table 1, and they represent the amount of prothrombin activity regenerated by the plasma fraction

Although the chromatographed fractions were distinguishable by immunoelectrophoresis, they all exhibited the capacity to generate prothrombin-*R* It may be postulated that the agent responsible for this capacity is an enzyme differentially distributed among the globulin fractions The major findings of

Table 1 PROTHROMBIN ACTIVITY GENERATED BY COHN PLASMA FRACTIONS AND FRACTIONS PURIFIED BY DEAE-CELLULOSE ANION EXCHANGE CHROMATOGRAPHY

Plasma fraction (0)	Protein concentration (μgm /ml) (1)	Prothrombin activity (min) 0 (2)	30 (3)	60 (4)	70 (5)	Maximum regeneration (per cent) (6)	Parabolic equation* (7)
Cohn II (rabbit)	12	552	600	648	576	15	$\hat{Y} = 283\ 6949 + 36\ 8866X - 0\ 6352X^2$
	35	552	570	880	880	59	
	50	528	552	552	510	18	
Cohn IV (rabbit)	250	581	672	800	720	41	$\hat{Y} = 70\ 9160 + 5{\cdot}1269X - 0\ 0073X^2$
	300	581	736	1,050	920	90	
	500	574	595	700	565	21	
Cohn V (rabbit)	150	534	576	602	532	10	$\hat{Y} = -374\ 1474 + 10\ 4031X - 0\ 0255X^2$
	200	564	672	672	525	28	
	250	525	560	525	504	9	
Purified α-globulin (man)	2 7	560	632	680	608	24	$\hat{Y} = -41\ 4997 + 336\ 1778X - 28\ 4487X^2$
	5 2	574	721	1,000	920	100	
	8 7	539	574	750	720	40	
Purified β-globulin (rabbit)	7 5	588	680	750	730	27	$\hat{Y} = 652\ 4374 + 17\ 2818X - 0\ 1511X^2$
	59 2	567	595	1,250	850	100	
	88 8	574	700	920	800	53	
Purified γ-globulin (rabbit)	5 3	574	736	960	850	91	$\hat{Y} = 824\ 7273 + 29\ 0289X - 1\ 0129X^2$
	10 6	552	623	1,000	950	100	
	31 8	574	576	720	700	35	
Purified albumin (rabbit)	45 6	560	600	750	700	42	$\hat{Y} = -381\ 0237 + 34\ 4901X - 0\ 2124X^2$
	91 2	574	680	1,000	920	100	
	114 0	574	648	790	770	50	

* $\hat{Y}$ = Expected prothrombin activity. X = Plasma fraction concentration

these experiments, as shown by Table 1, may be examined in terms of this hypothesis First, albumin was less effective than the globulin fractions in regenerating prothrombin activity Second, the commercially prepared Cohn fractions were uniformly less effective in regenerating prothrombin activity than the chromatographed fractions, both in terms of the percentage of prothrombin activity regenerated, and the protein concentration required to achieve the maximum observed regeneration This may have been due to the presence of multiple protein components in the Cohn fractions, some of which may have been acting as inhibitors of the active agent Third, the chromatographed globulin fractions exhibited differing amounts of activity, as indicated by the protein concentration required to achieve 100 per cent regeneration Fourth, as the parabolic equations indicate, activation of the prothrombin generating mechanism increases with the protein concentration of the plasma fraction, while inhibition of the mechanism increases with the squared concentration value As enzymes typically activate and inhibit reactions in this matter, the interpretation of the active agent as an enzyme is also compatible with the observed relationship The similarity observed for the globulin fractions in the regeneration of prothrombin activity is consistent with published findings that the globulin fractions are highly intercorrelated and exhibit a similar degree of relationship with serum cholesterol and serum inorganic phosphorus[17]

This work was carried out in the Department of Physiology and Pharmacology, Wayne State University College of Medicine, Detroit, Michigan, and was supported by research grant *H*–3447 from the National Institutes of Health, National Heart Institute I thank Dr Marion I Barnhart for cooperation and facilities

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SCRAPIE IN SHEEP: THE HEREDITARY COMPONENT IN A HIGH INCIDENCE ENVIRONMENT

By G J. DRAPER and H B PARRY

Unit of Biometry and Nuffield Institute for Medical Research, University of Oxford

IN a previous communication[1] one of us showed that natural scrapie in sheep was likely to be due to an autosomal recessive gene *s* A more comprehensive study[2] added further support for this genetic hypothesis and provided evidence against the supposition that environmental factors influence materially the clinical manifestation New evidence, consistent with these results, collected in a high-incidence flock among progenies of different sires has permitted a rigorous statistical analysis

The material and methods have been described[2,3] From flock *A*, in which the annual incidence of scrapie from 1957 to 1962 was 16 7, 7 5, 14 8, 10 9, 13 6 and 12 3 per cent, data on the attack-rate are available for (*a*) 14 females of the same breed and similar breeding introduced in 1959 from a flock free of scrapie then and since The 14 were aged 9 months to 6 years, after 2¾ years 12 are well and no sign of scrapie has appeared in any of the 14 or their progeny (*b*) 181 progeny born to the 10 stud rams used in the flock in 1957–58 and 1958–59, reared and kept in the flock, of which 75 have developed scrapie All females surviving to 9 months of age and able to breed have been retained, as well as all males of *ss* × *ss* matings Retention of males of other types of mating has not been complete, and, as the basis on which they were retained might introduce bias into the analysis, they have not been included, although the data for 7 such animals are consistent

Of the 10 stud rams the presumptive genotypes of 5 are *ss*, 4 being affected and 1 having both parents affected Three rams are unaffected and are presumptive *Ss* or *SS*, the genotype of two can be allotted on pedigree and other outside data, while that of the third is uncertain without using data from the 1958–59 birth-group, and the results of his 13 progeny have therefore been excluded, although they indicate this ram to be *Ss* Two rams died of competing risks and their 24 progeny have also been excluded However, the results on the progenies of these 3 rams are entirely consistent with those of the 7 The presumptive genotypes of the dams are also determined by reference to data other than that presented here

The effect on our conclusions if some of these presumptive genotypes are incorrect must be considered In the first part of the analysis here we show that the discrepancies between the observations and expectations based on the genetic hypothesis can be ascribed to chance It seems probable that mistakes in the genotyping would tend to increase these discrepancies, such mistakes would therefore not be in a direction to affect our conclusions Nor does it seem that any systematic bias could be introduced in this way which would vitiate the results of the second part of the analysis

The data may be analysed in two ways We may examine the observations to see whether they are consistent with the recessive gene hypothesis We may also try to determine whether there are any real differences in the proportions of affected progeny from different types of mating, to test various contagion hypotheses The results of both types of analysis support the genetic hypothesis

Table 1 gives data on the fate by age 3 years of the 137 sheep alive and well at age 9 months (This latter age is chosen to eliminate the special effects of normal wastage in early life) The first figure in each cell is the number of progeny, from the indicated type of mating, in which scrapie has manifested by the age of 3 years The second figure is the number which were either alive and free of scrapie at age 3 or had died from other causes by that age Similar data at age 4 years are presented in Table 2

Table 1 NUMBERS OF OFFSPRING* MANIFESTING SCRAPIE, AND THOSE REMAINING FREE OF IT†, AT AGE 3 YEARS, FROM VARIOUS TYPES OF MATING

		Ewe				
		Affected during gestation/ lactation	Affected later	Presumptive *ss*	Presumptive *Ss*	Presumptive *SS*
Ram	Affected	6, 6	9, 10	—	8, 37	0, 10
	Presumptive *ss*	3, 1	6, 3	1, 1	0, 3	0, 1
	Presumptive *Ss*	—	1, 2	—	0, 2	—
	Presumptive *SS*	0, 8	0, 10	—	0, 8	0, 1

* The first figure in each cell is the number of affected offspring and the second is the number free of the disease (all by age 3 years)

† The criteria for inclusion in the table and of assessment are given in the text

Table 2 NUMBERS OF OFFSPRING MANIFESTING SCRAPIE AND THOSE REMAINING FREE OF IT, AT AGE 4 YEARS, FROM VARIOUS TYPES OF MATING (SEE NOTES ON TABLE 1)

		Ewe				
		Affected during gestation/ lactation	Affected later	Presumptive *ss*	Presumptive *Ss*	Presumptive *SS*
Ram	Affected	2, 1	11, 4	—	8, 12	0, 4
	Presumptive *SS*	0, 3	0, 9	—	0, 7	0, 1

(1) We first examine the data to see whether they are consistent with the postulated genetic theory

The age of manifestation of the disease is highly variable The manifestation-level $\lambda(t)$, at age t, is defined here to be the proportion of all animals alive at age 9 months and homozygous for the postulated scrapie gene in which the disease actually appears by age t This definition differs from one used previously[1,2].

For some types of mating for which data on the offspring are available, it is possible to estimate $\lambda(t)$ if the genetic hypothesis is assumed true But it is not then possible to test the hypothesis itself If, however, data are available on the fate, by age t, of progeny from two or three different types of mating we may obtain a combined estimate of $\lambda(t)$ and then test the hypothesis using a χ^2 test which has one degree of freedom fewer than the number of types of family[4] More precisely, we estimate $\lambda(t)$ from the data, assuming the hypothesis, compute, using this estimated value, the expected number of affected (by age t) animals from each type of mating, and then use χ^2 to compare these expectations with the observed values

Data for estimating λ at age 3 years are available on three types of mating (Table 1) However, since there are only 2 progeny from matings of type *Ss* × *Ss* we cannot incorporate into the analysis the information which they provide (For the χ^2 test is invalid for such low numbers The value of λ is barely affected by this exclusion) The estimate of λ from the remaining two types of mating is $\lambda = 0\ 49$ On testing for 'goodness-of-fit' using this estimate we find $\chi^2 = 1\ 82$ with 1 d f Thus the data fit the hypothesis well

The fate of the male progeny born in the *ss* × *ss* group was very similar to that of the females This is also true of the few male animals from other matings which were kept under observation (but which are not included in the table)

Data for estimating λ at age 4 years are again available from two types of mating, all the progeny except one being female (Table 2) The combined estimate of λ at this age is 0 74 and the value of χ^2, with 1 d f, is 0 11, showing very good agreement between data and theory

Thus the recessivity hypothesis provides an adequate explanation of the observed data

(2) We next examine Tables 1 and 2 to ascertain whether differences in the proportions of affected animals from different types of mating are consistent with hypotheses based on transmission of the disease either by contagion or by one of the parents

The following discussion is based on the classification at age 3 years (that is, on Table 1) Less information is available regarding animals of 4 years, such conclusions as can be drawn are similar and are indicated later

On examining the first two columns of Table 1 and comparing, for each row separately, the pairs of figures in the two columns, it seems evident that there is no difference in the fate of the offspring of mothers affected by the disease during their gestation and/or suckling and those of mothers affected later

From the first two columns, taken separately or together, we may examine the proportions of affected progeny in matings involving rams of different presumptive genotype Taking both columns together, that is, considering the progeny from all affected females and comparing the proportions of affected animals arising from matings with different types of ram we find the following differences (ignoring the progeny of the presumptively *ss* ram) (Here and later we shall be using χ^2 to test for differences in the proportions of affected progeny from indicated pairs of matings)

Progeny from affected rams compared with progeny from presumptively *SS* ram, $\chi^2 = 10\ 38$

Progeny from affected rams compared with progeny from presumptively *SS* and *Ss* rams, $\chi^2 = 9\ 23$

Each of these χ^2's indicates a highly significant difference The probabilities that the observed differences are due to chance are respectively 1 in 700 and 1 in 400 Similar results are obtained, though at lower levels of significance, if we examine separately the results from the two types of affected ewes

From the parallel results for affected rams mated with various types of ewe similar conclusions may be drawn There are significant differences between the progeny born to affected ewes and those from either presumptive *Ss* or presumptive *SS* ewes If the latter two groups are taken together and compared with the progeny of affected ewes (all the rams being affected) the difference is significant at the 1 in 500 level Taking the two types of affected ewe separately we obtain similar results, but at a lower level of significance

Examination of the top two rows of Table 1 suggests there is no difference in the proportions of affected animals born to the ram believed on purely familial grounds to be of type *ss* and those born to rams actually affected

Similar conclusions may be drawn from Table 2

It should be noted that of 38 animals having a presumptive *SS* animal for a parent not one was affected by the disease

In assessing these results, two points should be borne in mind First, the various significance tests are not independent, this, however, does not vitiate the nature of the conclusions Secondly, the number of rams involved was small, and in particular there was only one presumptive *SS* ram However, the following conclusions may be drawn

(*a*) The hypothesis that the disease is controlled by an autosomal recessive gene provides an entirely adequate explanation of these data

(*b*) The transmission of the disease does not depend simply on whether the ewe is affected, but, for both ewes affected at the time and those affected later, depends also on the ram

(*c*) The chance of an affected ewe transmitting the disease to her progeny is the same whether she is affected at the time of their gestation and/or suckling, or does not become so until later

(*d*) The proportion of affected progeny from affected rams depends on the presumptive genotype of the mother No difference was observed between the progeny from later affected rams and that from one believed on purely familial grounds to be of the same genotype

(*e*) Paragraph (*b*) and the first part of (*d*) state that transmission of the disease does not depend on either parent alone However, the significance tests provide at least equally strong evidence that the probability of an animal becoming affected is not determined simply by the general environment

(*f*) The differences noted here are all in the direction which would be predicted on the genetic hypothesis

(*g*) In the dissemination of scrapie among sheep under natural conditions the hereditary component is paramount, and the role of contagion, even if it exists, for which we can find no evidence, is extremely small

(*h*) The control of scrapie could be effected by the use of rams of *SS* genotype Such a ram is the presumptive *SS* of Table 1, which may be considered to be of 'proved' homozygous dominant genotype, for the data suggest that the odds against this ram carrying the allele *s* are probably at least 100 to 1

This work was supported by the Nuffield Foundation, London, and the National Foundation for Neuromuscular Diseases, New York

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INCORPORATION OF ASSIMILATED CARBON INTO THE WALLS OF XYLEM ELEMENTS

By DR. H. JONES and J. E. EAGLES

Department of Botany, University College of Wales, Aberystwyth

MANY tracer studies with carbon-14 have shown that the carbon taken up by the leaves of a plant is soon distributed widely within it[1-3] Autoradiographs taken during such investigations have been concerned mainly with the demonstration of the translocatory channels and sinks However, autoradiographs of tissues where soluble carbon has been removed are also potentially useful in studies dealing with the growth of cell walls This is particularly so with respect to wood growth since the comparatively large amount of incorporation of carbon-14 into the massive walls of lignified xylem is especially noticeable in autoradiographs[4]

The distribution of carbon-14 in xylem walls in various parts of the tobacco plant following assimilation of $^{14}CO_2$ by one of the older leaves is being investigated An account of some of the preliminary findings follows

22 mg of $^{14}CO_2$ and $^{12}CO_2$ (about 80 μc) were circulated over a single illuminated leaf of each of three plants of tobacco (var White Burley) using similar apparatus and under similar conditions to those described by Jones *et al* [4] The $^{14}CO_2$ was assimilated in about 0 5 h, the leaf chamber remaining in position for about 2 h Afterwards the plants remained in light and normal air for the periods indicated in Table 1. After five days one of the plants was allowed to assimilate a second quantity of $^{14}CO_2$ (similar quantity and activity to the same leaf) Samples from stems and leaves of the three plants were killed and fixed in formalin/propionic acid/ethanol, then dehydrated through an ethanol series prior to embedding in wax Sections were then cut, dewaxed and taken down to water through a series of decreasing concentrations of ethanol At this point sections were either dried and exposed to Kodak X-ray film or exposed to Kodak *A R* 10 autoradiographic stripping film in the usual way. Sections prepared in this manner show no loss of activity on prolonged contact with water, and it is assumed that the remaining activity is located in the structural cytoplasmic and cell-wall constituents together with inclusions such as starch

Table 1

Plant	Period in light and normal air subsequent to $^{14}CO_2$ assimilation
A	5 days
B	10 days
C	5 days, then second $^{14}CO_2$ assimilation period followed by further 5 days in light and normal air

A marked localization of activity occurs in the petiole of the presentation leaf and of petioles of leaves above this point This results in tangential arcs of active xylem, often only 1 cell deep in a radial direction This feature is evident, though to a lesser extent, in the younger, rapidly growing parts of the stem. Below the presentation leaf (*n*), on the other hand, where the stem has finished extending and contains a large amount of wood, activity is more diffuse in a radial direction (Fig 1) The level of activity is also higher in the older stem

These differences are probably related to differences between the lignification of early formed wood and of that formed later In the former, wall growth appears to be a concentrated process whereby one element is dealt with completely before the next one is started, whereas, in the latter, wall growth is a more diffuse process, where many elements are simultaneously concerned It is possible that these differences are correlated in some way with the different types of lignified elements produced—that is, annular thickening on one hand (in the petioles) and pitted on the other with the inclusion of many fibres The fact that there is marked localization of carbon-14 in walls of xylem elements at a certain growth stage is particularly well seen in Fig 2c Here, the activity is concentrated in two tangential bands each mainly corresponding to a single arc of xylem elements separated by one or two relatively inactive elements Each of these arcs is related tó a single feeding period of $^{14}CO_2$ and corresponds to the arcs in Figs 2*a* and *b* In Fig 2*c*, where two inactive elements separate the active ones, the period of maximum deposition of carbon is probably between $1\frac{2}{3}$ and $1\frac{1}{4}$ days per element Where one inactive element interposes then the values lie between $1\frac{2}{3}$ and $2\frac{1}{2}$ In the stem below leaf $n - 2$, the two peaks of activity are separated by a distance which is between two and three times that between peaks in the petiole of leaf $n + 5$ The greater distance includes about 20 fibres (vessels are few and scattered) so that the time of maximal carbon deposition in any one fibre can be as short as 6 h This procedure, then, affords a method of investigating rates of xylem growth Fisher[5] used a similar method to label young leaf primordia at a certain stage of development and thus to calculate the plastochron

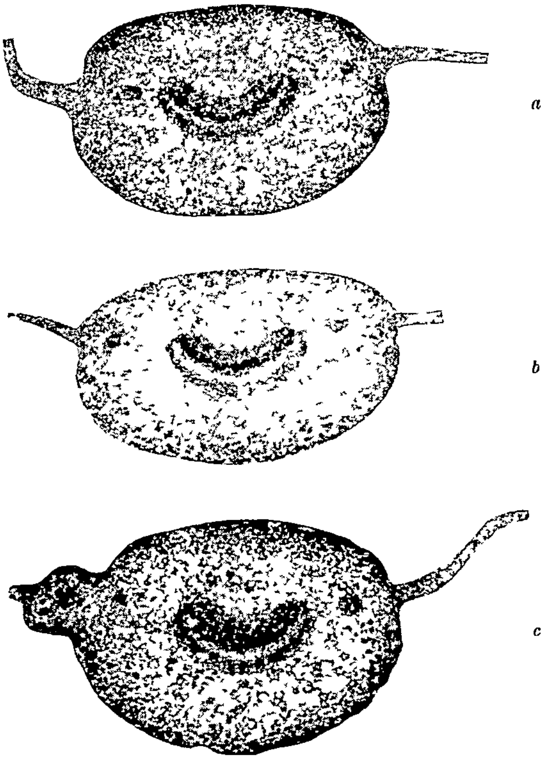

Fig 2 Autoradiographs on Kodak X-ray film of transverse sections of petioles of the fifth leaf above the presentation leaf of plants treated as indicated in Table 1 *c* shows two bands of radioactive xylem elements corresponding to the incorporation of carbon-14 from the two assimilation periods (× *c* 8 5)

A further point arises from the marked localization of the activity in the younger parts of the plant While this is certainly incorporated in the walls, the elements while they were growing must have contained appreciable amounts of soluble carbon-14 It has been clearly established[6] that the xylem transports soluble carbon Most of this material may well be carried in those elements which are undergoing rapid cell wall growth Hylmo[7] has suggested that movement of solutes (referring particularly to inorganic solutes) occurs solely through that part of the xylem which still has cytoplasm on the longitudinal walls but where the transverse walls have broken down Preston[8] with reference to trees has concluded "that the living xylem only is involved in water transport, that is, the xylem in the outermost annual ring only, and even only the outermost elements in it". The zone of maximal incorporation (in younger parts of the plant) may well be the zone of maximal water and solute uptake in the xylem This situation appears to differ markedly from that in the older parts of the tobacco stem There the wide zone of activity indicates that a considerable part of the xylem remains capable of synthesizing new wall material

This work forms part of a project supported by a grant from the Department of Scientific and Industrial Research, the assistance of which is gratefully acknowledged

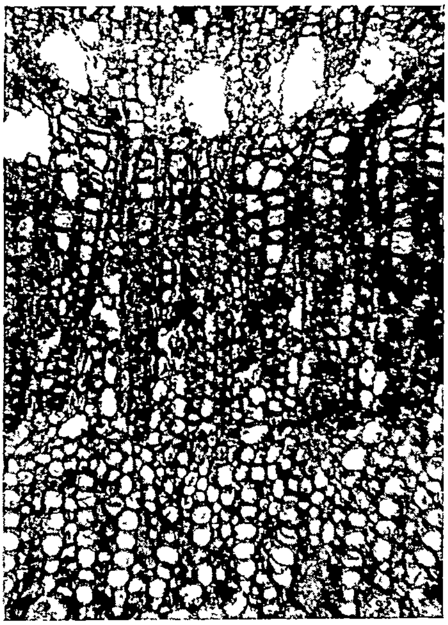

Fig 1 Kodak *A R* 10 stripping film autoradiograph superimposed on transverse section of tobacco stem (taken from the third internode below presentation leaf of plant *C* (Table 1)) The dark area is a wide band of xylem where the walls have incorporated carbon-14 (× *c* 93)

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INITIAL RESPONSE OF THE HEART-RATE OF A GASTROPOD, *ACMAEA LIMATULA*, TO ABRUPT CHANGES IN TEMPERATURE

By Prof EARL SEGAL
Department of Biology, Rice University, Houston, Texas

CONSIDERABLE information is available on the later stages of rate responses in poikilotherms to changes in temperatures, that is, from the period of steady state activity at the new temperature to the period of compensation or acclimatization[1-3] But, the initial response to temperature change is much neglected and little understood Many, but not all, investigators have reported that when various cold-blooded animals are subjected to a rapid increase or decrease in temperature they respond with an overshoot or undershoot in one or more activity rates before stabilizing at a rate characteristic of the new temperature Much of this work has been reviewed recently by Grainger[4]

In this investigation, changes in heart-rate after abrupt temperature steps were recorded in the aspidobranch gastropod *Acmaea limatula.* The experiments were carried out on populations of limpets from different habitats (Los Angeles and San Diego, California, and Punta Banda, Baja California) and at different times of the year Although individual differences in heart-rate due to size, season, previous thermal history, and general living conditions are known to be important[5], for the present study these differences have been masked by averaging the various response measurements for any given group under any given experimental conditions

The technique for revealing the heart has been reported on elsewhere[6] Limpets which had shown no post-operative effects after a minimum of 24 hr were placed two to a chamber, in a constant temperature water bath for a number of hours When the steady state of heart-rate was evident, the water in the animal chamber was poured off and replaced by water at the new temperature At the same time, the chamber was transferred to a second water bath at the new temperature The time taken to effect the change was 10–15 sec Controls were run by transferring animals to a second water bath at the same temperature as the first bath (29° C) The control animals[6] showed no greater variation in rate than was usual for that temperature Neither was there a greater variation in rate for a second control group (8 animals) which remained in the original water bath A third control group was maintained in constant temperature rooms at 18 2° and 26 5° C , then the water in the chambers was poured off There was no significant difference between the rates in water and in air Thus, it may be concluded that the experimental changes in heart-rate are due to the temperature change and not to the technique used to effect the change As a further control, heart-rates of two groups of limpets (10 and 14 animals) were counted at 15° and 24 5° C over a 48-hr period There was no discernible diurnal rhythm of heart-rate activity in either group Therefore, the time of day when the animals were subjected to the temperature changes did not affect the rate

Table 1. Overshoot and Undershoot (beats/min) and the Percentage Overshoot and Undershoot of *Acmaea limatula* subjected to abrupt Increases and Decreases of 4°, 9°, and 14° C.

Overshoot or undershoot is the difference, in beats/min , between the fastest or slowest rate achieved by an animal and its stabilized rate some hours after the temperature step

Temp step	No of animals	Overshoot and undershoot (beats/min) Mean	Overshoot and undershoot (per cent) Mean
Increase			
25–29° C	10	16 1	19 0
20–29	26	33 2	39 0
15–29	21	52 0	70 2
	*10	(41 0)	(48 2)
Decrease			
29–25° C	10	12 4	15 2
29–20	14	21 6	30 9
29–15	10	12 1	27 6
29–15	8	14 1	27 0

* Calculated on basis of stabilized rates of 10 animals which were permitted to remain at 29° C for 6 hr after the temperature change. Other animals in this group were subjected to a 14° C decrease 4 hr. after the initial temperature increase

Fig 1 shows the change in heart-rate, with time, of 3 individuals subjected to a 4°, 9° and 14° C. abrupt increase and decrease in temperature The response of these individuals is representative of the group at each experimental situation It can be seen that the response to an increase in temperature is an

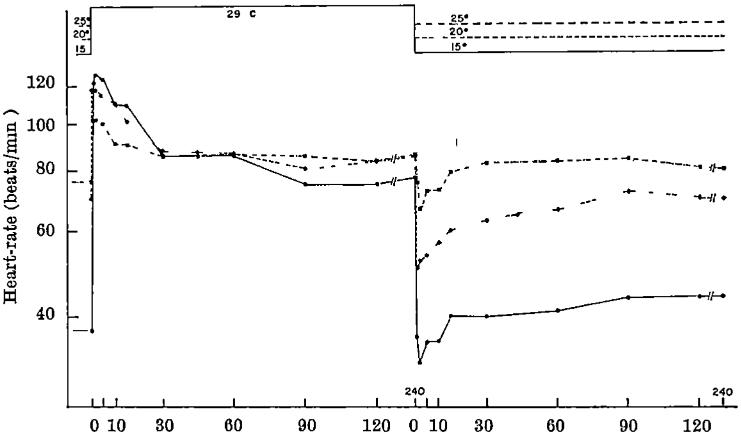

Fig 1 Semi-logarithmic plot of heart-rate (beats/min) as a function of time for three *Acmaea limatula* subjected to abrupt increase and decrease of 4° (■), 9° (◆), and 14° C (●) These individuals are representative of the group subjected to each of the temperature increments Stable heart-rates were recorded for 2 hr before initial temperature increase Animals obtained from rocky inter-tidal at Palos Verdes, California Experiments conducted a minimum of one day after collecting and trephining Animals kept in the meantime in running sea-water at 20° C

Table 2. OVERSHOOT (BEATS/MIN) AND PERCENTAGE OVERSHOOT OF 4 *Acmaea limatula* INDUCED INTO VIOLENT LOCOMOTOR ACTIVITY BY STIMULATION WITH ISOLATED TUBE FEET OF THE STARFISH *Pisaster ochraceous*

Overshoot is the difference between the fastest rate achieved by an animal and its stabilized rate some hours after stimulation Heart-rates could not be counted until 2 min after stimulation

Rate at 29° C (beats/min)	Overshoot (beats/min) (2 min)	Overshoot (per cent)
85 7	6 6	7 7
85 7	6 6	7 7
75 0	10 7	14 2
70 6	4 4	6 2
	Mean	8 9

overshoot in the heart-rate, the response to a decrease in temperature an undershoot in the heart-rate The peak frequency, over or under, is reached within 1 min , occasionally within 2, rarely over 2 The greater the temperature increment, the faster the frequency at peak of overshoot and the slower the frequency at peak of undershoot In general, the greater the temperature increment the longer it takes the heart-rate to stabilize at the new level It takes approximately 6 hr to return to a stable state after a 14° C increase in temperature In these animals the heart-rate shows a full cycle oscillation, the overshoot is followed by an undershoot before reaching a stable rate (Fig 1 and Table 1)

In this investigation the overshoot and undershoot is the difference, in beats/min , between the fastest or slowest rate achieved by an animal and its stabilized rate after the temperature step The greater the increment necessary to reach the same new higher temperature, the greater the overshoot In contrast, when animals at the same temperature are subjected to various lower temperatures (4°, 9° and 14° C), the undershoot in response to a 9° C drop is greater than that due to a 4° C drop, but the undershoot in response to a 14° C drop is not very different from the undershoot in response to a 9° C drop (Table 1)

To determine what portion of the overshoot may be due to the increased locomotor activity associated with the rise in temperature, 4 individuals at 29° C were induced into violent locomotor activity by being stimulated with isolated tube feet of the starfish *Pisaster ochraceous*[6] Heart-rates were arhythmic, steady rates could not be obtained until 2 min after stimulation The mean per cent increase in heart-rate was 8 9 per cent (Table 2), which was less than half shown for the animals subjected to a 4° C rise in temperature After stimulation it took approximately 15 min for the rates to return to normal although the animals were still active At first it appears that for the same temperature increments 'overshoots' are greater than 'undershoots', however, when the rate increase due to locomotor activity is subtracted from the overshoot, overshoot and undershoot to a 4° and 9° C increment are practically mirror images. This is not true for the 14° C increment

Another facet of the overshoot phenomenon is that the same temperature increment causes a greater overshoot at higher temperatures (Table 3) Thus a 9° C increase between 11° and 20° C. produces a 10 per cent overshoot while the same increment between 20° and 29° C produces a 39 per cent overshoot

Table 3 OVERSHOOT (BEATS/MIN) OF *Acmaea limatula* SUBJECTED TO AN ABRUPT INCREASE OF 9° OR 14° C. OVER DIFFERENT PORTIONS OF THE TEMPERATURE SCALE

Overshoot is the difference between the fastest rate achieved by an animal and its stabilized rate some hours after the temperature step

Temp step	No of animals	Overshoot (beats/min) Mean	Overshoot (per cent) Mean
20–29°	26	33 2	39 0
17–26	10	28 1	39 5
16–25	12	21 1	30 2
15–24	7	22 0	27 1
11–20	10	6 0	10 5
15–29	21	52 0	70 0
	*10	*(41 0)	*(48 2)
11–25	34	18 1	25 8
10–24	10	18 5	24 3

* Stabilized rate after 6 hr at the new temperature For all other animals figures are for stabilized rates after 3–4 hr at the new temperature

Nothing is known about the cardioregulatory nerves in *Acmaea* Nor can a distinction be made between the direct effect of rapid temperature change on the heart and any indirect effects resulting from an alteration in the physiological state of the animals A physiological explanation for the overshoot awaits further examination However, it is difficult to see how the overshoot or undershoot can have any functional significance in terms of temperature adaptation It is more probable that an abrupt temperature change produces a temporary imbalance in certain physiological systems (nervous, and less likely, hormonal) and the overshoot or undershoot are merely incidental to the adjustments that are forced on the animal

This work was supported by a grant from the National Science Foundation

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RELATIONSHIP BETWEEN THE LETTUCE BIG-VEIN VIRUS AND ITS VECTOR, *OLPIDIUM BRASSICAE*

By PROF R N CAMPBELL

Department of Plant Pathology, University of California, Davis

BIG vein of lettuce (*Lactuca sativa* L) is caused by the big-vein virus[1–3] that is transmitted by a chytrid fungus, *Olpidium brassicae* (Wor) Dang [2,3], or by grafting[1,3], but not by mechanical methods[2,3] It has been shown that an isolate of *Olpidium* that could not transmit the causal agent of big vein could acquire what I consider to be the big-vein virus from the roots of graft-inoculated plants and transmit it to healthy plants[4] The reported survival of big-vein virus in air-dry soil for 8 years[5] could be due to survival of big-vein virus within *Olpidium* resting sporangia, to adsorption of big-vein virus on clay particles, root tissues or resting sporangia, or to accidental contamination by *Olpidium* and big-vein virus[3] This article presents evidence that the big-vein virus survives within the resting sporangia of *Olpidium* and does not multiply within its vector An abstract is in the press[6]

Roots of *Olpidium*–big-vein-infected plants grown in sand culture were washed free of sand, air dried and stored in the laboratory for 2–5 months Resting sporangia were extracted from the roots and treated as follows The dry roots that contained numerous resting sporangia were pulverized in a mortar, tap water added and the fine particles were washed successively through 100-, 250- and 400-mesh screens The material remaining on the 100-mesh screen was re-ground in the mortar and washed through the same screens

The resting sporangia in the material which passed the 400-mesh screen were concentrated by centrifugation at 5,000 r p m for 10 min in either the *GSA* or *SS*34 rotors of a Servall centrifuge, re-suspended in a small volume of tap water, and divided into three aliquots that were given these treatments (*a*) untreated checks, (*b*) acidified with 1 0 *N* or concentrated hydrochloric acid to *p*H 0 5 for 2 hr (*p*H determined with a Beckman zeromatic *p*H meter), (*c*) incubated for 30 min in an equal volume of 20 per cent aqueous trisodium phosphate (w/v) The *p*H of each suspension was checked near the end of the treatment period, it ranged from *p*H 0 6 to 0 8 for the acid treatment, *p*H 7 2–8 1 for the untreated checks, and *p*H 11 3–12 3 for the trisodium phosphate treatment These treatments were selected because they are known to inactivate tobacco mosaic virus, one of the most stable plant viruses, in the seedcoats of tomato seeds (*Lycopersicon esculentum* Mill)[7]

The tubes containing the resting sporangia treatments were incubated at room temperature with occasional stirring and, at the end of the specified treatment time, the resting sporangia were sedimented by centrifugation and re-suspended in 20 ml of tap-water The resting sporangia from each treatment were poured on to a single pot of pasteurized quartz sand in which many lettuce seeds were sown immediately An un-inoculated seedling pot was planted as a check for each pot inoculated with resting sporangia All the pots were placed on the same greenhouse bench with the un-inoculated check pots interspersed among the inoculated pots The cultural methods have been described[3]

Three weeks after the seeds were sown, the seedlings were removed from each pot and four randomly selected seedlings were transplanted individually to pots These are referred to as 'transplanted seedlings' Root washings were made from the roots of the remainder of the seedlings, examined for *Olpidium* zoospores[3], and poured around two healthy lettuce seedlings in individual pots These are referred to as 'root washing-inoculated plants' Similar healthy seedlings inoculated at the same time with *Olpidium* zoospores from big-vein infected plants served as positive checks *Olpidium* zoospores were abundant in those root washings in which they were present, it is estimated that each plant inoculated with these root washings received more than 1 × 10^6 zoospores At the end of the experiment each plant was examined for the presence of *Olpidium* by the previously described method[3]

Two experiments were done at different times experiment I consisted of one replicate, experiment II consisted of two replicates that gave the same results and have been combined (Table 1) *Olpidium* survived and transmitted big-vein virus in all three replications of the trisodium phosphate treatment Although *Olpidium* survived the acid treatment and transmitted big-vein virus in experiment I, this treatment eliminated the fungus in both replications of experiment II The reason for this inconsistency in the acid treatment is unknown but could relate to the temperature at which the treatment was incubated A higher temperature in experiment II may have caused faster hydrolysis and more complete dissolution of the walls of resting sporangia *Olpidium* zoospores were present in the root washings made at the time of transplanting in all treatments that afterwards developed big-vein symptoms and were absent from all other treatments At the end of each experiment *Olpidium* was present in the roots of all plants that developed big-vein symptoms and absent from all un-inoculated checks Two plants in experiment II were *Olpidium*-infected but symptomless, whereas the other four plants in the same replicate had symptoms Zoospore transfers are being made to determine whether big-vein virus can be transmitted from these plants

The first symptoms of big-vein appeared in 21 days on the plants inoculated with zoospores in root washings This period was about a week shorter than usually required for symptom development and is due to inoculation with large numbers of zoospores and near-optimum conditions for disease development The first symptoms on the seedlings transplanted from pots inoculated with resting sporangia appeared in 38 days on the fifth or sixth true leaf This corresponds to the pattern of symptom development in the field[8] The increase in the time for symptom expression with resting sporangia inoculum probably is due to the delay in *Olpidium* infection Abundant infections occur with zoospore inoculations within a few hours With resting sporangia inoculations, however, additional time is required for seed germination and for germination of resting sporangia Examination of seedlings from pots inoculated with resting sporangia showed no *Olpidium* infection one week after sowing, but many infections after two weeks Because of the rapid and uniform appearance of symptoms the experiments were terminated 54 days after sowing the seeds or 32 days after inoculation of seedlings with zoospores in root washings

These results show that *Olpidium* and big-vein virus survive in roots air-dried for as long as five months and subjected to strong chemical treatments, and demonstrate again the close relationship between *Olpidium* and big-vein virus transmission It seems

Table 1 SURVIVAL OF *Olpidium* AND BIG-VEIN VIRUS (BVV) IN RESTING SPORANGIAL PREPARATIONS AFTER TREATMENT WITH ACID OR TRISODIUM PHOSPHATE

Experiment	Treatments	Big-vein symptoms* Transplanted seedlings	Big-vein symptoms* Plants inoculated with root washings	Total No of plants with *Olpidium**
Expt I†	Acid	4/4	2/2	6/6
	Na_3PO_4	4/4	2/2	6/6
	Untreated	4/4	2/2	6/6
	Un-inoculated check	0/12	0/6	0/18
	BVV–*Olpidium* check	—	2/2	2/2
Expt II‡	Acid	0/8	0/4	0/12
	Na_3PO_4	7/8	3/4	12/12
	Untreated	8/8	4/4	12/12
	Un-inoculated check	0/24	0/12	0/36
	BVV–*Olpidium* check	—	3/3	3/3

* No of plants with big-vein symptoms or *Olpidium* at end of each experiment/number of plants transplanted and/or inoculated with root washings See text for description of plants

† Each treatment was done on resting sporangia extracted from 1 gm (air-dry weight) of roots after storage for 2 months

‡ Each replicate of each treatment was done on resting sporangia extracted from 0 5 gm (air-dry weight) of roots after storage for 5 months

likely that having survived for five months the fungus and virus could survive for years, thus accounting for the survival in air-dry soil for eight years[5] It seems most likely that *Olpidium* survives these adverse conditions as thick-walled resting sporangia rather than as thin-walled vegetative sporangia or zoospores Since big-vein virus survived the strong chemical treatments along with *Olpidium*, it appears most probable that big-vein virus was within the thick-walled resting sporangia If big-vein virus had been external to the resting sporangia it seems likely that it would have been inactivated by treatments that have been shown to inactivate tobacco mosaic virus in tomato seed coats but not in the endosperms[7] Until the nature of the big-vein virus is better known, however, the possibility that the virus itself is in some way resistant cannot be ruled out If this is the case, then big-vein virus could have survived as particles adsorbed on host cell residues or on the exterior of resting sporangia Since the air-dried roots were obtained from plants grown in quartz sand the possibility of virus adsorption and survival on clay particles was obviated Vacuum infiltration of the preparations of resting sporangia by the chemicals was not done and seems unnecessary since the chemicals have strong penetrating action as evidenced by the penetration of dry seed coats of tomato seeds and the destruction of tobacco mosaic virus[7] Therefore, it is concluded that the resting sporangia of *Olpidium* can withstand drying and strong chemical treatments and that the big-vein virus survives within the resting sporangia presumably within the fungus protoplasm Both *Olpidium* and big-vein virus undoubtedly survive in the same manner in the field

In order to study multiplication of the big-vein virus in its vector, *Olpidium* zoospores from the big-vein virus–*Olpidium* culture that had been maintained on lettuce roots for more than one year were transferred to sugar beet (*Beta vulgaris* L cultivar U S 75) seedlings At intervals root washings were made, examined for the presence of zoospores, and re-inoculated to healthy sugar beets and to two lettuce seedlings Big-vein virus was transmitted to both inoculated lettuce plants by the third transfer of zoospores and to one of two plants inoculated with the fourth transfer, but not by the fifth or sixth transfers (Table 2) Other experiments have shown that the zoospores from the first and second transfers would consistently transmit big-vein virus to inoculated lettuce plants After symptomless plants inoculated with the fourth to sixth transfers of zoospores had been incubated for 2–3 months, root washings were made from each plant and transferred to two individual healthy seedlings (the second generation lettuce plants) None of these plants developed big-vein symptoms during a 57-day incubation period whereas both the big-vein virus–*Olpidium* checks inoculated at the same time developed big-vein symptoms within 32 days

Table 2 THE LOSS OF ABILITY OF *Olpidium* TO TRANSMIT BIG VEIN VIRUS AFTER 6 CONSECUTIVE TRANSFERS ON SUGAR BEETS

No of transfers on sugar beets	Incubation period in sugar beets (wk)	Big-vein symptoms on lettuce 1st generation*	 2nd generation†
1	4	not tested	
2	4	not tested	
3	2	2/2	
4	2	1/2	0/2
5	2	0/2	0/4
6	4	0/2	0/4

* 2 plants inoculated with root washings containing zoospores directly from sugar beets

† 2 plants inoculated with root washings containing zoospores from each symptomless plant of the first generation

Although *Olpidium* from lettuce readily infects and reproduces on sugar beet roots, the virus apparently was lost in repeated transfers of the vectors on beet, whereas it has been consistently perpetuated under similar conditions in lettuce These results indicate that sugar beet is probably not susceptible to big-vein virus and that the virus probably does not multiply in the fungus vector, at least not to the extent necessary to perpetuate itself during fungus multiplication It should be noted, however, that big-vein virus was maintained in an infectious state for 12 weeks by actively multiplying *Olpidium* on sugar beets This fact, plus the survival of the virus for 5 months in dry resting sporangia, indicates a high degree of persistence in *Olpidium*

I thank Prof R G Grogan for help in the preparation of this article

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EFFECT OF MATERNAL ISOANTIBODIES ON THE QUANTITATIVE EXPRESSION OF TWO ALLELIC GENES CONTROLLING γ-GLOBULIN ALLOTYPIC SPECIFICITIES

By DR SHELDON DRAY

Laboratory of Immunology, National Institute of Allergy and Infectious Diseases,
National Institutes of Health, Bethesda 14, Maryland

ALLOTYPES are serum proteins which differ in part of their antigenic specificity from one individual to another in the same animal species Allotypes were discovered in domestic rabbits by Oudin in 1956[1] Serum protein allotypes of indivdual rabbits are antigenic for other rabbits and yield precipitating antibodies, these isoantibodies can then be used to identify the allotypes by agar-gel immunochemical methods[1-3] Allotypes have been found among the α-, β- and γ-globulins[2], several $7S$ γ-globulin allotypes have been identified and their genetic control investigated[1-8] The maternal-fœtal barrier of rabbits has been shown to be permeable to these γ-globulin allotypes[3,6,8].

One purpose of this article is to show that, by genetic manipulation, γ-globulin allotypes may serve as natural markers for the disappearance of passively transferred maternal γ-globulin and for the appearance of newly synthesized γ-globulin in the serum of neonatal rabbits A second purpose is to show that maternal isoantibodies to an allotype of the progeny interfere substantially with the ability of the young to exhibit this allotype in its serum and that at the same time there occurs a compensatory increase of the allotype controlled by the allelic gene

The γ-globulin allotypes A5 and A4 (formerly called *RGG* I and II)* are controlled by a single pair of autosomal genes with both specificities exhibited by the heterozygote[4,6,8] Heterozygote A_b^5/A_b^4 dams (with A5 and A4 in their sera) were back crossed to homozygote A_b^5/A_b^5 sires The offspring were either heterozygotes or A5 homozygotes. The A5 homozygotes do not synthesize A4, and thus A4 transferred during pregnancy from the mother serves as a marker for the decay of maternal γ-globulin in the neonatal rabbits

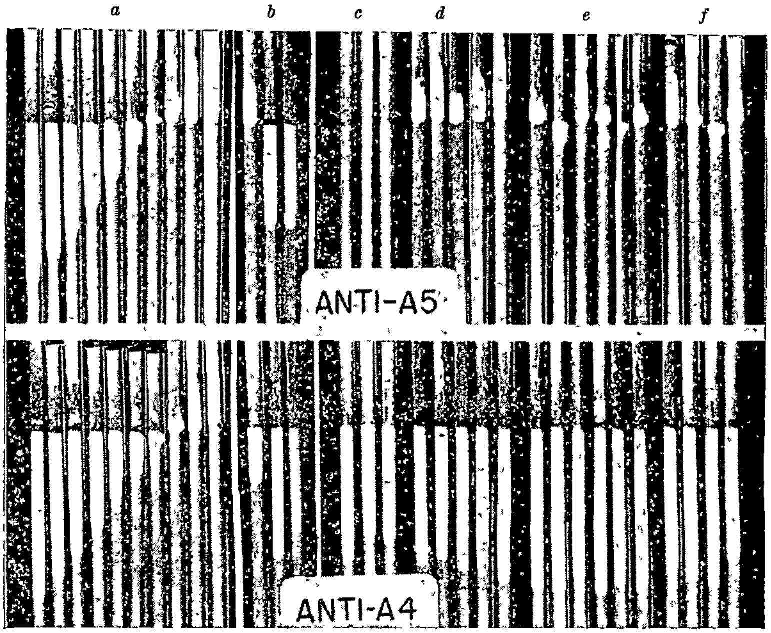

Fig 1 Agar-gel tube analysis with constant concentrations of anti-A5 and of A4 in the bottom layer Top layer as follows *a*, two-fold dilutions of each reference serum with its respective antiserum, *b*, sera from a 4-, 24-, and 56-week-old offspring from non-immunized homozygous mothers, *c*, *d*, *e* and *f*, sera of 9½-, 23-, 42-, and 45-week old litters from immunized mothers

To examine newly synthesized γ-globulin allotypes, male rabbits homozygous for A5 were mated to female rabbits homozygous for A4 All the offspring were heterozygotes exhibiting allotypes A5 and A4 in their sera A5, being absent in the mother, is present in the offspring only as a result of synthesis, but not by passive transfer of maternal γ-globulins as occurs with A4 Finally, homozygous A_b^4/A_b^4 dams after immunization with A5 were also crossed with homozygous A_b^5/A_b^5 sires During pregnancy, the anti-A5 in the mother's circulation is passively transferred to the heterozygous offspring and it is therefore possible to study the effect of anti-A5 on the developing progeny, which genetically would be expected to synthesize A5 This kind of experiment is somewhat analogous to the Rh maternal-fœtal incompatibility in humans which results in erythroblastosis fœtalis

Serum was obtained from the progeny of the aforementioned matings The relative concentration of A5 and of A4 in the sera of the progeny at different ages was determined by Oudin agar-gel tube analysis using an antiserum specific for A5 and one specific for A4 The total γ-globulins were measured with guinea pig anti-rabbit γ-globulin The antisera were used at the highest dilution which provided clearly visible precipitin bands Antisera and antigens were mixed with equal volumes of 0 6 per cent (Noble) agar before layering into Oudin tubes. The tubes were sealed, maintained for seven days at 28° C, and then photographed[10]

Sera from a rabbit homozygous for A5 and one homozygous for A4 were selected for use as reference reagents These two reference sera had the same total γ-globulin concentration Two-fold serial dilutions of each reference serum were examined The migration of precipitin bands (Fig 1*a*) was plotted against the serum dilutions to obtain the two standard curves The concentration of each allotype in the serum of neonatal rabbits relative to the amount of the allotype in the appropriate reference serum was derived from the band migration and the standard curve

Fig 2 shows the relative quantities of A5 and A4 in heterozygote offspring of non-immunized and immunized homozygous A_b^4/A_b^4 mothers Each point on this curve represents the average of 4–7 rabbits The offspring of non-immunized mothers (solid line) show the following (1) A5 is not found in the serum on the first day, is barely detectable at about three weeks and it then rises rapidly in concentration until about 24 weeks when the level remains the same at 22 per cent of the quantity present in the reference A5 homozygote, (2) A4 is present in large quantities in the serum on the first day as a result of maternal transfer, then drops to a minimum concentration at about 5 weeks and, finally, rises rapidly to a concentration about 46 per cent of the reference A4 homozygote, (3) the increase in concentration of A5 and of A4 occurs at the same time In contrast, the offspring of immunized mothers (broken line) show that (1) the appearance of A5 is delayed until 24 weeks, 21 weeks later than normally, (2) once A5 does appear, its concentration remains low for a long period so that even at 44 weeks the amount of A5 is only about 5 per cent of the controls; (3) the concentration of A4 is greater, approximately 1 8 times the amount in the controls

Although the total amounts of γ-globulin are the same in the two reference homozygotes, the absolute amounts of A5 and A4 are not known so that a direct comparison of the amounts of A5 and A4 cannot be obtained from the data presented If it were found (as is likely) that the A5 reference homozygote had about the same quantity of A5 as the amount of A4 and in the A4 homozygote, then the 44-week-old offspring of non-immunized mothers shown in Fig. 2

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* A paper entitled "The Nomenclature of Allotypy", by S Dray, S Dubiski, A. Kelus, E S Lennox and J Oudin, has been submitted for publication This paper presents a uniform nomenclature that will be used by these authors henceforth Two of the allotypes were designated A4 and A5, A4 was assigned to the *b* gene locus

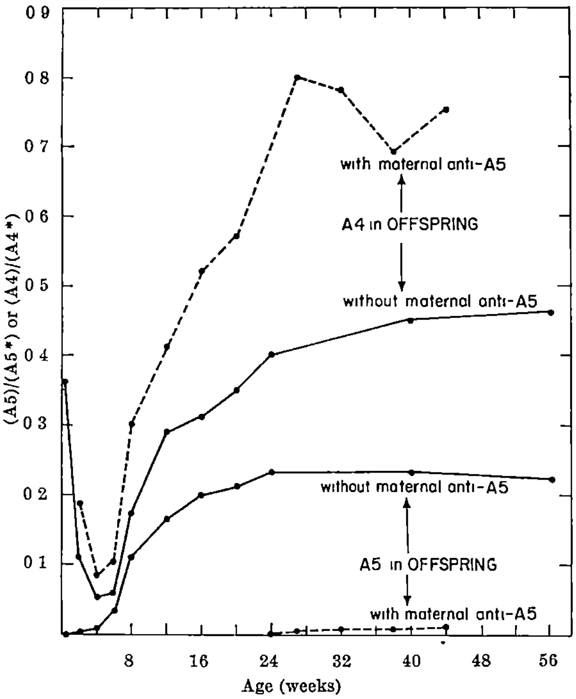

Fig. 2. Relative quantities of A5 and A4 in heterozygote offspring of non-immunized (solid line) and of immunized (broken line) A4 homozygous mothers. A4* and A5* are the amounts of these allotypes in the sera of the two reference homozygotes

would have approximately twice as much A4 as A5 whereas, at the same age, heterozygote offspring of immunized mothers would have approximately 72 times as much A4 as A5.

In another experiment, the sera of 11 heterozygous offspring (age 16 weeks), from the same parents before and after immunization of the mothers, were analysed for allotypes A5 and A4 and for total rabbit γ-globulin. This experiment also showed that A5 was not detectable and that there was a compensatory increase of A4. No significant difference was found in the total γ-globulin level of these two groups.

To date, this phenomenon occurred in all 18 heterozygote rabbits from two immunized homozygous mothers. Fig. 1c–f shows the migration of precipitin bands with uniform concentrations of anti-A5 (upper half) and of anti-A4 (lower half) for the sera of these 18 rabbits from 4 litters (9½, 23, 42 and 45 weeks of age). Fig. 1c shows that the sera of three progeny at 9½ weeks did not even react with anti-A5 whereas they did show substantial precipitin band migration with anti-A4. Most of the 15 reactions shown in Fig. 1*d–f* with anti-A5 occurred in the antigen layer, only with *e*-2, *e*-5 and *f*-3 was there sufficient A5 to migrate into the antibody layer. As also shown in Fig. 1*d–f*, the 15 sera reacted with anti-A4 to give substantial band migration. For comparison, Fig. 1*b* shows typical examples of the migration bands from the sera of 4, 24 and 56 week old offspring from non-immunized mothers.

In the experiments designed to study the disappearance of maternal allotype, it was found that for the first few weeks the curve was similar to that shown for A4 (Fig. 2) except that instead of the concentration increasing at 6–8 weeks, it continued to decrease to the point where the allotype could no longer be detected at about 8 weeks.

For simplification, this article focuses primarily on the two allotypes, Ab5 and Ab4, where *b* designates the gene locus. However, two other γ-globulin allotypes the specificities of which are controlled by alleles at a second gene locus, namely, the *a* locus, could also be followed in the same progeny[7,8]. Thus, in addition to Ab5 and Ab4, the sera of all the progeny were analysed also for Aa1 and Aa2 to ascertain whether these allotypes would serve as a second marker for passively transferred and newly synthesized γ-globulins in neonatal rabbits. The curves were similar when allotypes Aa2 and Ab5 were used as markers for newly synthesized γ-globulins, also the curves were similar when allotypes Aa1 and Ab4 were used as markers for the disappearance of passively transferred γ-globulins. In the studies of progeny from immunized mothers, whereas anti-Ab5 suppressed synthesis of Ab5, it did not suppress synthesis of allotypes controlled at the *a* locus. Double heterozygote progeny, from immunized mothers homozygous at both loci (A^1_a/A^1_a, A^4_b/A^4_b) and with anti-Aa2 as well as anti-Ab5, are under observation to ascertain whether the phenomenon of suppression will occur simultaneously with respect to the allotypic specificities controlled at the two gene loci *a* and *b*.

That maternal antibodies can effect an alteration of the quantitative expression of two allelic genes for such a prolonged period of time is a novel and unexpected finding[11]. The delay in the appearance of A5 as a result of maternal transfer of anti-A5 might superficially appear to be due simply to the reaction of maternal anti-A5 with the neonatal A5 until sufficient A5 is synthesized to neutralize all the transferred antibody. Since maternal γ-globulin was not found in the serum after about 8 weeks, A5 should appear at this time or shortly thereafter if there were no interference with A5 synthesis. Instead, as shown in Fig. 2, A5 appears much later at about 24 weeks. Furthermore, when all the antibody is neutralized, the concentration of A5 would be expected to increase sharply similar to what occurs during the third to twenty-fourth week in the offspring from non-immunized rabbits. This does not occur. Instead, the concentration of A5 remains very low until 44 weeks. Whether the concentration of A5 will eventually reach the normal level of offspring from non-immunized mothers is yet to be determined.

Two alternative hypotheses are being explored. If A5 and A4 were synthesized by the same cell, then antibody would act by suppressing the synthesis of A5 without interfering with the function of the same cell in its synthesis of A4. Moreover, the compensatory increase of A4 would suggest that the substrate available in the cell for the synthesis of A5 has been diverted toward the synthesis of A4. On the other hand, if A5 and A4 are synthesized by different cells, antibody may act by suppressing the proliferation of those cells capable of synthesizing A5. The compensatory increase of A4 could then be explained as an increase in cells which synthesize A4. These alternatives are subject to experimental investigation. For example, preliminary studies[12] have already shown that fluorescent-labelled anti-A5 and anti-A4 will specifically localize A5 and A4 in the tissues. Only A5 was found in the lymph nodes or spleen of A5 homozygotes, only A4, in A4 homozygotes, and both A5 and A4, in heterozygotes.

In a 9-week heterozygote offspring from an immunized mother, the Coons fluorescent antibody technique revealed A4 being actively produced in lymph nodes and spleen, but no production of A5 γ-globulins was demonstrable In two older 'suppressed' heterozygotes, immunofluorescent histological studies showed diminished A5 production consistent with the low A5 serum concentration These preliminary studies[12] support the concept that this suppression is not merely a result of neutralization by antibody but rather a more fundamental suppression of allotype production In addition to further investigation of these heterozygotes, the critical question whether one γ-globulin-producing cell synthesizes both A4 and A5 or just one allotype is also being studied

There are many facets of the maternal antibody effect which require further study, for example, the quantity of antibody needed, the period of time that it must be present, and how long it persists in the tissues Furthermore, would greater quantities of antibody administered for a longer period of time result in complete suppression of A5 or lead to some disease analogous to erythroblastosis fœtalis [9] Should the timing be found restricted to fœtal or neonatal life, the phenomenon could be related to immunological tolerance with the role of antigen and antibody reversed The question whether persistence of antibody is necessary for maintaining the decreased yield of A5 is somewhat analogous to the problem whether antigen must be present to maintain immunological tolerance, a problem which can be attacked by simultaneous genetic manipulation at two gene loci in order to follow the disappearance of maternal γ-globulin and the appearance of neonatal γ-globulin in the same rabbit One also wonders whether the phenomenon can be terminated at any time by injection of antigen or by other means The phenomenon of allotype 'suppression' and associated 'compensation' is interwoven with those of immunological tolerance, protein synthesis, and antibody formation Experiments devised to examine these phenomena are mutually useful

In conclusion, allotypes of γ-globulin may serve as natural markers for the disappearance of passively transferred maternal γ-globulin and for the appearance of newly synthesized γ-globulin in the serum of neonatal rabbits Maternal isoantibodies to an allotype of the progeny greatly interfere with the ability of the young to exhibit the particular allotype in its serum, at the same time there occurs a compensatory increase of the allotype controlled by the allelic gene This effect is still present in rabbits ten months after birth

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DEPENDENCE ON TEMPERATURE OF THE INTERLAYER SPACING OF IRRADIATED GRAPHITES

By B P JACKETS and D J DYSON

The General Electric Company, Limited, Hirst Research Centre, Wembley, Middlesex

MEASUREMENTS of the interlayer spacing, at temperatures between room temperature and − 192° C , of three (unirradiated) graphites of differing crystal perfection, and of one lightly neutron-irradiated *A*-grade graphite have been reported[1] The rate of contraction of each material showed a 'levelling off' with decreasing temperature The graphs of temperature versus interlayer spacing for the various samples were essentially similar but translated parallel to the spacing axis, the less perfect graphites having the larger interlayer spacings at corresponding temperatures

When graphite is irradiated by fast neutrons, changes occur in both the bulk dimensions and the crystal lattice parameters Simmons[2] has developed a relationship between the bulk and crystal dimensional changes, which applies both to thermal expansion and irradiation growth and shrinkage Extension of this relationship to more highly damaged material requires a knowledge of the changes in the crystal thermal expansion coefficient induced by different irradiation doses The present X-ray investigation was designed to determine whether or not the crystal expansion coefficients of heavily irradiated graphites differ from the crystal expansion coefficients of lightly irradiated or unirradiated materials As spacing measurements at high temperatures are not possible (heating above 150° C produces irreversible changes in the lattice parameters owing to thermal annealing of the neutron damage) the lattice contraction on cooling to liquid nitrogen temperature has been studied Owing to the diffuse nature of the diffraction maxima the measurements have had to be confined to determining the comparatively large changes in the interlayer spacing

Three specimens of graphite were irradiated in rigs in the *Dido* Materials Testing Reactor at the Atomic Energy Research Establishment, Harwell The doses experienced by the specimens were determined by the activity produced in nickel by an (*n,p*) reaction Details of the rigs and the relation between nickel dose and damage dose have been given previously by Bell *et al* [3] The irradiation conditions of the samples are given in Table 1

All measurements were made with a Philips diffractometer, type *PW* 1050, fitted with a proportional counter , nickel-filtered copper *K*α radiation was used The diffractometer low-temperature attachment used has been described elsewhere[4]

In Fig 1 the contraction of the interlayer spacing of each sample is shown as a function of temperature , it is evident that the magnitude of the contraction, over the temperature range examined, is substantially

Table 1

Sample code	Irradiation temperature (°C)	Nickel irradiation dose (n v t)
KB/B/BC	200	0 89 × 10^{20}
KB/B/AL	200	1 67 × 10^{20}
KP/B/DI	200	3 72 × 10^{20}

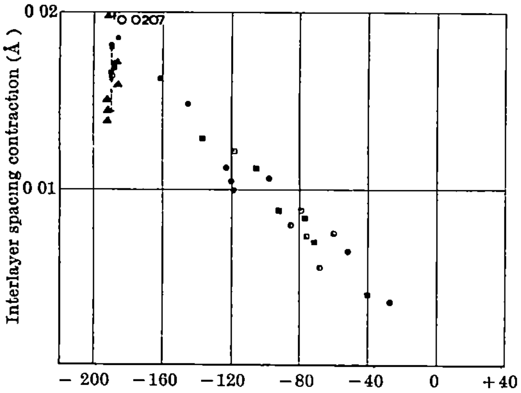

Fig. 1. Contraction of the interlayer spacing of irradiated graphites between 21° C and − 192° C. △, *KP/B/DI*, □ *KB/B/AL*, ○ *KB/B/BC*

the same, within the limits of experimental error, for all three samples. Consideration of only the results obtained at approximately − 190° C reveals, however, a tendency for the contraction to decrease slightly with increasing neutron irradiation dose; this is particularly so if the contraction value of 0.0207 for sample *KP/B/DI* is ignored. This contraction value is appreciably higher than the other five values obtained at this temperature with this sample.

The mean coefficient of expansion in the *c*-direction over the temperature-range from − 190° to 21° C has been calculated for each sample and the values are given in Table 2. Again, if the particularly large value for the contraction of sample *KP/B/DI* is ignored, a small decrease in the expansion coefficient with increasing irradiation dose is found.

An essential similarity is apparent in Fig. 2 between the results for the present irradiated graphite samples and the previously examined[1] unirradiated graphites. It may be seen that the rate of interlayer spacing contraction of all the samples below room temperature is less than the rate of expansion of the unirradiated samples above room temperature.

Table 2. Expansion Coefficients in the *c*-direction over the Temperature-range from about − 190° to 21° C

$$a = \frac{2}{d_1 + d_2} \times \left(\frac{d_1 - d_2}{t_1 - t_2}\right)$$

Sample code	Expansion coefficient (per ° C.)
KB/B/BC	$(25.2 \pm 0.8) \times 10^{-6}$
KB/B/AL	$(23.2 \pm 0.4) \times 10^{-6}$
*KP/B/DI**	$(22.4 \pm 2.8) \times 10^{-6}$
KP/B/DI†	$(21.1 \pm 1.3) \times 10^{-6}$

* Value calculated from the six results obtained for this sample.
† Value calculated omitting the particularly high 0.0207 result.

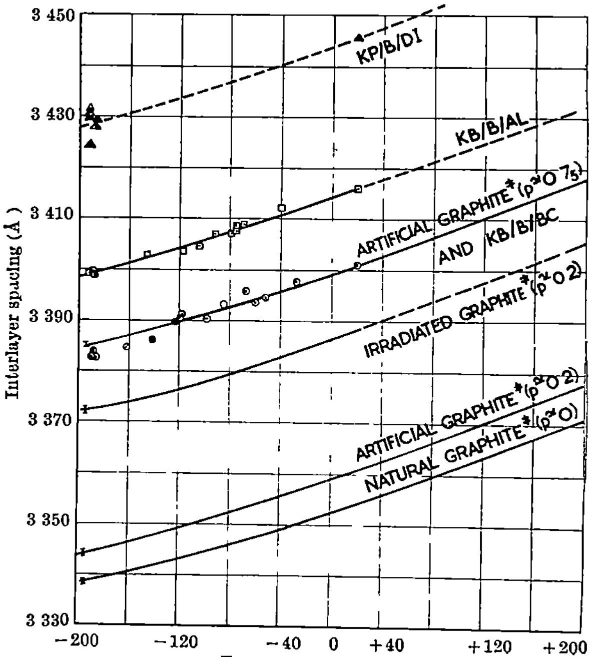

Fig. 2. Interlayer spacings of irradiated and unirradiated graphites as a function of temperature. ‡ Previously examined samples (ref. 1)

Possible differences between the samples in the change in *a*-spacing on cooling have not been investigated, the *a*-spacing change for each sample would be expected to be very much smaller than the *c*-spacing change, because of the much greater bonding forces within the layer planes than between them. Figs. 1 and 2 indicate that there are no marked differences in the magnitude of the *c*-spacing contraction with increasing neutron dose to a nickel-dose of 3.7×10^{20} n.v.t. There is therefore as yet no firm indication that the observed changes[2] in the bulk properties of graphites on irradiation are dependent on changes in the crystal expansion coefficients.

We thank the Reactor Materials Laboratory, Culcheth, U.K. Atomic Energy Authority, for suggesting the investigation and supporting the work. This article is published by permission of the Managing Director of the Reactor Group of the Authority.

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PARENT BODY HYPOTHESIS FOR ORIGIN OF TEKTITES

By ERNST W. ADAMS and ROBERT M. HUFFAKER

Geo. C. Marshall Space Flight Center, National Aeronautics and Space Administration, Huntsville, Alabama

According to Chapman *et al.*[1], observation of australites shows that they were derived from bodies of revolution and in the majority of cases from spheres. These authors presented conclusive experimental proof that glass spheres, when placed in an electric arc-jet tunnel and exposed to heating-rates of the order of those experienced in hypersonic flight become strikingly similar to button-type australites. This similarity comprises, first, a peculiar system of ring waves on the ablated front face, secondly, a coiled circumferential flange made of solidified melt, and finally, opposite to the stagnation point and enclosed by the flange, a spherical remainder of the original surface shape The anterior surface of button-type australites was thus formed by aerodynamic ablation acting on initially cold glassy spheres which, therefore, entered the atmosphere of the Earth at high altitude

According to observed mass loss-rates and according to results of trajectory and ablation analysis[2], this entry took place in a nearly horizontal direction with velocities between 6 5 and 11 2 km /sec in case of average-size buttons (11 gm initial weight[3]) Trajectory analysis indicates that bodies as small as tektites hurled up from sea-level altitude cannot traverse the atmosphere to re-enter under these conditions A simple calculation yields an initial deceleration of 96,000g for a solid glass sphere of 1 3 cm radius leaving the surface of the Earth at 8 km /sec This proves that ejecta hurled up by impact on the surface of the Earth cannot form a tektite cluster Because of the presence of nickel–iron spherules in some tektites[4], meteor impact and not volcanic eruption caused tektite flight Ejecta hurled up by impact on any extra-terrestrial body in the solar system generally stay in space for very long periods of time and, if approaching the Earth, do not arrive in a dense and short-lasting shower[5], as is demanded by the existence of a few and relatively small tektite-strewn fields These arguments show that tektite showers could not have originated as clusters of individual tektites by impact on the Earth or any other celestial body

The existence of limited tektite-strewn fields could be explained if tektites were released as liquid fragments from the ablating surface of a hypothetical parent body in hypersonic flight in the Earth's atmosphere Hardcastle[6] and Hanuš[7] stipulated that the surface of a stony meteor melted during hypersonic flight in the atmosphere and sprayed liquid droplets, which gave rise to the characteristic forms of tektites while cooling off In general, however, aerodynamic heating-rates in high-speed flight are so great that the molten layer on the surface of a stony meteor is extremely thin, because of the low thermal diffusivity of stone and the almost immediate evaporation of the melt O'Keefe[8] pointed out that the evaporation-rate of the melt might become sufficiently small if a grazing orbit with small instantaneous heat transfer-rates is considered, like that of the Cyrillid shower

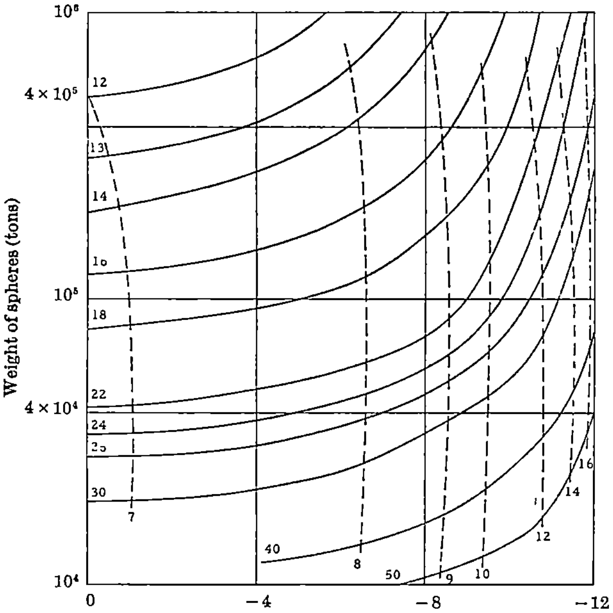
Aerodynamic Heating and Ablation of a Parent Body

By applying a calculation method (ref 9 and unpublished reports), we have examined the ablation of stony and glassy objects in high-speed flight in the atmosphere of the Earth The physical aspects of the underlying ablation processes are summarized as follows Since the thickness of the hot gas layer between the shock front and the surface increases together with the cross-section of a body in supersonic flight, the obviously large parent bodies are predominantly heated by radiation emitted from the hot gas layer, whereas bodies as small as tektites essentially experience convective heating only Detailed calculations show that the extremely high radiation-rates of as much as 10^4–10^6 kcal /m ^{2} sec , which are incident at the surface of a large parent body in hypersonic flight, cause the surface to boil If the material is opaque to thermal radiation, the heated layer beneath the rapidly receding surface has a thickness of considerably less than 1 mm , obviously, only negligible amounts of liquid material could then be released from the parent body The calculations show, however, that a layer of several cm thickness reaches temperatures of 3,000°–5,000° K if the material is sufficiently transparent to thermal radiation In one special case, the thickness of the radiatively heated layer turned out to be 5 cm for the absorption coefficient $\alpha = 100$ (1/m) of clear window glass and 0 6 cm for $\alpha = 1,000$ (1/m) Vapour nuclei are then formed at non-homogeneous spots in the glassy melt Unless strong temperature gradients normal to the surface exist in the melt, there is no force present which drives the bubbles towards the surface The outer portions of the radiatively heated layer, therefore, consist of a spongy glass melt which offers little resistance to being removed from the parent body by the air forces acting in hypersonic flight Since this mechanism rests on the existence of sufficiently high temperatures in the spongy layer, the detached fragments possess low viscosity and break up readily in the air stream The outlined process implies that the parent body consists of a glassy material the chemical composition of which is similar to the one of tektites This glassy substance cannot be generated by fusion of siliceous stone due to aerodynamic heating, because the transparency of stone is too low as to allow a radiatively heated layer of sufficient thickness (see the numbers already quoted)

Terrestrial Origin of Parent Body Flight

If tektites became detached from an ablating glassy parent body which was hurled up by some violent natural event from the Earth's surface, the parent body must have been sufficiently rigid at the time of its departure in order to avoid immediate disintegration The initial speed must have been sufficiently high and the initial trajectory angle sufficiently shallow if an area of the size of Australia is to be covered by fragments of this body or a cluster of large bodies hurled up by the same event Fig 1 presents exit speed and exit angle at 150 km altitude above sea-level for non-ablating spheres of specific weight 2 4 gm /cm ^{3}, which depart from sea-level altitude at an angle of 5° relative to the horizon The ascending spheres must exit from the atmosphere with at least the previously derived minimum entry speed[2], 6 5 km /sec , of button-type australites if average-size buttons are released from these spheres According to Fig 1, the exit speed of 8 km /sec requires an initial speed of 24 km /sec for a body of 46,000 tons and 12 km /sec for a body of 1 05 × 10^6 tons Solid fragments hurled up from terrestrial volcanic eruptions or impact craters are known to be considerably smaller than 10^4 tons and to have travelled only short distances Since initial speeds at sea-level altitude in excess of 12 km /sec cause very high ablative mass loss-rates, the parent body shrinks rapidly

As compared with the results in Fig 1, which pertains to non-ablating bodies, considerably larger initial weights and velocities are necessary to obtain an exit speed $\geqslant$ 6 5 km /sec at 150 km altitude The extremely large initial acceleration-rates, going together with the initial flight speeds under discussion, subject the impact-ejecta to surface and internal stresses which cause immediate disintegration, regardless of whether the material is in the liquid or in the solid state The presented arguments show that individual tektites and their hypothetical parent



Exit angle (deg.) relative to Earth's horizon at altitude H = 150 km. The trajectory angle θ is negative for ascending flight period

Fig. 1. Exit velocity and exit angle of non-ablating spheres starting from sea-level altitude at the initial angle $\theta = -5°$ relative to horizon. —, Curves of constant initial velocity (km./sec.) at sea-level (H = 0 km.), – – –, curves of constant exit velocity (km./sec.) at exit altitude (H = 150 km.)

bodies could not have been hurled up from the surface of any planet covered by a dense atmosphere, unless one stipulates an extremely violent natural event of such magnitude as to cause the atmosphere to be essentially 'blown out'. Urey[5] has suggested a comet–Earth collision as being a possible origin of the tektites.

Extraterrestrial Origin of Parent Body Flight

Characteristic features of the Indomalayan and Australian tektite-strewn fields can be explained if a parent body of extraterrestrial origin in hypersonic skipping flight in the atmosphere of the Earth is considered. The vapour bubbles, in spongy droplets which skip together with the parent body, expand continuously in ascending flight because of the decrease of the external pressure. The bubbles, correspondingly, tend to burst under this condition, and surface tension converts sufficiently small droplets into relatively compact glassy spheres. Since the majority of the australites were derived from glassy spheres[1,3], the described ablation mechanism implies that the parent body carried out a skipping flight pass in the Earth's atmosphere and released the australites during the ascending flight phase so that they could exit together with the parent body.

The droplets, which became detached from the parent body and did not exit from the atmosphere, remained in the liquid state until they slowed sufficiently in the denser layers of the atmosphere. Because of the increasing pressure forces exerted by the air stream in descending flight, surface tension did not shape these droplets which, at the same time, remained spongy. Due to a lack of well-defined shape, the non-skipping droplets in general did not possess stable flight attitude so that traces of previous melt flow should appear only in exceptional cases on the solidified surfaces. Since the relatively frothy indochinites and philippinites usually do not possess any well-defined shape and scarcely any markings of melt flow, it may be concluded that these groups of tektites from the Indomalayan strewn field became detached from the parent body and descended immediately without a preceding skipping phase.

Liquid droplets released during the ascending flight phase of a skipping parent body may exit from the atmosphere with any vertical velocity component between zero and the one of the parent body. The droplets take spherical shape only if they fly outside the atmosphere for a sufficient period of time. The necessary minimum flight time outside of the atmosphere decreases together with the size of the droplets, and thus also the necessary vertical component of exit speed.

The mass loss of the smallest discovered button-type australites (1.4 gm. initial weight[3]) correspondingly varies in the limits 20–80 per cent. Fig. 2 of ref. 2 indicates that a mass loss of 20 per cent in case of a button of 1.38 gm. initial weight requires an entry speed of less than 5–7 km./sec. This entry speed is considerably smaller than the minimum velocity at which an extraterrestrial object arrives at the Earth. In addition to the previously presented argument pertaining to the size of the strewn fields, the existence of small button-type australites with mass losses of ⩾20 per cent confirms the parent body hypothesis and rules out, for example, direct departure of a cluster of spheres from the Moon. Droplets which are too large to be shaped by surface tension, or which do not stay outside of the atmosphere long enough, cannot take spherical shape and may account for the australites which were derived from general bodies of revolution, for example, the canoe-shaped forms[3].

The droplets were closely spaced in the parent body's wake at the time of their release. The pressure fields surrounding the individual droplets, therefore, induced velocity components normal to the flight direction of the parent body. This, together with turbulence in the wake of the parent body, as pointed out by O'Keefe[8], explains why droplets released from a parent body can cover strewn corridors of some lateral extension along the projection of the parent body's trajectory. The width of the Indomalayan strewn field possibly cannot be explained by these two effects. It may be necessary, therefore, to assume that the parent body broke up during a first skipping pass through the atmosphere.

Conclusions

Experiment[1] and analysis[2] indicate that button-type australites were derived by aerodynamic heating from initially cold glassy spheres which, in case of average-size specimens, entered the atmosphere in a nearly horizontal direction between 6.5 and 11.2 km./sec., the lower limit, 6.5 km./sec., is demanded by the observed mass loss, 70–90 per cent of average-size buttons. Trajectory analysis shows that terrestrial origin of the spheres is impossible. Since the smallest discovered buttons[3], according to ablation analysis[2], must have entered at less than 7 km./sec., extraterrestrial origin of such a cluster is impossible. The existence of limited tektite-strewn fields can be explained if tektites were released as liquid droplets from the ablating surface of a hypothetical parent body in skipping flight through the Earth's atmosphere. Ablation analysis shows that this parent body must consist of a glassy substance, which cannot be generated by fusion of siliceous stone due to aerodynamic heating Terrestrial origin of such a parent body, even when the material is not in the liquid but in the solid state, would require a violent event of such magnitude as to be very unlikely to have ever occurred

We thank Dr John A O'Keefe of the Goddard Space Flight Center (National Aeronautics and Space Administration) and Mr Werner K Dahm of the Marshall Space Flight Center (National Aeronautics and Space Administration) for useful discussions The assistance of Mr Verkuel N Eubanks for the 7090 computer programming is gratefully acknowledged

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APPARENT SOLAR CONTROL OF THE EFFECTIVE CAPACITY OF A 110-V. 170 AH LEAD-ACID STORAGE BATTERY IN AN 11-YEAR CYCLE

By D R BARBER

formerly Superintendent, Norman Lockyer Observatory, University of Exeter

IN March 1914 a Tudor storage battery of 60 cells—each of 170 Ah nominal capacity—was installed at the Norman Lockyer Observatory to supply direct current for lighting and instrumental control The cells are housed within a stone-walled building, with asbestos-tiled roof, the windows of which on the south and west walls are glazed with painted glass (Fig 1) Current is fed to the buildings and telescopes via an overhead grid of approximately L-shaped outline, the lengths of the major and minor arms being 84 and 45 yards respectively

During the 40-year period of its operation, both sets of plates were replaced in 1933, and positive plates again in 1952 Apart from these two interruptions and a period of inactivity during the First World War, the battery has been operated continuously during the whole of its life to date under reasonably constant and light loading conditions

Each unit cell has three positive and four negative plates of dimensions 18 × 30 cm Charging is by means of a direct-coupled Diesel generator, and the battery is re-charged whenever the specific gravity of the pilot cell has fallen to 20 points below the fully charged value The above range of acid density represents about one-half of the maximum permissible drop for this type of cell

Since 1921 a continuous log has been kept of the date of each re-charge , and subsequent to 1946 detailed records of duration of charge, average charging current, and generator voltage are available also No detailed records of the loading are kept, the control board not being equipped with integrating meters It can be stated, however, that prior to December 1938, when a small 110-V 250-W refrigerator was installed, the demand on the battery had been exceptionally light , and apart from intermittent extra loading up to a maximum of 5 amp during night observing, the daily requirements are very constant also Thus throughout its long service the battery has been consistently under-loaded, the actual discharge-rate being such as to simulate 'shelf-life' conditions for this type of cell This point is emphasized as being particularly important in relation to the effects now to be described

Casual reference to the engine-room log book (by Mr A J Denner, attendant in charge of the electrical equipment) in 1946 brought to light what appeared to be a periodic fluctuation in the total number of charges given to the battery in successive years The amplitude was surprisingly large, the ratio of extreme maximum to minimum annual totals being as high as 3 1 On a closer inspection of the data it was seen that the period of fluctuation was close to 11 years, the annual total appearing to show a similar trend to the Zurich sunspot number, R_Z, with a lag of 1–2 years

Fig 2*b* depicts the sequence of annual charge numbers over the 40-year period, 1921–60 The upper inset (*a*) shows the corresponding march of

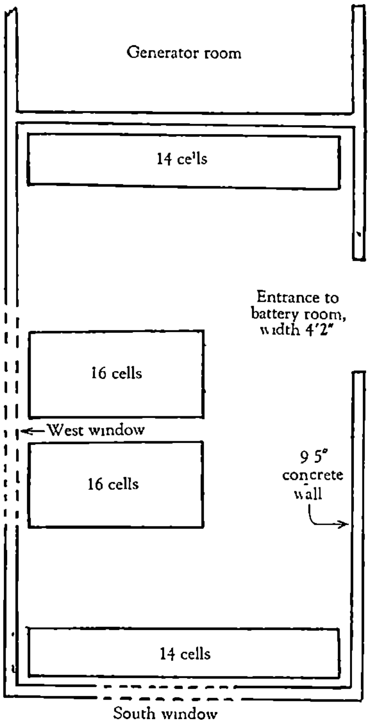

Fig 1 Sketch plan of battery room layout Each window measures 4 ft 2 in by 3 ft and has 18 glass panes 5 in by 10 in covered with white paint At certain times of year some cells near the windows are exposed to diffused sunlight The roof is covered with corrugated asbestos sheet underlaid with 'Ruberoid'

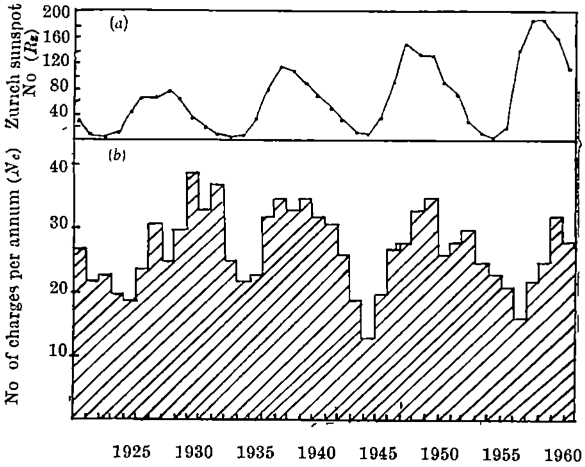

Fig 2 Comparison of curve of annual sunspot numbers (*a*) with secular variation of annual charge number (*b*)

sunspot (R_z) numbers for the same interval, covering approximately three and one-half 11-year cycles It will be noticed that the charge number lags behind sunspot activity by 1 or 2 years Taking the latter into account, the general similarity between the two plots is indeed striking Such an effect appearing most unlikely, it was decided to undertake a rigorous statistical treatment of the material to ascertain whether or not the apparent parallelism of the two curves was really significant

Coefficients of correlation, with their probable errors, were computed for a series of phase differences (relative to sunspot activity) of 0, 1, 1 5, 2 and 3 years The results are give in Table 1

Table 1 ANNUAL CHARGE NUMBER VERSUS ZURICH SUNSPOT NUMBER CORRELATION COEFFICIENTS, AND PROBABLE ERRORS FOR FIVE DIFFERENT TIME LAGS

Time lag (years)	0	1 0	1 5	2 0	3 0
Coefficient *r*	+ 0 25	0 46	0 56	0 46	0 30
P E	±0 10	0 09	0 08	0 08	0 10

It is evident that the best fit of the two parameters occurs for an assumed lag of 1 5 years with a maximum value for the correlation coefficient of + 0 56 ± 0 08 The corresponding values for lags of 0 and 3 years are each about one-half of the maximum

Since the available data cover a sufficiently long period to yield 38 degrees of freedom, the correlation obtained by assuming a $1\frac{1}{2}$ year lag is statistically significant for $P < 0\ 001$, whereas the values of *r* for both 0- and 3-year lags are barely significant at the 0 05 level Thus, it can be said with some confidence that the observed association between the annual trends of frequency of battery charges and sunspot number is highly significant when account is taken of the eighteen-month lag in the battery response Nevertheless it should be stated that there is no implication of a causal relationship between battery response and sunspot activity Indeed, it is highly improbable that any solar relationship to the observed effect will be a direct one A more likely explanation is that the mechanism responsible for the effect is itself dependent on some geophysical phenomenon—for example, some form of ionospheric control the magnitude of which is governed by the prevalent level of sunspot activity

Confirmation of the reality of the mean phase-lag of $1\frac{1}{2}$ years was obtained by first averaging the annual charge totals for years falling at approximately the same phase of each 11-year sunspot cycle The mean values of charge number so obtained were then plotted against corresponding mean values of Zurich number to form a time sequence The resultant plot, shown in Fig 3, reveals a distinct hysteresis loop (broken curve), the charge number for a given value of sunspot number ($R_z = 57\ 5$) being higher during the declining period of spot activity by about 10 charges (Fig 3, *A*, *B*) If now the monthly totals of battery charges from which the annual totals were computed are now re-grouped to allow for the assumed $1\frac{1}{2}$-yr lag, it is found that the dispersion of the observations into two branches disappears, the points now falling on a single curve the equation of which is

$$N_R = N_0\ (1 + a\ R_z + b\ R_z^2)$$

where N_0 is the mean annual charge number for Zurich number, $R_z = 0$, and *a* and *b* are empirical constants A 'least squares' fit of the observed values gives $N_0 = 15\ 7, a = 4\ 0 \times 10^{-1}$, and $b = -2\ 4 \times 10^{-3}$

Since the single curve passes through a flat maximum at $R \simeq 85$, the computed annual charge number in years for which the Zurich number exceeds this value will be ≤ 32 From 1948 onwards successful predictions of N_R from estimates of R_z have been made to within ± 3 charges without exceptions

These results suggest that the ultimate effect of the external controlling factor—whatever it may later prove to be—is to diminish the effective capacity of the electrical storage system during periods of maximum solar activity by an amount equivalent to about 36 Ah, or ~ 30 per cent of the effective capacity of the cells under the conditions of usage stated previously

An additional feature shown in Fig 2*b* is the association of *decreasing* maximum values of charge number in each sunspot cycle with *increasing* maximum sunspot number This is evident when these maximum values are tabulated in sequence as in Table 2

Table 2 SUNSPOT CYCLE*

No	14*b*	15*a*	15*b*	16*a*
Max R_z	77 8	114 4	151 6	(189 5)†
Max N_c	39	35	35	(32)†

* See *Observatory*, 67, 183 (1947) † Provisional values only.

This result again illustrates the diminished effect of the external factor on the battery at levels of sunspot activity in excess of $R_z \simeq 85$, and may indeed aid in discovering the ultimate nature of the mechanism responsible for the effect

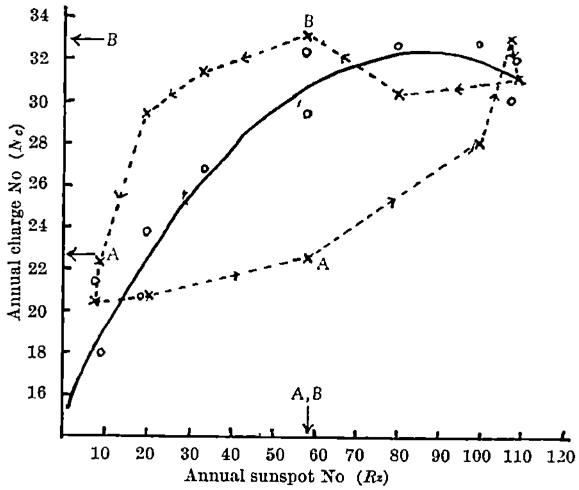

Fig 3 Plots of mean annual charge number versus mean annual sunspot number for three sunspot cycles, 1923–54 ×, uncorrected charge numbers, O, 'least-squares' values after correction for $1\frac{1}{2}$-yr lag of charge number behind sunspot number

It would be of interest to arrive at some quantitative estimate of the actual amount of additional energy imported to the storage battery during years of high solar activity Although no data other than charging frequency exist for the interval 1921–46, it is fortunate that from this latter year onwards detailed records are available for each year up to the present Thus the total annual quantity of electricity generated is known to within ± 0 05 kAh

The situation in regard to the supply side of the system is less satisfactory In the absence of recording meters, no quantities are directly available from which to ascertain annual loads However, the major contribution in each year since 1939 is the load taken intermittently by the 110-V 250-W refrigerator , and tests of this unit have been made which enable its annual load to be computed within close limits To this must be added the load imposed by lighting, light power, and heating circuits that seldom exceeds *in totem* that consumed by the refrigerator alone

Thus it has been possible to assess the supply loading, Q_c, for each year subsequent to 1954 with an uncertainty of ± 0 1 kAh These data, with the corresponding quantities of electricity generated, Q_g, appear in Table 3 In the third row of Table 3 there is entered the nett excess Q_e for each year from 1946 to 1960

Table 3 N L O 110-V 170 AH STORAGE BATTERY OF 60 CELLS
Electrical balance sheet, 1946-60
(Unit = 1 kAh)

Year	1946	1947	1948	1949	1950	1951	1952	1953
Q_g	3 32	3 82	4 36	4 28	3 32	3 70	3 96	3 49
Q_c	2 75	2 95	2 99	3 06	2 49	2 96	2 98	3 01
Q_e	0 57	0 87	1 37	1 22	0 83	0 74	0 98	0 48

Year	1954	1955	1956	1957	1958	1959	1960
Q_g	3 42	3 06	2 48	3 32	3 57	5 63	5 10
Q_c	2 78	2 97	2 29	3 02	2 93	4 69	4 24
Q_e	0 64	0 09	0 19	0 30	0 64	0 94	0 86

This period includes the 1947 maximum and 1954 minimum of sunspot activity , and the years 1952–53 when the positive plates of all cells were renewed, and 'booster charges' afterwards given The latter addition has been allowed for in estimating the tabulated values of Q_g entered in Table 3 It is of further interest to note that the re-platal carried out in 1952, like that of 1933, does not appear to have affected the run of annual charge number over the relevant solar cycle Indeed no trace of any discontinuity appears in either instance

The striking similarity in the trends of excess electricity generated per annum, and annual sunspot number is clearly revealed in the above data The Q_e values first rise to a maximum of 1 37 kAh one year after the 1947 sunspot maximum, afterwards falling, with superposed minor fluctuations, to a minimum value of 0 1 kAh in 1955, one year after sunspot minimum It is uncertain whether the secondary maxima that occur in 1952 and 1954 are evidence of peak values in the external controlling factor, or are internal effects associated with the renewal of the positive plates

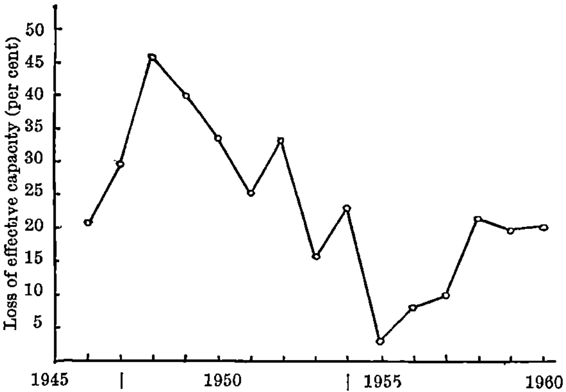

Fig 4 Plot of annual percentage loss of effective cell capacity, 1946–60, exhibiting increased storage efficiency with waning solar activity The period includes the sunspot maximum of 1947 and minimum of 1954

As is clearly shown in Table 3, the fact that in years of high solar activity an appreciable excess of electricity is required at each charge to restore the cells to a fully charged condition implies a loss of effective capacity that appears to be controlled by some unknown and intermediary factor

One useful way of defining this loss is in terms of the ratio (excess electricity supplied/electricity consumed), making use of the relation, percentage loss of effective capacity = 100 (Q_e/Q_c) Annual values of the percentage loss so computed, when plotted versus epoch as in Fig 4, strikingly reveal the changes just described

These detailed results thus confirm the general conclusion derived from the earlier examination of the charge frequency data, namely, that in years of high solar activity the battery loses ~ 30 per cent of its electrical capacity.

The question now arises, does this loss of storage capacity reveal the presence of an internal 'self-discharge' subject to a cyclic variation in step with the 11-yr sunspot cycle ? To attempt an answer, we may first note that in a year of maximum loss of capacity (1948) a total of 1 37 kAh was required to maintain the battery in operation A simple calculation then shows that this corresponds to a continuously flowing leakage current of ~ 0 25 amp or ~ 2 μamp per sq cm plate surface for each cell Considered as an external leakage current, this value is quite unrealistic since careful checks have established that, even under conditions of 100 per cent relative humidity, with heavy and continuous rain falling, the leakage current from the transmission poles to ground never exceeds 1,000 μamp , while in normal conditions (relative humidity <95 per cent) this value falls to $\leqslant 100$ μamp Thus it is obvious that external leakage alone cannot possibly account for the cyclic fluctuation in effective capacity actually observed

Consequently we are forced to look elsewhere for a mechanism capable of providing the observational facts

To this end a large number of experiments have been carried out with small capacity (40 Ah and 4 Ah) lead-acid cells in glass boxes Some of these have included tests with lead, and other forms of direct shielding , and others have been designed to investigate the possible role of incident radio-frequency fluxes on the cells In all instances to date, the results can be regarded as inconclusive evidence The exact nature of the control thus remains unknown but certain of the results may yet prove fruitful in further attempts to elucidate the problem

For example, in some experiments carried out at the suggestion of Dr C E R Bruce (Electrical Research Association) a 2-V cell was first fully charged and then discharged through a constant load of 21 ohms (this value was chosen deliberately so as to give an integrating time of approximately the same length, 2–3 weeks, as that for the large battery) while connected through small capacitors to the Observatory's 110-V positive supply line, and to

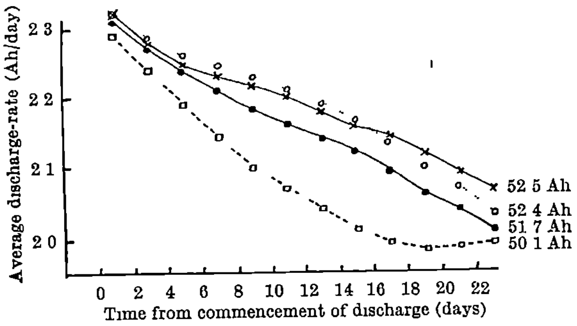

Fig 5 Representative daily discharge curves for 2 V 40 Ah cells under different test conditions ●, normal operation, cell in air, ×, cell shielded with 1-in -thick lead castle, ☐, cell in air, but connected to 110-V line 'aerial', ○, shielded cell connected to 'aerial' (Effective amp -hr capacities under test conditions are entered alongside each curve)

earth If the transmission lines act as a resonant receiving system for very-low-frequency radio-waves, small differences in the effective capacity of the cell might result These would be apparent as differences in the shape of the voltage-time discharge curves when the cell is alternately connected to, and disconnected from, the line 'antenna' Six separate tests gave variable results In three of these a positive result was obtained, the effective capacity decreasing by ~4 per cent when the discharging cell was connected to the 110 V line In two other tests no significant difference was noted, while the sixth test gave a negative result—the capacity was slightly increased when the cell was discharged while connected to the line 'aerial' This sequence of differential tests extended over the period June–September 1960, and it may be argued that the differences shown in the results might well have been due to a seasonal variation of the external controlling factor the constancy of which was assumed throughout each test

A second series of 'aerial' tests, similar to those already described, but with the cell shielded with lead, gave effects of smaller magnitude

In an earlier series of experiments with the same type of cell (1957–59) the test cell was enclosed in a castle of lead having walls 2 5 cm thickness A control cell was operated alongside, in air Several experiments, using the same cell, both screened and unscreened, indicated a gain of a few per cent in the electrical capacity when screened while discharging through a 21-ohm resistor as compared with the discharge curve in air But similar tests of equal weight showed either no significant capacity change, or an actual loss of effective capacity when screened with lead Again, since it was not possible to eliminate the effect of any seasonal variation in the controlling factor, no firm conclusions can be drawn from these experiments They do, however, suggest that in general the effect of radiation screening is to increase the effective capacity of the discharging cell

Tests were also made with very small cells (4 Ah nominal capacity) with, and without, lead screens In this instance it was impracticable to use the same integration time as was used in the other tests, and each experiment was restricted to an overall discharge time of 48 hr No significant differences were found as between screened and unscreened cells This negative result implies that the integration time was in all cases insufficient to allow the effect to build up

Some typical results of the tests made with the 40 Ah cells are depicted graphically in Fig 5 The four curves illustrate the behaviour of the cells under the various conditions cited, and convey an idea of the magnitudes of the effects observed From the results of the foregoing laboratory tests, it would appear that screening from incident low-energy particle radiation may lead, at times, to an increase of the normal cell capacity, while the absorption of very-low-frequency radio radiation may effect the reverse Both effects, however, must obviously be associated with exceedingly small energy exchanges, and it is not easy to visualize any electrochemical reaction capable of producing a sufficiently large change in the electrical capacity of the system to yield an amplification of at least 10^5

However, Piccardi[1] has reported the existence of a world-wide cosmic influence on the rate of precipitation of a suspension of bismuth oxychloride in distilled water He has found also that the cosmic effect is quite similar to that obtained when the precipitation vessel is subjected to a flux of 10 kc /s radiation from a nearby laboratory transmitter

If indeed we are concerned here with a variant of the Piccardi effect, perhaps attributable to radio-noise of terrestrial origin, it is not unreasonable to postulate an indirect solar control of the flux intensity via the lower ionosphere, with an approximately 11-yr periodicity Nevertheless, any approach along the lines just indicated must be regarded as purely speculative in the absence of definite evidence of a specific controlling factor

Summing up the more important features of the observations reported here, four facts appear very pertinent (1) a statistically significant and cyclic variation of the effective capacity of the Observatory's 110-V 170-Ah storage battery with a mean period of 11 yr is evident throughout the forty-year period for which records are available, (2) the fluctuation is seen to follow the 11-yr sunspot cycle with a mean lag of 1½ yr, the effective storage capacity decreasing with rising sunspot number to a minimum at around $R_Z = 85$, thereafter increasing slowly, (3) the loss of electrical capacity, which in 1948 amounted to nearly 50 per cent of the nominal value, would appear to be controlled by an external factor, the exact nature of which is not known A long integration time—at least two to three weeks—coupled with very light loading of the battery appears necessary to reveal the effect, (4) experiments with small capacity (40 Ah) cells, discharged at rates comparable with that of the large storage system, have been made with the cells screened with lead, and coupled to a line 'aerial' system tuned to very-low-frequency radio waves Although the results cannot be regarded as conclusive they point to the possibility that both particle and wave radiations may be involved in the unknown factor controlling the performance of the 110-V battery

Clearly much more experimental work is necessary to elucidate this novel problem But it is felt that publication of the facts already available is justified, for the following reasons

First, a search of the likely literature, coupled with numerous inquiries, has failed to bring to light a prior publication of results similar to those described here, and secondly, it is hoped that publication at this stage may encourage other workers—perhaps more familiar with the complex physico-chemical reactions of the lead-acid cell—to pursue the problem to a satisfying conclusion

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MEASUREMENT OF ELECTRON DENSITIES IN THE IONOSPHERIC D-REGION AT THE TIME OF A 2+ SOLAR FLARE

By J. S. BELROSE and E. CETINER*

Defence Research Telecommunications Establishment, Ottawa

A NUMBER of workers[1,2] have computed ionospheric *D*-region electron densities at the time of sudden ionospheric disturbances. The intensity distribution of the ionizing solar flare radiations causing the sudden ionospheric disturbance is not known precisely, and there are uncertainties in the values of ionospheric ionization and loss coefficients, as well as in the knowledge of the *D*-region constituents. This article reports a measurement of electron densities in the *D*-region made near the peak of a sudden ionospheric disturbance, during a class 2+ flare on March 1, 1962. The sudden cosmic noise absorption effect observed on a 30 Mc./s. polar riometer at Ottawa is shown in Fig. 1. The noise burst associated with the flare partially obscured the absorption, which is, however, clearly evident. From the sudden cosmic noise absorption record, the timing and amount of absorption can be clearly established, but only indirectly can the height where the absorption occurs be inferred. The sudden ionospheric disturbance began at 16 38 U.T. (11 38 E.S.T.), reached a maximum at 16 47 and finished by 17 50.

It is difficult to devise ground-based experiments which give direct evidence about electron densities and collision frequencies in the *D*-region. In this investigation, the Gardner and Pawsey[3] experiment was used. When pulses of frequency greater than about 1 Mc./s. or so are returned vertically from the ionosphere, they are usually reflected from above the *D*-region. If, however, the power is very great, it is possible to detect weak echoes (reflexion coefficients of 10^{-3}–10^{-7}) from heights of 50–100 km. These echoes are usually, but not invariably, accompanied by much stronger echoes from greater heights. Previous workers[3-5], who have observed such reflexions, have experienced difficulties because of insufficient transmitter power, lack of discrimination between magneto-ionic components, and oblique reflexions. At this establishment, an attempt was made to overcome these limitations by using high-transmitter powers and directive antenna systems. Two frequencies are used: at 2.66 Mc./s. four-dipole circularly polarized antennæ are used for transmission and reception, and a transmitter power of 1,000 kW.; at 6.275 Mc./s. a similar antenna system is used for transmission, but for reception a 128-dipole circularly polarized antenna array is used covering 9 acres of ground, and a transmitter power of 100 kW.

The amplitude of radio waves partially reflected from *D*-region ionization irregularities is determined partly by the reflexion coefficient of the scatterers, and partly by absorption in the region below the scatterers. Thus, if R is the amplitude of the extraordinary or ordinary wave reflected from a scatterer at height h, and A is the effective reflexion coefficient of the scatterer, then

$$\frac{R_e}{R_0} = \frac{A_e}{A_0}\exp[-2\int_0^h (K_e - K_0)\mathrm{d}h] \qquad (1)$$

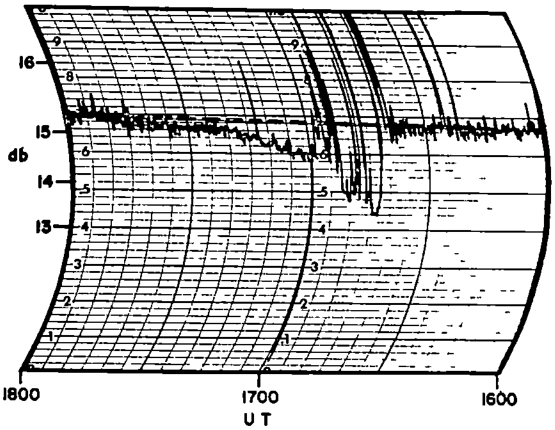

Fig. 1. Record of the sudden ionospheric disturbance absorption (sudden cosmic noise absorption) observed at 30 Mc./s. using a polar-directed antenna

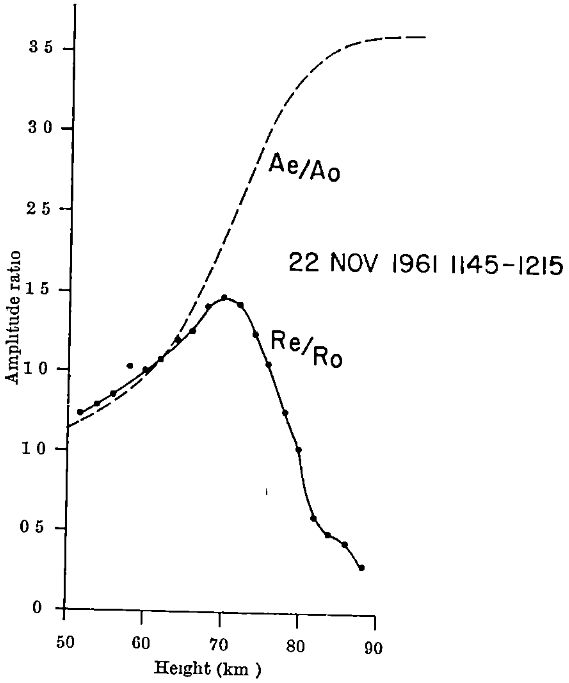

Fig. 2. Function A_e/A_0 and R_e/R_0 with height for 2.66 Mc./s. for a quiet day, November 22, 1961. Average results for 11 45–12 15 E.S.T.

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* On leave from Directorate of Signal Factory, Ankara, Turkey.

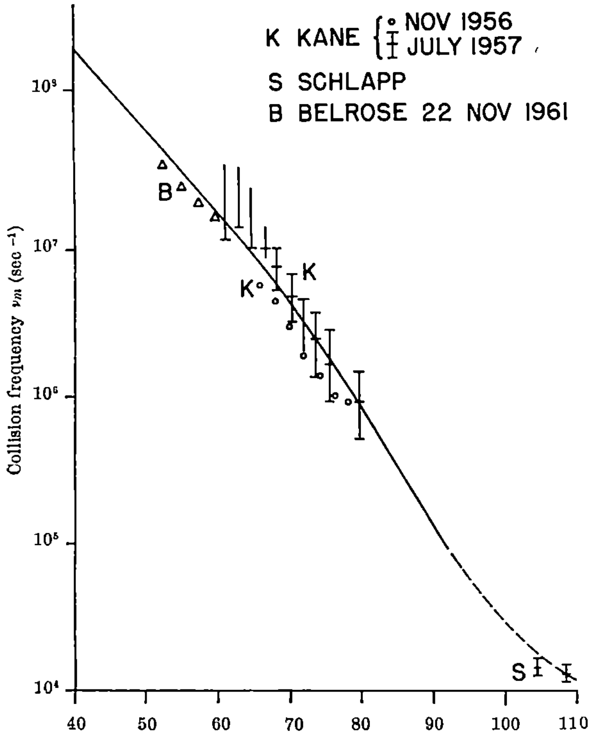

Fig. 3. Collision frequency of electrons ν_m (collision frequency of monoenergetic electrons) plotted against height for the best theoretical and experimental data available

where K is the absorption coefficient integrated up to the scatterer and back. Making certain simplifying assumptions, the ratio A_e/A_0 depends only on the wave frequency ω, the gyro-frequency ω_H, and the electron collision frequency ν_m[6]. Hence the integrated differential absorption can be determined as a function of height, and electron density profiles can be computed, once the variation of ν_m with height has been established. The generalized Appleton–Hartree formula of Sen and Wyller[7] was used, with the aid of C-script tables computed by Burke and Hara[8].

When the integrated differential absorption is small

$$\frac{R_e}{R_0} \simeq \frac{A_e}{A_0} \qquad (2)$$

and from the measured echo amplitude ratios a measure of ν_m can be obtained. From Fig. 2 it appears that at 2.66 Mc/s this approximation is reasonable for a small range of heights near the bottom of the ionosphere. The A_e/A_0 curve of Fig. 2 was calculated from the collisional frequency curve shown in Fig. 3. These values, which are also tabulated in Table 1, have been calculated from a formula given by Phelps[9], based on laboratory measurements, and the COSPAR International Reference Atmosphere 1961 model atmosphere[10]. Also shown in Fig. 3 are values measured by Kane[11] using rocket techniques at Churchill under polar cap absorption conditions, and values measured by Schlapp[12] at Cambridge using the group retardation method. Both the results of Schlapp and Kane have been re-evaluated using the generalized Appleton–Hartree formula.

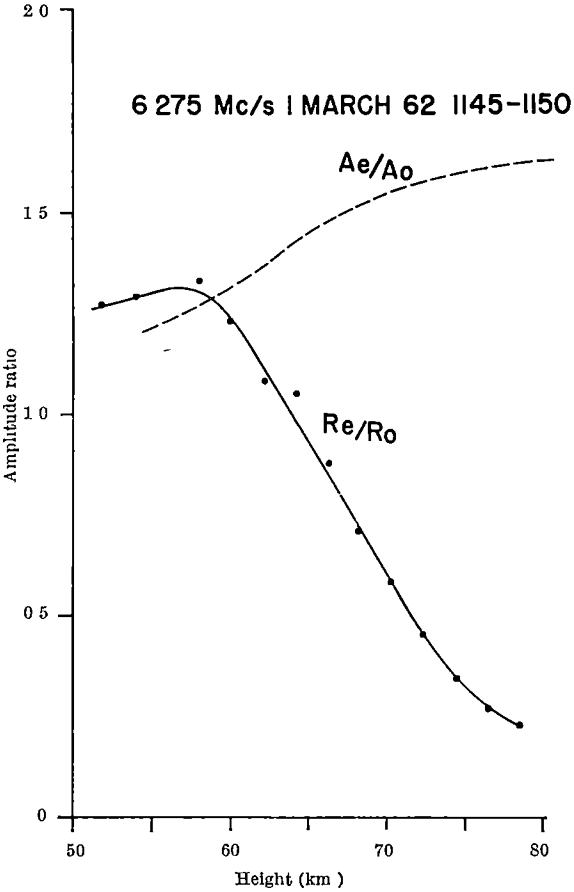

Fig. 4. Function A_e/A_0 and R_e/R_0 with height for 6 275 Mc/s for March 1, 1962 (5-min. average near the peak of a sudden ionospheric disturbance, time shown is E.S.T.)

Table 1

Height (km.)	ν_m (sec $^{-1}$)
30	9.7×10^8
40	2.3×10^8
50	6.3×10^7
60	1.8×10^7
70	4.5×10^6
80	8.4×10^5
90	1.3×10^5

The amplitude ratios obtained during the sudden ionospheric disturbance of March 1, 1962, at 6 275 Mc/s are shown in Fig. 4. Note that differential

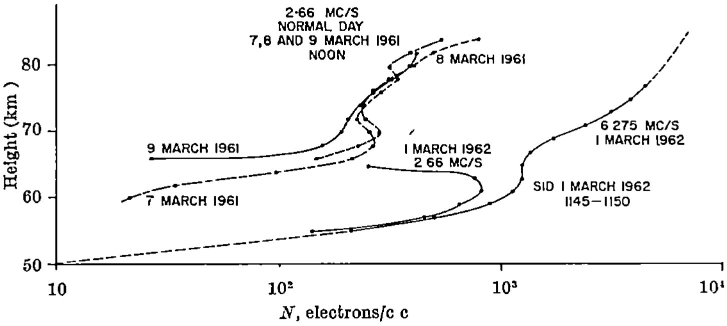

Fig. 5. $N(h)$ curves derived from observations at Ottawa at the frequencies marked. Time shown is E.S.T.

absorption appears at a height of about 57 5 km On quiet days the absorption at this frequency is negligible below about 85 km The values of R_e/R_0 at low heights are in reasonable agreement with the calculated values for A_e/A_0 In any event, the collision frequency at these heights was not greater than usual, since to bring the R_e/R_0 values into closer agreement with the A_e/A_0 values would require lower collision frequencies than shown by the curve of Fig 3 The electron densities deduced from the results of Fig 4 are shown in Fig 5, together with values deduced from 2 66 Mc /s observations for the same sudden ionospheric disturbance and for quiet days in March, 1961 Note that the data at the two frequencies are in reasonable agreement at low heights, 55–60 km , but are not above this height This is attributed to the effect of oblique reflexions at the lower frequency At the higher frequency a much more directive antenna array was used, and, moreover, there is less absorption

The absorptions that a 30 and 60 Mc /s wave would suffer in passing once vertically through the sudden ionospheric disturbance layer(s) obtained from the 6 275 Mc /s observations were computed for comparison with riometer data The calculated absorption profile at 30 Mc /s is shown in Fig 6 The $N(h)$ curve was extrapolated to higher and lower height for this purpose Neglect of these extrapolations reduces the calculated absorption at 30 Mc /s by slightly more than 0 1 db The calculated and measured absorptions are compared in Table 2

Table 2

Frequency (Mc /s)	Calculated absorption (db)	Measured absorption (db)
30	1	1 16
60	0 3	0 3

The phase and amplitude of low-frequency transmissions (80 kc) was also being recorded at Churchill from a frequency stable transmitter at Ottawa The amplitude increased by 5 db (the normal amplitude change from day-to-night amounts to about 9 db at this time of the year), and the phase lag decreased by 0 17 cycle (which is interpreted as being due to a decrease in the height of reflexion by about 2 5 km)

In conclusion, the sudden ionospheric disturbance ionization associated with the class 2^+ flare of March 1, 1962, resulted in an increase in the electron densities in the 60–75 km region by about a factor of ten,

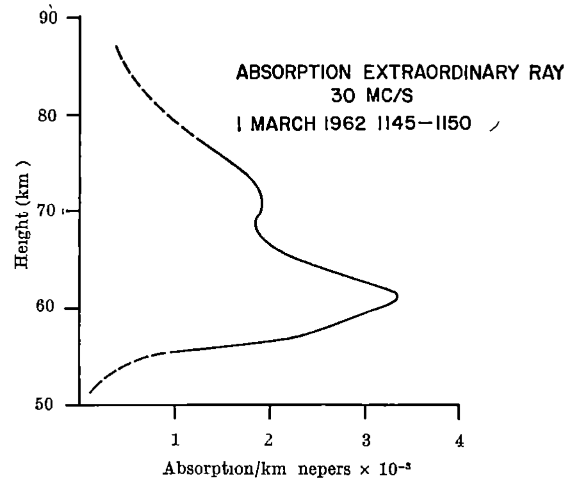

Fig 6 Absorption profile for the extraordinary ray at 30 Mc /s for the sudden ionospheric disturbance curve of Fig 5

and in a lowering of the base of the ionosphere The electron-density gradient at the base of the ionosphere, although not very accurately obtained on most quiet days, was increased over normal by about a factor of two These results are consistent with absorption changes measured by riometers, and phase and amplitude changes observed on low-frequency transmissions The change in the rate of increase of electron density with height near 60 km suggests that more than one ionization process is involved The partial reflexion observations also show that the collision frequencies at low heights during the flare are not greater than normal

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FORMATION OF UNSATURATION AND CROSS-LINKS IN THE RADIOLYSIS OF *n*-ALKANES AND POLYETHYLENE

By Dr B J LYONS*

British Insulated Callender's Cables, Ltd , Research Organization, London, W 12

THERE seems little doubt that most of the organic products (including dimers and polymers) of the radiolysis of *n*-alkanes and cyclo-alkanes are formed by free radical ractions This conclusion follows from measurements of yields of free radical using chemical determination or electron paramagnetic resonance measurements[1] Moreover, Charlesby *et al* [2] have shown that the decay of anthracene in

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* Present address Chemistry Department, Northwestern University, Evanston, Illinois

cyclo-hexane solution shows a dependence on the square root of the ionizing radiation intensity, indicating that the radical combination reaction follows bimolecular kinetics Dewhurst, however, while postulating a free mechanism for the radiolysis of the *n*-alkanes, suggests that the dimerization may actually occur as an ion-molecule reaction[3].

In accounting for the cross-linking of polymers by a free radical mechanism, allowance must be made for the observation that the radical decay-rates in these systems follow a first-order law This has been confirmed by electron paramagnetic resonance[4-6], and hydrogen exchange[7,8] studies on polyethylene and by analysis of the decay-rates of anthracene in polydimethyl siloxane[9] To explain this first-order dependence it has been suggested that radicals in polymers react with the neutral environment[9] or that they are formed in pairs, the pairs being distributed at random[4,5,7]

Pearson[10-12] has proposed a theory of cross-linking of polyethylene based solely on the reaction of unsaturated groups with radicals Dole and co-workers[13-15] have proposed an alternative mechanism based on activation of unsaturated groups through absorption of energy of excitation followed by reaction with the neutral environment to give end-linking or cross-linking Crook and Lyons[16] have suggested that unsaturated groups in polyethylene react with radicals (produced by C—H scission) through a chain mechanism (usually of very short length)

The observation of Snow and Moyer[17], that radiation-induced unsaturation in a paraffin wax (largely C_{26}–C_{27} and initially containing only small amounts of unsaturation) is more likely to be found in the cross-linked fraction, led them to postulate that, though unsaturation may be involved in cross-linking, it does not decay in the process They suggest

$$\text{—CH}_2\text{-CH}_2\text{—CH}_2\text{-CH}_2\text{—}\rightsquigarrow \text{—CH}_2\text{—CH} = \text{CH—CH}_2\text{—}$$

$$\text{—CH}_2\text{—CH} = \text{CH—CH}_2\text{—} + R \longrightarrow \text{—CH}_2\text{—CH} = \text{CH—}\dot{\text{C}}\text{H—}$$

$$\text{—CH}_2\text{—CH} = \text{CH—}\dot{\text{C}}\text{H—} + R' \longrightarrow \text{cross-linking}$$

It is the purpose of this article to show that the results of Snow and Moyer can be reconciled with the hypothesis that unsaturation decays by cross-linking if unsaturation formation and C—H scission are conceived, not as separate radiation events but as successive stages in the same radiation event

Williams[18], in suggesting that *trans*-vinylene unsaturation is formed by loss of molecular hydrogen from an alkane ion to give an olefine ion, points out that such a reaction is slightly exothermic Collinson, Dainton and Walker[19] have pointed out that a positive charge formed in the radiolysis of *n*-hexadecene-1 will probably rapidly migrate along the chain to the vinyl double bond, association with which would represent for it a more stable energy state, they suggest that such migration is followed by an ionic polymerization, and not by neutralization and radical formation It is probable that the positive charge would likewise remain associated with the trans-vinylene group in an olefine ion once it is formed from an alkyl ion A subsequent hydrogen abstraction reaction, similar to that suggested by the above workers, would lead to a loss of the unsaturated group and not its retention in the cross-linked molecules However, neutralization of such an olefine ion would release sufficient energy of excitation (approximately 10 eV) to break at least two C—H bonds and if, as seems likely, hydrogen abstraction reactions follow, thus form at least four radicals in close proximity to the unsaturated group In all probability two of these radical sites would be initially on the olefine molecule itself In a low molecular weight hydrocarbon, molecular diffusion would result in the radical decay following bimolecular kinetics, and in solid systems the absence of such diffusion would result in apparent unimolecular kinetics While intermolecular combination results in cross-linking, intramolecular migration and combination would result in the formation of additional *trans*-vinylene groups A slight post-irradiation increase in *trans*-vinylene unsaturation in a high density polyethylene has been noted by Fydelor and Pearson[12]

The foregoing mechanism would indicate that provided the unsaturation is fairly unreactive towards radical attack a higher proportion of radiation-induced unsaturation would be found in the cross-linked fraction of both low and high molecular weight hydrocarbons

The increased probability of finding positive charge, and hence of forming radicals, in the vicinity of *trans*-vinylene groups does explain the favoured nature of the *trans*-vinylene decay reaction, if this occurs through reaction with radicals A similar process may explain the marked dependence of chain fracture and cross-linking rates in polypropylene on the concentration of radiation-induced vinylidine unsaturation[20]

It is not suggested that all radicals formed by C—H scission in hydrocarbons result from neutralization of olefine ions, though the extrapolated lower limit of concentration of unsaturation in the polymer fraction of the paraffin wax studied by Snow and Moyer suggests that a high proportion of the radicals formed may arise through this process

I thank Dr R M Black for his advice and Dr L G Brazier, director of research, British Insulated Callender's Cables, Ltd , for permission to publish this article

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LETTERS TO THE EDITOR

RADIOPHYSICS

A Correlation between the Spectra of Non-thermal Radio Sources and their Brightness Temperatures

RECENT observations at nine frequencies between 38 and 3,200 Mc/s have been compiled and used to determine the spectra of 160 discrete radio sources[1] Although, in general, the spectra can be represented by a simple power law, at least for frequencies below 1,420 Mc/s, in a few cases the spectral index shows a considerable dependence on frequency

Fig 1 shows the spectra of the eleven sources which show a marked deviation from a simple power law spectrum Of these, Cygnus *A* has long been known to have a spectrum of this type[2,3]

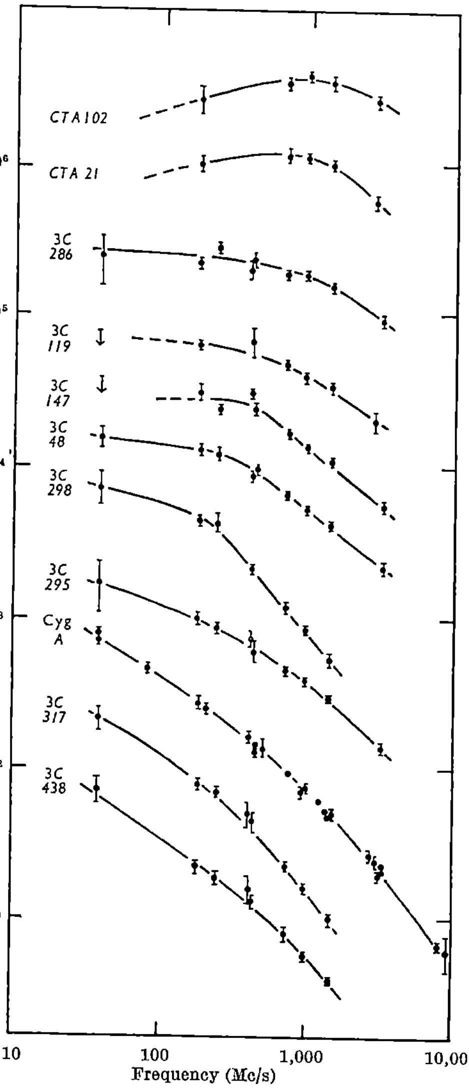

Fig 1 Spectra which have been found to show considerable curvature The sources *CTA* 21 and *CTA* 102 are taken from the list of Harris and Roberts (ref 4), while the remainder are taken from the third Cambridge catalogue (ref 5) (3*C*) The points shown for Cygnus *A* are taken from a large number of observers (ref 1)

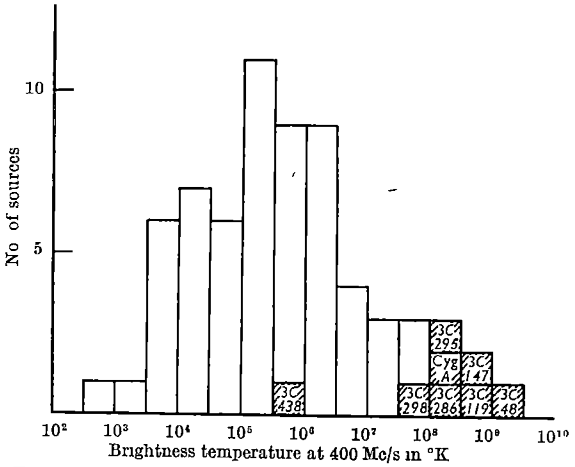

Fig 2 Histogram of the distribution of brightness temperatures Sources shown shaded have markedly curved spectra

Apart from the radio spectrum, the surface brightness is the only other intrinsic property that (apart from possible red shift corrections) can be measured without a knowledge of the distance to the source It is therefore interesting to compare these two quantities

A histogram of the distribution of apparent brightness temperatures for 66 sources is shown in Fig 2 These have been estimated from recent interferometric measurements of angular size[6-9], and include only those sources for which adequate measurements of spectra and angular size are available

On this histogram the sources with distinctly curved spectra are shown shaded (3*C* 317, *CTA* 21, and *CTA* 102, which are of this type, are not included in Fig 2 because of the lack of long spacing interferometer measurements) It is evident that there is a correlation between distinctly curved spectra and very high brightness temperatures This result is somewhat surprising in view of the fact that two of the sources with curved spectra, Cygnus *A* and 3*C* 295 (ref 10) have been identified with distant galaxies, while 3*C* 48 (ref 11) seems to be associated with an object of stellar appearance It would appear, therefore, that quite similar conditions may exist in radio sources of vastly different luminosity and linear size

It is generally accepted that radiation from non-thermal radio sources is due to synchrotron radiation The observed spectrum is then related to the energy distribution among the relativistic electrons and the mechanisms by which their energy is dissipated The observed spectrum can also be affected by a red shift in the radiation from a very distant source or by absorption along the line of sight to the source

If we assume, however, that the effects of red shift and absorption are not of primary importance, the correlation of curvature and high brightness temperature suggests that evolution within radio sources can account for the observed variety of spectra The intensity of the synchrotron radiation depends both on the energy of the relativistic electrons and on the strength of the magnetic field Thus, under conditions of minimum total energy, the existence of high surface brightness implies the presence of a strong magnetic field[12] It therefore follows that the radio spectrum of a source will become steeper with time at higher frequencies as a result of the rapid loss of energy from high-energy electrons by synchrotron emission[13] A source, therefore, with an initially flat spectrum will soon show a spectrum that is markedly curved, and will finally have a spectrum of the type observed for the majority of sources

A full discussion of such an evolutionary sequence would have to consider changes in the magnetic field and in the rate of production of relativistic electrons, and other mechanisms of energy-loss such as ionization and bremsstrahlung[14] and the physical expansion of the source

This work forms part of a joint investigation of the spectra of radio sources by the California Institute of Technology Radio Observatory, the Mullard Radio Astronomy Observatory, and the Nuffield Radio Astronomy Laboratories We thank Dr R G Conway for helpful discussions

K I KELLERMANN
California Institute of
Technology Radio Observatory,
Pasadena, California

R J LONG
Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
Cambridge

L R ALLEN
M MORAN
Nuffield Radio Astronomy Laboratories,
Jodrell Bank

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PHYSICS

Spectra of Amine-Chloranil Complexes

SMITH[1] has shown that the spectra of some methyl substituted aniline-chloranil charge-transfer complexes contain two absorption bands not exhibited by the individual components of the complexes One of the absorption bands is an intense broad band with maximum absorption in the region of 600–800 mμ This band is thought to be the charge-transfer band of the complex The other band is an intense band with a well-defined maximum in the region of 320–380 mμ McGlynn[2] has interpreted this band as being a charge-transfer band of the complex also and has suggested that methyl-substituted aromatic molecules have two similar ionization potentials which give rise to two charge-transfer bands Evans[3] has shown that the spectrum of oxygen-perturbed dimethylaniline has a shoulder with maximum absorption at 380 mμ. He has interpreted this shoulder as being due to the superposition of two charge-transfer bands corresponding to the two ionization potentials of the methyl-substituted aromatic molecule suggested by McGlynn

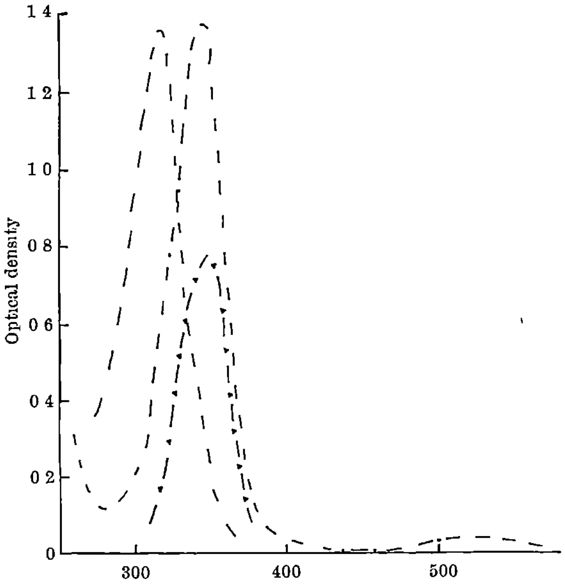

Fig 1 Absorption spectra of chloranil in *a*, triethylamine (— — —), *b*, 50 per cent aqueous diethylamine (– ▲ —), *c*, (5 *M*) ethylamine in 50 per cent aqueous ethanol (—●—)

I[4] have shown that the spectrum of oxygen-perturbed tryptophan has a shoulder with maximum absorption at 380 mμ This shoulder has been inter-

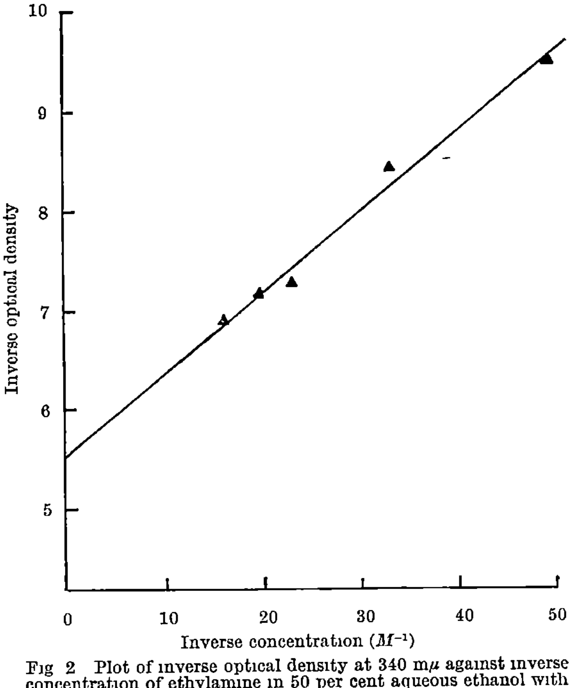

Fig 2 Plot of inverse optical density at 340 mμ against inverse concentration of ethylamine in 50 per cent aqueous ethanol with constant concentration (2×10^{-5} *M*) of chloranil

preted as being due to the ground-state to first triplet state transition of tryptophan

The spectra of some aliphatic amine-chloranil mixtures have been investigated These are shown in Fig 1 It is seen that the ultra-violet absorption band of chloranil in aliphatic amines is shifted to longer wave-length as compared with its position in inert solvents such as n-heptane and ethanol, etc [5], where the peak of the chloranil absorption band occurs at about 285 mμ No strong absorption bands are observed in the visible region such as occur in the aromatic amine-chloranil complexes[1] The faint band observed at 540 mμ is similar to that displayed by old solutions of chloranil in ethanol and is probably due to a small amount of chloranilic acid formed from chloranil in the presence of ethanol

A study has been made of chloranil–ethylamine mixtures in 50 per cent aqueous ethanol The optical absorption of the chloranil-ethylamine mixtures was found to be reversible and to obey the Benesi–Hildebrand equation for 1 1 complexes[6] A plot of the inverse optical density at 340 mμ against the inverse concentration of ethylamine with a constant concentration of chloranil $(2 \times 10^{-5} M)$ is shown in Fig 2 The equilibrium constant of the complex derived from this plot is 7 5 l /mole at 20° C

The position of the peak in the chloranil-ethylamine spectra is quite independent of concentration The aliphatic amines do appear to form 1 1 charge-transfer complexes with chloranil These charge-transfer complexes must be of the n–π type, charge-transfer occurring from the lone-pair electrons of the nitrogen in the amino-group

It appears that the intense absorption band, in the region of 320–380 mμ, observed in the spectra of the methyl substituted aromatic amine-chloranil complexes is due to the amino part of the molecule and not to the aromatic system as suggested by McGlynn[2] Tsubomura[7], on considering the structure of dimethylaniline, has suggested that dimethylaniline acts as an n-donor rather than a π-donor and should therefore behave more like an aliphatic amine than a methylated benzene in forming charge-transfer complexes One feature of n–π complexing is the shifting of the ultra-violet absorption bands of the acceptor molecule on complexing[8] The intense band in the region of 315–380 mμ in the spectra of the amine-chloranil complexes does not resemble the charge-transfer bands normally observed with chloranil complexes[1] but is very similar to the original chloranil band Thus in ethanol, chloranil has a maximum absorption at 290 mμ (34,500 cm^{-1}) and a minimum at 245 mμ (40,800 cm^{-1}) The absorption in the ethylamine-chloranil complex is a maximum at 350 mμ (28,600 cm^{-1}) and a minimum at 285 mμ (35,200 cm^{-1}) The differences between the two maxima and the two minima are 5,600 cm^{-1} and 5,900 cm^{-1} respectively It can be concluded that the intense band in the region of 315 mμ–380 mμ observed in the spectra of amine-chloranil complexes is the absorption band of chloranil shifted to longer wave-length on complexing and is not a charge-transfer band

Evans's explanation of the oxygen-dimethylaniline spectrum thus appears to be incorrect The shoulder observed at 380 mμ (3 26 eV) in the dimethylaniline-oxygen spectrum[3] could well be the first singlet–triplet transition of the dimethylaniline The first singlet state of dimethylaniline is at 4 25 eV [9] This gives a singlet–triplet energy separation of 1 eV This is comparable with the singlet–triplet energy separations of the polycyclic aromatic hydrocarbons[10]

I acknowledge the tenure of a Department of Scientific and Industrial Research fellowship

M A SLIFKIN

Physical Laboratories,
University of Manchester

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Effects of Nuclear Radiation on Electrical Conductivity of Chromic Oxide

EXPERIMENTS performed in the past few years on nuclear radiation effects on oxidic semiconductors[1-5] have shown that their electrical conductivity is strongly influenced by irradiation The results so far reported refer mainly to oxidic semiconductors with relatively large initial charge carrier concentration In order to obtain further information about radiation effects on oxidic semiconductors with small initial charge carrier concentration, we chose for our experiments pure chromic oxide (Cr_2O_3), known as a p-type semiconductor with small hole concentration at ordinary temperatures

For measurements, compacts were prepared by compressing Cr_2O_3 powder (A R grade, 'Chinoin'), at 2,000 kgm /cm^2, into cylinders (8 mm diameter, 8 mm height) and sintering for 10 hr in air at 1,150° C Irradiations of the samples (inserted in quartz ampoules) were performed in an experimental hole of the 2 *MW* enriched uranium reactor at this Institute

The following electrical measurements were made

(*a*) Temperature-dependence of the electrical conductivity of the samples in the range 350°–950° K , before and after exposure in the reactor to an integrated fast neutron flux of $\sim 4 \times 10^{17}$ neutrons/cm^2

(b) Time-dependence of electrical conductivity of the samples during in-pile exposure up to an integrated fast neutron flux of $\sim 2 \times 10^{17}$ neutrons/cm^2

In Fig 1 log σ versus $10^3/T$ is plotted for a sample of chromic oxide before (curve 1) and after (curve 2) exposure in the reactor to a fast neutron dose of $\sim 4 \times 10^{17}$ neutrons/cm^2 It is seen that the conductivity is much smaller after irradiation, but up to 700° K (below which the temperature-dependence of σ is reversible) the activation energy is very little affected by irradiation The fact that exposure in the reactor does not change the activation energy, while the conductivity drops drastically, shows that irradiation produces mainly hole-traps which lead to a decrease in the hold concentration but does not affect its temperature dependence (in the range considered)

By heating the irradiated sample to higher temperatures (above 700° K) its conductivity tends more and more toward the values it had before irradiation (Fig 1) and, at about 800° K , curve 2 overlaps with curve 1 When the sample is now cooled, the value and the temperature-dependence of its conductivity are the same as before exposure to radiation It may be concluded that at temperatures above 700° K

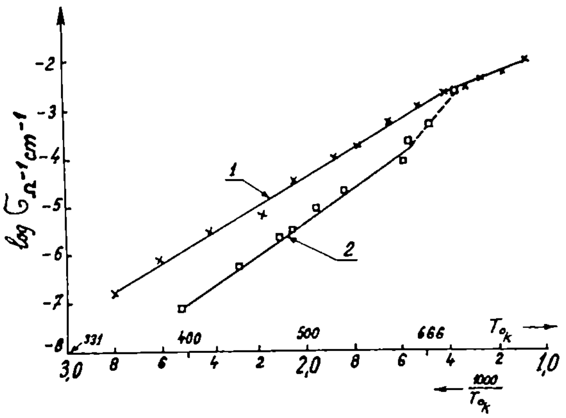

Fig 1 Temperature-dependence of electrical conductivity of chromic oxide before (curve 1) and after (curve 2) irradiation in an integrated fast neutron flux of ~ 4 × 10^{17} neutrons/cm^2

a fast annealing of the defects occurs and the electrical conductivity of the sample returns to the values before irradiation

To determine how the conductivity of chromic oxide varies with the fast neutron dose, the time and consequently the irradiation dose-dependence of the electrical conductivity of a chromic oxide sample was recorded during exposure in the pile (Fig 2) The temperature of the sample being irradiated was always kept at 343° K, so that all conductivity values (including those in absence of the radiation field) were given for this temperature

From Fig 2 it is seen that introducing the sample in the reactor is accompanied by a quick rise in conductivity, however, when the chain-reaction is suddenly stopped, the intensity of ionizing radiation decreases very rapidly and the conductivity decreases as well This shows that the initial rise in conductivity is due to ionization caused by reactor radiations After this initial rise the conductivity soon reaches a maximum, and a subsequent decrease, due to lattice displacements under fast neutron irradiation, occurs

The initial portion of the curve is a straight line, but at higher doses there seems to be a saturation effect As we have seen, these defects are permanent at temperatures below 700° K, so that after irradiation the conductivity of the sample will have a much lower value than before

The following conclusions may be drawn from these results

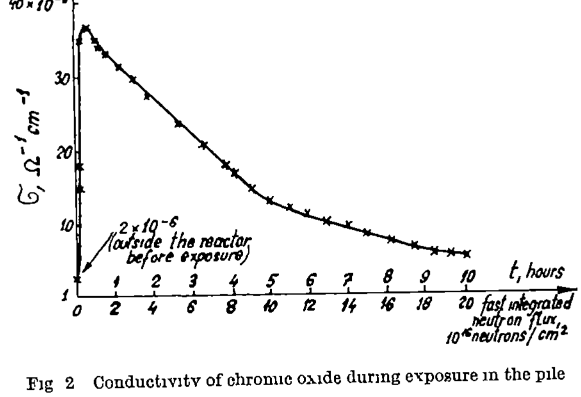

Fig 2 Conductivity of chromic oxide during exposure in the pile

(a) Nuclear radiations cause two kinds of effects in chromic oxide which manifest themselves in changes in its electrical conductivity (1) Ionization effects, which lead to a rise in the conductivity due to the generation of charge carriers in excess, these effects are not permanent, they disappear after cessation of radiation (2) Displacement effects which lead to a decrease of the conductivity due to formation of trapping centres in the chromic oxide lattice, these effects are permanent at room temperature but can be annealed at higher temperatures (b) Below annealing temperature, the temperature-dependence of the electrical conductivity of chromic oxide is not affected by irradiation

M NACHMAN
L COJOCARU

Institute of Atomic Physics,
Bucharest

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CHEMICAL ENGINEERING

Anomalous Excitation Processes in Flames and Detonations

SPECTROSCOPIC study of flames, particularly those of hydrocarbons, has revealed abnormally strong emission from their reaction zones[1] The radicals produced during the combustion process itself such as OH, CH and C_2 all show the effect and so do the non-resonance lines of added impurities such as iron and lead Excitation energies up to 173 kcal /mol have been observed but the processes responsible have remained obscure

In order to examine the combustion process in more detail I have used a brightness and emissivity method[2] to measure the electronic temperature in detonation waves Equimolar oxy-acetylene, sometimes with a trace of cyanogen, and oxy-cyanogen have been used as fuels These basic mixtures were further diluted with hydrogen, carbon monoxide, argon, or nitrogen Both the C_2 and CN radicals have been investigated

To simplify the analyses, the detonation waves were all initiated by a shock wave from a bursting diaphragm and overdriven The initial pressures were in general below 1 cm mercury and the diaphragm was burst with about 8 atm of hydrogen The detonations were carried out in a 'Pyrex' tube of 2 in diameter and 5 ft long With a sufficiently long driver section the pressure behind the detonation front will be constant

The temperature-histories obtained all showed a high value at the front which relaxed to a constant value a little behind the front A typical record is shown in Fig 1 Only in detonations through oxy-cyanogen initially at pressures greater than 1 cm mercury was this temperature spike not observed

The value obtained for the temperature in the plateau agreed very well with that calculated allowing for chemical and thermodynamic equilibrium

When hydrogen was present it appeared in the equilibrium gases partly dissociated together with carbon monoxide and/or nitrogen and argon as the

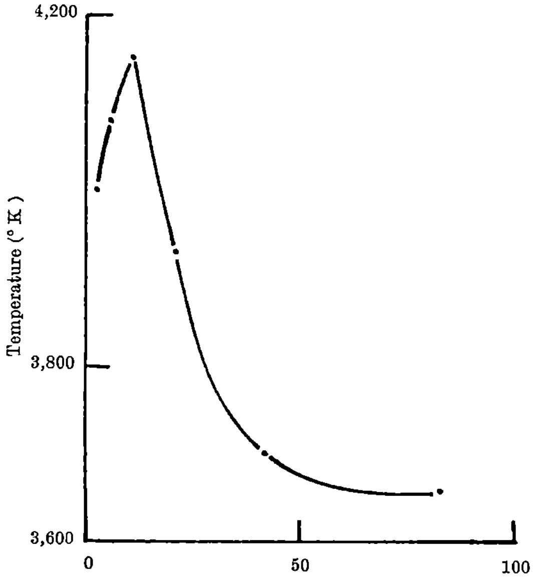

Fig. 1. Temperature measured in CN violet (0,0) band in a $C_2N_2 + O_2 + H_2$ detonation. Initial pressure 9 mm mercury, final pressure 540 mm mercury. Driver pressure 8.2 atm hydrogen. Shock speed, 2.58 mm/μs.

case might be. It was at first considered that the falling temperature was caused by the dissociation of the hydrogen, but the presence of the spike in cyanogen–air and oxy-cyanogen plus carbon monoxide showed that it was not necessarily associated with the hydrogen.

The duration of the spike was measured from the records and used to derive (1) the vibrational relaxation time of carbon monoxide as a function of the equilibrium temperature, (2) the rate constants for the dissociation reactions $M + H_2 \rightarrow M + 2H$ where M can be H_2, N_2, CO, H or A.

The relaxation times of the spike were found to correlate very well with the carbon monoxide times given by Windsor, Davidson and Taylor[3] but gave scattered rate constants for hydrogen rather lower than those of Rink[4].

These results clearly indicate that vibrationally excited carbon monoxide is among the products of the combustion of both acetylene and cyanogen. There are a number of possible reactions capable of producing this activated carbon monoxide.

The vibrational relaxation time of carbon monoxide is very long, and so it is quite likely that it can act as a carrier of excess energy. It is becoming clear that vibrational-electronic energy exchanges are important routes to the production of excited species in hot gases[5] and so the vibrationally excited carbon monoxide may be responsible for the anomalous effects observed in the combustion of hydrocarbons.

A. R. FAIRBAIRN

Department of Chemical Engineering,
Imperial College of Science and Technology,
London, S.W.7

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CHEMISTRY

Chromatographic Identification of Complex Mixtures of High-Boiling Compounds

GAS-LIQUID chromatography[1] is a commonly accepted method for the identification of substances in their mixtures. For these purposes a comparison of retention data on non-polar and selected-polar stationary phases is used with advantage.

By applying this method on a mixture of substances we have to admit that in the chromatogram transference and overlapping of components occur. Without chemical simplification of the mixture, the chromatogram is difficult to read. Further, by applying high temperatures for identification of the high-boiling compounds the choice of polar stationary phases, as well as their selective effect, decrease considerably.

In the case of complex mixtures such as coal tar fractions or substances of natural origin we mostly encounter overlapping of gas-liquid chromatography peaks, which makes identification sometimes impossible. In connexion with these facts, the amount of information about the composition[2] of the mentioned materials decreases with their increasing boiling points. This disadvantage has been overcome by a new method in which the high-separating performance of gas-liquid chromatography is connected with the high polarity of solid adsorbents, when thin-layer chromatography is used. The scheme can be demonstrated by the following example.

About 10 mgm. of anthracene oil from coal tar is separated in a high-temperature chromatograph with katharometer on the non-polar stationary phase ('Apiezon *L*') at a temperature of 250°–270° C. The anthracene–phenanthrene peak to be examined is trapped in a capillary ice-cooled U-tube. The trapped mixture is dissolved in 1 or 2 drops of warm benzene and the solution applied in the usual way[3] on a plate coated with free silica gel (grain size 0.03–0.08 mm., thickness 0.5 mm.). The chromatogram is developed by means of *n*-hexane/benzene mixture (1:1 vol.) and after evaporation of the solvent the plate is inspected under ultra-violet light. According to the situation of the luminescent spots the areas on the plate containing the separated substances are marked. Silica gel from the marked areas is transferred into the capillary tube with a small glass wool tampon using an exhausted air stream. From the column of adsorbent detained in the capillary tube the substance is displaced by several 0.1 ml. methanol or pyridine. The substance might be contained in the first 2–3 drops of solvent which break through the column. A part of this solution is generally used directly for the injection into the chromatograph with an ionization detector. It serves for quantitative analysis or for the further examination on the same or different stationary phase. In this way, without any other preparation, it is possible to separate and detect components up to 0.1 per cent weight in the original mixture. In the peak of anthracene and phenanthrene examined the impurities of carbazole and acridine were easily estimated. Retention ratios are shown in Table 1.

I would like to point out that the adsorbent polarity is higher than any of the polar stationary phases used in gas-liquid chromatograhy[4]. Therefore the separation runs in groups according to the type of the function groups. This means that in, or near, the zone of the first member of the series even

Table 1. Relative Retention Characteristics

Compound	Gas-liquid chromatography 'Apiezon L', 260° C.	Thin-layer chromatography, silica gel/n-hexane/benzene, 1 : 1 v. (R_F)	Type of function group
Anthracene	1·00	0·88	hydrocarbon
Carbazole	1·02	0·55	—NH—
Acridine	1·01	0·06	—N=
6-Methyl quinoline	0·15	0·07	—N=
5-Methyl indole	0·23	0·52	—NH—
Fluorene	0·49	0·93	hydrocarbon
1-Methyl anthracene	1·50	0·89	hydrocarbon
1-Methyl acridine	1·52	0·06	—N=
Benzcarbazole	>10	0·58	—NH—

its homologues can be found. This method can be successfully used for separation and identification not only of alkyl homologues of anthracene, phenanthrene, carbazole or acridine, but also other types of structurally similar substances as, for example, benzcarbazole and other condensed heterocyclic compounds.

This method has proved to be commonly applicable for identification of mixtures of high-boiling substances. The method can be further developed by using other adsorbents, which have not been known in thin-layer chromatography up to now (see ref. 3), for example, Linde molecular sieves, substituted montmorillonites ('Bentone 34 and 38', by Abbey Chemicals, Ltd., London), modified silica gels, etc. Interesting results can be achieved by applying development solvents, which cannot enter the pores of the molecular sieves.

Jaroslav Janák

Laboratory for Gas Analysis
Czechoslovak Academy of Science,
Brno.

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Acrylamide Gel as an Electrophoresis Medium

The use of acrylamide gel as a supporting medium for electrophoretic separation was forecast by Ornstein[1] and by Raymond and Weintraub[2], and was rendered feasible by the work of Dr. S. Raymond[3] of the William Pepper Laboratory, University of Pennsylvania, with Wang[4], Nakamichi[5], and Nakamichi, Brodsky and Bryant[6]. At present, the procedure is as follows. 'Cyanogum 41$^{(R)}$' monomer mixture, 5 per cent dissolved in *tris*-ethylenediamine tetracetic acid-borate buffer[4] is mixed with 0·2 per cent catalyst as described by Raymond and Wang[4] and poured into a special electrophoresis cell (Fig. 1) which was devised by Raymond and is to be described in detail elsewhere[3]. However, the *E–C* Apparatus Corporation of Swarthmore, Pennsylvania, has recently marketed the apparatus and materials for the application of this technique, which this corporation calls 'vertical gel electrophoresis'.

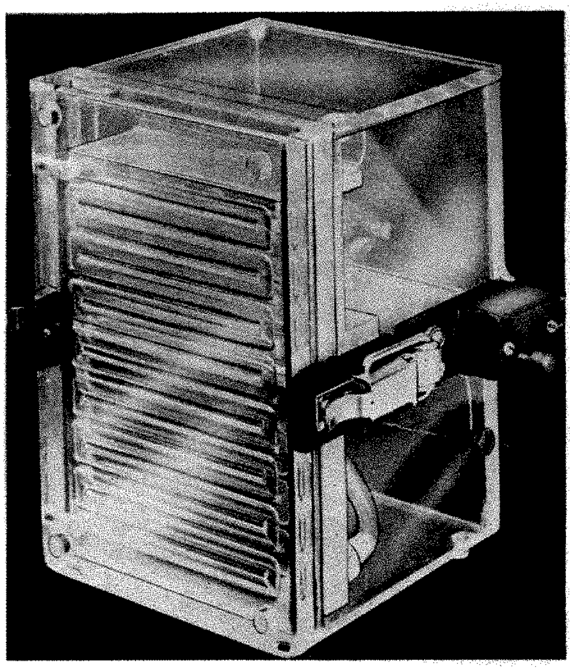

Fig. 1. Vertical gel electrophoresis cell, showing upper and lower electrode chambers; cooling water channels embedded in the walls of the gel compartment, electrical connector, and clamps holding the two parts of the cell together

After polymerization of the gel is complete the apparatus is placed in a vertical position and both electrode chambers are filled with a buffer solution. The sample in appropriate buffer solution (unbuffered serum can be used directly) is layered into premoulded slots in the top edge of the gel and subjected to electrophoresis. For usual types of protein at alkaline pH the lower electrode is the anode and the upper one is the cathode. A current of 100–150 m.amp. is applied (300–400 V.) for 2 hr. to obtain migration of albumin 10 cm. downward through the gel. During electrophoresis, cooling water is circulated through the plates adjacent to the gel slab for temperature control. After electrophoresis the gel is stained 30 min. with amido black and destained in 10 per cent acetic acid solution.

Because of absence of electrosmosis in acrylamide gel medium, all serum proteins, including γ-globulin,

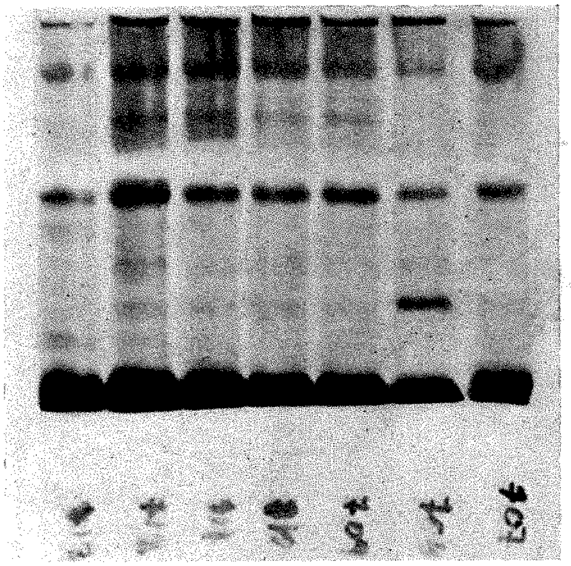

Fig. 2. Serum protein electrophoretic patterns of a series of seven random samples after simultaneous electrophoretic resolution in the vertical gel apparatus of Fig. 1; gel concentration 5 per cent, buffer *tris*-EDTA pH 9·2, field strength 15 V./m., duration 2 hr., stained with amido black

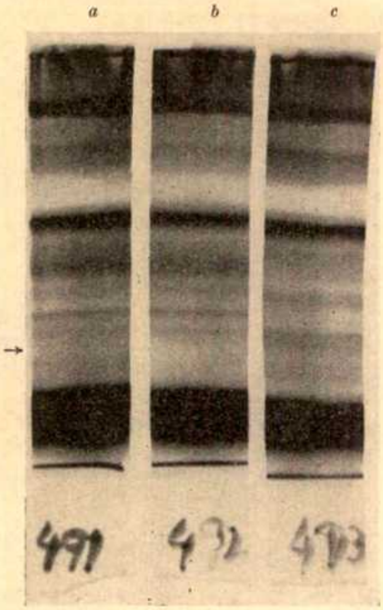

Fig. 3. Serum protein patterns on 5 per cent gel; 491 and 493 are native sera; 492 was a portion of 491 heated at 56° for 30 min. before electrophoresis. A component just behind the broad albumin band in *a* and *c* shows more clearly on the original gel than in the photograph

move from the point of application toward the anode. During the initial period of the electrophoresis, the proteins in the sample migrate downward through the free solution to form a thin, concentrated layer at the surface of the gel, which then enters the gel as a thin, sharply defined zone. The resulting patterns, Fig. 2, show much improved resolution of serum proteins as compared with usual techniques. Hæmoglobins are also well resolved in this technique.

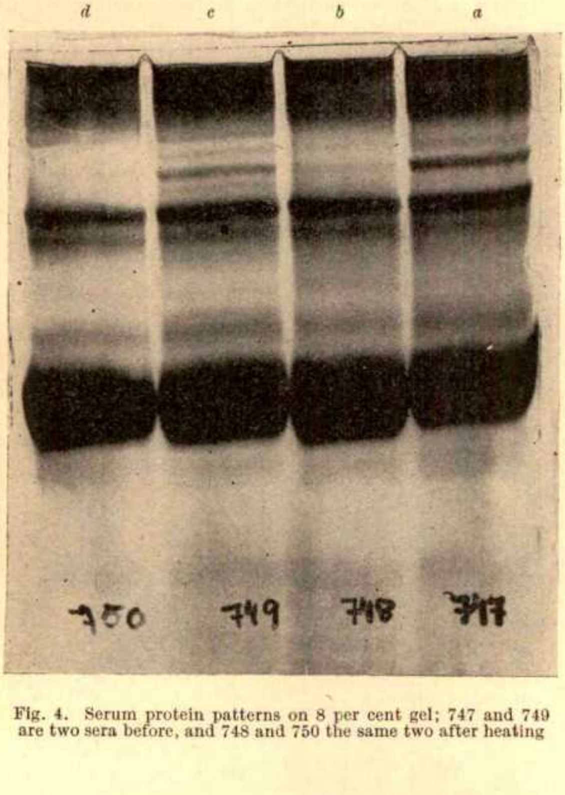

Fig. 4. Serum protein patterns on 8 per cent gel; 747 and 749 are two sera before, and 748 and 750 the same two after heating

The technique described has advantages over the technique described by Chang[7]. Several protein samples (4–12 in the apparatus described) can be intercompared directly on a single gel slab under identical conditions of gel polymerization, temperature, buffer composition, field strength, and duration of electrophoresis. Larger protein samples, for example, 2 ml. of 10 per cent protein solution, can be applied for semi-micro preparative electrophoresis; individual bands can be eluted from the gel by extraction with buffer or by electrophoresis. The apparatus has also been applied effectively to studies of gel structure in relation to electrophoresis effects.

As an example of the latter, Figs. 3 and 4 demonstrate the effect of gel concentration on the resolution of serum components. Fig. 3 *a* and *c* shows two individual sera resolved on a 5 per cent 'Cyanogum' gel, *b* being the same serum sample as *a*, incubated at 56° C. for 30 min. before subjecting it to electrophoresis. The disappearance of the component indicated by the arrow is typical of all serum samples studied in this procedure. Fig. 4 similarly shows two individual sera subjected to electrophoretic resolution in 8 per cent gel before (*a* and *c*) and after (*b* and *d*) heating. At this gel concentration the pattern is qualitatively different. The component just behind albumin does not disappear on heating, but instead, a new system of bands, which have appeared between the origin and the β-band now, are those which disappear after incubation.

We believe that the effect of gel concentration is related to the pore size of the gel, of which a theoretical analysis is in preparation.

This work was supported by grants from Institute for Cooperative Research, University of Pennsylvania, and from the U.S. National Institutes of Health.

SAMUEL RAYMOND
MASUMI NAKAMICHI
BARBRO AURELL

William Pepper Laboratory,
Medical School,
University of Pennsylvania,
Philadelphia 4, Pa.

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Solution Hydrates of Aqueous Non-electrolytes

FRANKS and Johnson[1] recently developed a computer method for the accurate determination of partial molar quantities from the measured total properties of binary systems. In their treatment of the volume of aqueous ethyl alcohol solutions, an unexpectedly complex and apparently anomalous variation of the ethyl alcohol partial molar volume was revealed at 0·06 alcohol mole fraction over the temperature-range 0°–30° C. In addition to the known[2] increase of the water solvent partial molar volume and the decrease of the alcohol partial molar volume to a minimum at about 0·08 alcohol mole fraction, the alcohol partial molar volume–concentration contour furnished[1] a hitherto unobserved change in course at 0 06 alcohol mole fraction appearing as a shoulder at 10° C which progressively developed with increasing temperature to furnish a small but definite peak at 30° C.

Elsewhere[3], the increase of the solvent water partial molar volume has been interpreted as arising from the orientation of hydration shell water adjacent to the ethyl group of the alcohol It is now proposed that the alcohol partial molar volume anomaly at 0 06 alcohol mole fraction derives directly from, and provides direct evidence for, the existence of a three-dimensionally hydrogen-bonded liquid hydrate of composition C_2H_5OH 17H_2O Such a liquid hydrate would involve 28-fold co-ordination of the alcohol molecule by water with the alcoholic-OH group hydrogen-bonded to the water cage, the solution structure being likened in respect to short-range order to a broken-down structure II gas-hydrate[4] of formula M 17H_2O

This interpretation provides a direct structural basis of the older hydrate theory of aqueous solution as developed by Pickering[5] in 1893, provided that the aqueous solutes are limited to non-electrolytes and that the meaning of a molecule of hydrate in solution is understood in the general crystallographic sense as the composition of the solution repeat unit, bearing in mind that the solution repeat unit composition does not define the hydration number of the solute but is structurally and geometrically related to it The solid gas hydrates[4] of dimethyl ether, ethylene oxide and of acetone, lead one to expect that the molar volume anomaly presented by aqueous ethyl alcohol will not be limited to ethyl alcohol alone, but will be generally exhibited in dilute aqueous solutions of many of the smaller, soluble, organic non-electrolytes

D N GLEW

Exploratory Research Laboratory,
Dow Chemical of Canada, Ltd ,
Sarnia, Ontario

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Fluorescence of Quinine in an Alkaline Medium and in Absolute Ethanol

PAPERS dealing with fluorescence of quinine only give the characteristics of this compound in an acid medium[1-4] Some publications suggest that quinine does not fluoresce at all in an alkaline medium[5,6]

In our experiments with quinine we discovered some fluorescent characteristics of quinine in an alkaline medium and in absolute ethanol which so far as we know have not been mentioned previously (Table 1) The maxima in an acid medium are given for comparison The ratio of intensity of the fluorescence in the media mentioned is approximately 1 10 60 for alkali, alcohol and acid respectively

Table 1 EXCITATION AND FLUORESCENCE MAXIMA OF QUININE (2 μg/ml) IN DIFFERENT MEDIA
Figures between brackets indicate that there is no distinct maximum but only a shoulder

Medium	Excitation maxima (mμ) as recorded	Fluorescence maximum (mμ) as recorded
0 1 N sulphuric acid	250, [310–320], 350	442
Absolute ethanol	[230–250], [280–290], 327	350
0 01 N sodium hydroxide	[230–250], [280–290], 327	365

Table 2 ABSORPTION OF QUININE (10 μg/ml) IN DIFFERENT MEDIA THE OPTICAL DENSITY IS GIVEN BETWEEN BRACKETS

Medium	Wave-lengths of maximum absorption (mμ)
0 1 N sulphuric acid	250 [0 900], 316 [0 125], 348 [0 165]
Absolute ethanol	234 [0 860], 280 [0 125], 319 [0 145], 333 [0 160]
0 01 N sodium hydroxide	231 [0 940], 282 [0 115], 327 [0 145]

We used a Farrand spectrophotofluorometer equipped with a 1P28 photomultiplier tube, a pen-and-ink recorder (Micrograph from Kipp, Delft) and a 10 × 20 × 50 mm Farrand quartz cuvette The entrance slits were 2 mm, the exit slits 0 5 mm for both monochromators

The differences in excitation maxima might be due to artefacts, however, the ultra-violet absorption spectra of quinine (10 μg/ml) in the foregoing media showed that the wave-lengths of maximum absorption (Table 2) were in good agreement with the excitation maxima in Table 1 Such an agreement between excitation and absorption maxima has already been demonstrated for quinine in an acid medium[3]

The fact that the fluorescent characteristics of quinine in different media are quite distinct may be used for a better identification of this compound in qualitative analysis

H A LINNEWIEL
B J VISSER

Pharmaco-Therapeutic Laboratory,
University of Amsterdam

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Influence of Deoxyribonucleic Acid on the Intermolecular Structure of Water

SEVERAL years ago Jacobson[1] directed attention to the fact that the helical structure of deoxyribonucleic acid (DNA) proposed by Watson and Crick[2] showed a remarkable relationship to the intermolecular structure of liquid water, which has been postulated to be quasi-crystalline and only slightly different from ice[3] Jacobson proposed that when DNA is dissolved in water, an increased ordering of the water structure results and further suggested this increased ordering of the intermolecular water structure accounts for the high viscosity and dielectric constant of DNA solutions In later work Jacobson *et al* [4] studied the proton resonance spectra of DNA solutions and interpreted the results of their experiments as offering further support of the above-mentioned proposals Jacobson[5] then made a detailed study of the dielectric constants of DNA solutions and interpreted the results as further evidence of stabilization of the normal water structure by the formation of hydration shells with diameters at least one-quarter the length of the dissolved macromolecules, he also cited certain X-ray studies[6] for further support of his hypothesis The sodium salt of deoxyribonucleic acid (sodium thymonucleate) was used in all Jacobson's experimental work

The work recorded here was undertaken with the view of obtaining infra-red data on the nature of the stabilized lattice structure reported by Jacobson In their early work on structure of water, Bernal and Fowler[7] suggested that the extent of the liquid water structure was greatest at low temperatures and decreased as the boiling point was approached, later work[3] on the quasi-crystalline nature of water has differed in details from the original Bernal–Fowler work but has not altered their general view that the intermolecular structure is most extensive at low temperatures Bernal and Fowler also discussed the influence of ionic solutes on the intermolecular structure of water

The infra-red spectrum of water was investigated by Ganz[8], who found shifts in the positions of major bands as the water temperature was raised from 17° C to 84° C Williams *et al* [9] examined the effects of various ionic solutes on the water spectrum, their results indicated that, in general, large ions have effects similar to those produced by increasing the water temperature as predicted by Bernal and Fowler, whereas small ions, notably F^-, have the reverse effect Hence, if DNA has the influence proposed by Jacobson, the solvent portion of the infra-red spectrum of a DNA solution at room temperature would correspond to that of pure water at a lower temperature

Although most of the characteristic water bands are overlapped by characteristic bands in the spectrum of DNA itself, the 'associational' water band at 4 7μ is in a spectral region not overlapped by characteristic DNA bands Ganz's work[8] had shown that this band shifts to shorter wave-lengths and becomes more intense as the temperature is reduced, thus, if Jacobson's hypothesis is valid, the 4 7μ band would be expected to shift to shorter wave-lengths when DNA is dissolved in water

In the course of the present work, the 4 7μ water band was examined in pure water and in a series of solutions of DNA varying in concentration from 6 per cent by weight to highly concentrated gels A Perkin-Elmer 21 spectrograph with a lithium fluoride prism was used, the absorption cells were fitted with calcium fluoride windows The DNA material used was sodium deoxyribonucleate prepared from calf thymus One set of solutions was prepared from DNA material with an intrinsic viscosity of 0 064 dl/g and molecular weight of the order of 10^5, which was supplied by the Light Company, another set of solutions was prepared from material having an intrinsic viscosity of 11 4 dl/g and molecular weight of approximately 3×10^6, which was prepared by Worthington Although, on the basis of Jacobson's claims, even more dilute DNA solutions would have been expected to produce marked shifts of the 4 7μ band, no shifts were observed in any of the solutions In order to check the present instrumentation, some of the earlier work on solutions of electrolytes was repeated with positive results in agreement with those obtained earlier If extensive intermolecular ordering of the type proposed by Jacobson had existed, the resulting shift of the water band could easily have been observed Thus, on the basis of the present infra-red work, it may be concluded that any influence DNA may have on the structure of water is quite different from those produced by temperature variations and by strongly ionized solutes

In view of the negative results afforded by the infra-red investigations, it was decided that, owing to the improved techniques now available, it might be well to check the early nuclear magnetic resonance data reported by Jacobson. The present nuclear magnetic resonance experiments were performed by Dr J Smidt at the Central Laboratory of the Staatsmijnen (The Netherlands) on numerous samples supplied from our laboratory and prepared from the highly polymerized DNA described above, the measurements were made at 40 Mc/sec in a highly homogeneous magnetic field, the percentage by weight of solutions and gels, both in pure water and in 0 05 N sodium chloride, ranged from 0 3 to 19 per cent

On the basis of the early work[4], which used photographic recording of oscilloscope traces, marked broadening of the proton line would have been expected even at lower concentrations than those utilized in the present experiments However, no broadening of the water resonance peak, which could not be accounted for by ordinary viscosity effects, has been noted in the present set of measurements, the results of which are summarized in Table 1, for the sake of comparison, we have also tabulated the figures observed for solutions of simple salts It is seen that as regards line widths the ionic solutes give an effect parallel to that observed in infra-red, that is, large ions raise the structure temperature thus leading to a narrower line, whereas small ions decrease this temperature giving the converse result[10]

Table 1 OBSERVED PROTON LINE WIDTH IN AQUEOUS SOLUTIONS

Solutions	Percentage by weight	Line-width (mG)
DNA in pure water	0 5	1 3
	1 5	1 5
	19 0	4 8
DNA in 0 05 N sodium chloride	0 3	1 4
	1 9	1 5
	16 8	5 0
Sodium chloride	0 05 N	1 2
Sodium fluoride	Saturated	1 4
Potassium iodide	Saturated	1 0

It seems thus well possible that there may have been some unrecognized instrumental difficulties involved in the earlier investigations A parallel study of the dielectric behaviour of water adsorbed on DNA materials and proteins is now in progress and a full account of this will be given elsewhere

In view of the negative infra-red results obtained in the present work, together with the lack of agreement between the present nuclear magnetic resonance results and the early experimental results of Jacobson, grave doubt must be raised as to the validity of Jacobson's quite attractive hypothesis regarding the influence of DNA on the water structure

We thank Prof J Duchesne for directing our attention to this problem

JOSEPH DEPIREUX *
DUDLEY WILLIAMS †

Institut d'Astrophysique,
Université de Liège

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* Chercheur à l'Institut Interuniversitaire des Sciences Nucléaires

† Professor of Physics, Ohio State University, National Science Foundation Senior Postdoctoral Fellow, 1961–62

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Esterification of Fatty Acids with Glycerol

Biswas and Ganguly[1] have recently published observations on the esterification of fatty acids with glycerol which are not in full agreement with the results of the work carried out at present in this Laboratory It is hoped to publish the details of this work elsewhere, but it might be of interest to discuss a few salient points here

Biswas and Ganguly state that since esterification of fatty acids with glycerol and glycerolysis of fats yield appreciable amounts of β-monoglycerides the ignoring of their presence could lead to a wrong computation of diglycerides and hence also of triglycerides based on the estimation of monoglycerides and hydroxyl values (Incidentally the results for β-monoglyceride contents obtained by Biswas and Ganguly in the esterification of oleic acid, namely, 0 04 per cent, differ greatly from the value of 3 per cent obtained under similar conditions in this Laboratory, and are probably much too low) The occurrence of β-monoesters in technical monoglycerides prepared by alkali-catalysed glycerolysis of fats has been repeatedly reported in the past few years[2–4] and Biswas and Ganguly's advice as to the need of accounting for their presence appears reasonable However, there is here a curious anomaly Some years ago Feuge and Bailey[5] suggested that glycerolysis proceeds according to chance inasmuch as the amounts of mono-, di- and tri-glycerides found experimentally in equilibrated products follow closely compositions calculated on the basis of random distribution of acyl groups These results were confirmed recently by Choudhury[6] in a number of oils In his investigation the conclusions were based on the estimation of α-monoglycerides by the periodate method and on hydroxyl values, the presence of β-monoglycerides being dismissed as insignificant In reality β-monoesters in freshly prepared products may amount to as much as 10 per cent of total monoglycerides and normally to about 3 per cent absolute Since on the basis of hydroxyl values 1 per cent of monoglyceride with an average hydroxyl value of 320 is equivalent to 3 5 per cent of diglyceride with an average hydroxyl value of 90 it would follow that Choudhury's conclusions regarding glycerolysis were wrong If in his calculations the hydroxyl values due to β-monoglycerides were taken into consideration, the contents of diglycerides would, in most cases, be reduced by some 10 per cent and the contents of triglycerides increased by a similar amount The results would be incompatible with random esterification

However, recent investigations[4,7] in which total mono- di- and tri-glycerides were determined chromatographically point clearly towards random or near random esterification It would seem therefore justifiable to suggest that Choudhury's estimations of α-monoglycerides or more likely of hydroxyl values were inexact, and that this error led to the correct assumption of random esterification The computation of di- and tri-glycerides from hydroxyl values is thus open to criticism whether the amount of β-monoglycerides is known or not A direct estimation of mono-, di- and tri-glycerides seems to be required

Another issue raised by Biswas and Ganguly is the esterification-rate of various fatty acids Their experiments show that fatty acids of medium molecular weight (such as lauric) and unsaturated acids (oleic, linoleic) react with glycerol faster than high molecular saturated acids (stearic) They attribute these differences to the higher solubility of glycerol in lauric and oleic acids than in stearic acid This opinion and similar views on the effects of the solubility of glycerol in fats[8] are based on a fallacy Admittedly, on the weight basis the solubility of glycerol in fats rich in stearic acid is lower by about one-tenth than in fats rich in lauric acid[9] On the basis of molar equivalents, however—and this is the basis of Biswas and Ganguly's own kinetic measurements—the solubility of glycerol in stearic acid is by one-tenth higher than in lauric and equal to that in oleic acid, which seems to dispose of Biswas and Ganguly's explanation According to earlier investigations[10,11], there is no appreciable difference between the reactivities of various fatty acids with methanol except those with less than four carbon atoms This has now been confirmed in this Laboratory in the case of glycerol by carrying out the esterification in high-boiling inert solvents The different rates of esterification of various fatty acids with glycerol in the absence of co-solvents appear thus to be due to factors other than the reactivity of fatty acids The investigation of these factors is in progress

L Hartman

Fats Research Laboratory,
Department of Scientific
and Industrial Research,
Wellington, New Zealand.

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BIOCHEMISTRY

Actinonin : an Antibiotic Substance produced by an Actinomycete

During a programme of screening of soil and other materials to select antibiotically active microorganisms, Drs R Green and R Bhagwan Singh, of the Institute for Medical Research, Kuala Lumpur, isolated a new species of an actinomycete, later classified under the serial number *Streptomyces* Cutter *C*/2 (N C I B 8845) It was active against a number of bacterial strains, including *Mycobacterium phlei* and *Staphylococcus aureus*

We have found that the organism grows well in deep culture at 30°–32° in a medium containing a source of nitrogen (for example, dried autolysed yeast) and a carbohydrate (for example, starch) in stirred, aerated flasks or fermentation vessels; such cultures show the same type of antibacterial activity as on plate culture, the activity being present practically exclusively in the medium

Isolation of the active substance (named 'actinonin') on a large scale (that is, from 300 l. of culture) proved to be a relatively straightforward matter. The material was completely transferable from the culture filtrate into *n*-butanol from which, after concentration, it was further purified by a series of partitions between aqueous solution and chloroform at different pH values, to give a final chloroform concentrate Solid actinonin was precipitated from this by addition of diethyl ether at 0° Treatment of the substance in hot ethanol solution with active carbon, followed by crystallization from ethanol-ether, gave fine white needles or colourless rods, m p 148°–149° (uncorr)

Actinonin is a faintly acid substance of molecular formula $C_{19}H_{35}O_5N_3$ It is stable in cold alkali, and not readily affected by cold dilute acid It is soluble in water, alcohols and pyridine, and is active in broth dilution against a number of Gram-positive and Gram-negative bacteria, including the following *St aureus* G, *Bacillus anthracis* 0/15, *Salmonella enteritidis*, *S typhosa*, *Streptococcus pyogenes* 0/8, *Klebsiella pneumoniae* 0/10, *M smegmatis*, *B C G*, *M phlei* 0/17 A F, *M phlei* 0/14 Minimum concentrations necessary to inhibit growth vary between 20 and 100 μg/ml It also inhibits the phages of a number of strains of *St aureus* at concentrations down to 0 25 μg/ml It has no apparent toxicity to mice in doses up to 400 mg/kg body-weight

J J GORDON*
B K KELLY†
G A MILLER‡

Medical Research Council,
Antibiotics Research Station,
Clevedon, Somerset

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* Chemical Defence Experimental Establishment, Porton Down, Salisbury, Wilts

† Medical Research Council Statistical Research Unit, London, W C 1

‡ Microbiological Research Establishment, Porton Down, Salisbury, Wilts

Inorganic Phosphate and the Rate of Glycolysis in Insect Muscle

CHANGES in the level of inorganic phosphate are believed to be responsible for the Pasteur effect in various cells metabolizing glucose, for example, in yeast[1], ascites tumour cells[2], and bacteria[3] A similar mechanism is, however, difficult to accept for insect thoracic musculature where the anaerobic increase of glycogen breakdown was shown to occur without any simultaneous change in the level of inorganic phosphate[4] In experiments reported here, evidence was found that the level of inorganic phosphate is rate-limiting for the anaerobic glycolysis in this tissue

In preparations of the intact metathoracic musculature of the cockroach *Periplaneta americana*[4], changes in the acid-soluble phosphorus compounds were followed during anaerobiosis and in subsequent recovery in oxygen Phosphorus compounds were labelled by injecting the animals with phosphorus-32 a week prior to the experiment, after this period, the labelling of all soluble phosphorus compounds was practically uniform[5] The neutralized perchloric acid extract was subjected to paper ionophoresis and the radioactivity of the spots of various phosphorus compounds was taken to be proportional to their absolute amounts Determination of the absolute content of a single compound (colorimetric determination of inorganic phosphate with the butanol extraction method[4] or colorimetric determination of arginine phosphate[6]) allowed calculation of the absolute amounts of all other soluble phosphorus compounds

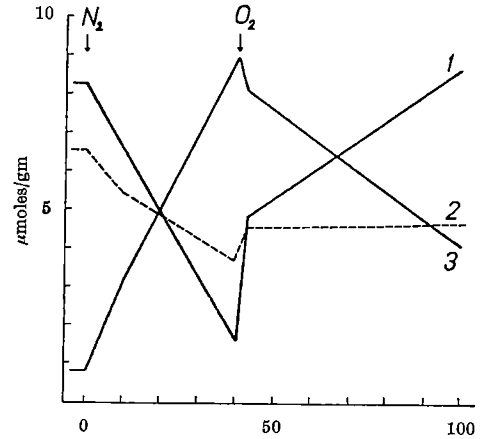

Fig 1 Changes in the level of arginine phosphate (1), adenosine triphosphate (2), and glycerophosphate (3), in metathoracic musculature of *Periplaneta* in anaerobiosis and after oxygen re-admission (marked by arrow)

It was confirmed that the total amount of soluble phosphorus remains unchanged during 40 min anaerobiosis and that the main change in these conditions is the breakdown of arginine phosphate and adenosine triphosphate and synthesis of glycerophosphate[7] (Fig 1) The amount of glycerophosphate synthesized is exactly equivalent to the amount of the energy-rich compounds broken down and the level of inorganic phosphate does not change significantly after transition into anaerobiosis (Fig 2)

After re-admission of oxygen, arginine phosphate is rapidly resynthetized, the resynthesis being complete after 1 hr The resynthesis of adenosine triphosphate is incomplete, evidently because only a small amount of adenosine diphosphate accumulates in the tissue during anaerobiosis most of it appears to be converted via adenosine monophosphate to inosine monophosphate and further dephosphorylated The re-oxidation of glycerophosphate in the tissue was found to be a rather slow process, thus little phosphate is liberated from it in the first

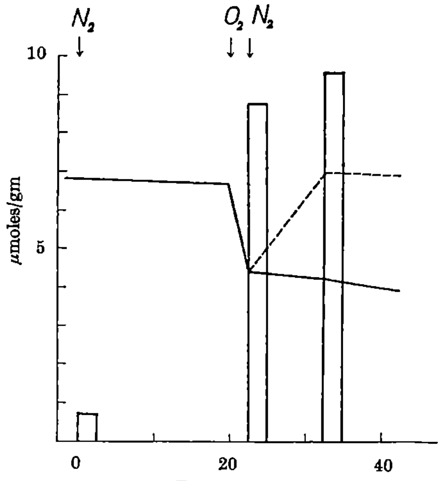

Fig 2 The level of inorganic phosphate in metathoracic musculature of *Periplaneta* in anaerobiosis and after re-admission of oxygen (full line) and in repeated anaerobiosis (broken line) Columns represent the respective levels of glycerophosphate in the experiment with repeated anaerobiosis

minutes after oxygen re-admission and the synthesis of arginine phosphate in this time occurs at the cost of inorganic phosphate, which sinks rapidly considerably below the original level (Fig 2) This shows that a considerable amount of it is available for metabolism, at least for oxidative phosphorylation

Further experiments were designed to show whether glycolysis in this tissue can proceed at this lowered level of inorganic phosphate After 20 min anaerobiosis (at this point the process of glycolysis is still approximately linear) the preparations were transferred for 2 5 min into oxygen and then into nitrogen again The level of inorganic phosphate returned rapidly to the original level, whereas in oxygen it remained lowered for at least 20 min , this shows that in the first 10 min of the second exposition to nitrogen the breakdown of the phosphorus compounds exceeded their synthesis, that is, that glycolysis was slowed In accordance with this, the rate of glycerophosphate accumulation decreased considerably in this period (Fig 2)

The rapid return of inorganic phosphate exactly to the original level favours the hypothesis that inorganic phosphate is the rate-limiting factor of anaerobic glycolysis in this tissue After transition into anaerobiosis the tissue cannot synthesize glycerophosphate at the expense of inorganic phosphate, and therefore the rate of glycolysis is limited by the rate of breakdown of the macroergic phosphorus compounds, the level of inorganic phosphate remains constant If anaerobiosis is introduced at the moment when the inorganic phosphate is lowered, glycolysis is slowed and the splitting of the macroergic phosphates exceeds glycerophosphate synthesis until the inorganic phosphate reaches its original level, in this moment, the steady state is again established and the level of inorganic phosphate then remains unchanged

The assumption that inorganic phosphate is the rate-limiting factor of anaerobic glycolysis in normal muscle is further supported by experiments with 2,4-dinitrophenol This substance is known to enhance the anaerobic glycogen breakdown in this tissue twice[8], this observation eliminates the possibility that in normal muscle some of the glycolytic enzymes are rate-limiting, since dinitrophenol is not known to interact with any of the glycolytic enzymes[9] The anaerobic increase of the glycolytic rate after application of dinitrophenol was found to be accompanied by an increase of the level of inorganic phosphate from 7 0 to 8 9 μmoles/gm as a result of accelerated dephosphorylation of macroergic phosphates, this is in agreement with the hypothesis suggested here

VÁCLAV KUBIŠTA
MILIČ FOUSTKA

Zoological Institute,
Charles University,
Prague

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Liver Ribonuclease and Deoxyribonuclease Activity of Vitamin B_{12}-Deficient Chicks: Effects of Orotic Acid and Methionine

One of the most interesting aspects of the nutritional and metabolic relationship between vitamin B_{12}, orotic acid and methionine observed in chicks[1] concerns the participation of these factors in nucleotide metabolism In fact, at the level of this metabolism, both orotic acid and methionine have effects quite similar to those of vitamin B_{12} in chicks deficient of this vitamin increase of the liver RNA content and of the nucleotide content of the acid-soluble fraction[2], as well as increase in rate of turnover of these structures[3] These results induced us to consider nucleotide metabolism as a common field of action of these three substances and to try to explain the nutritional relationships observed between them

To get a better idea of the mechanisms through which vitamin B_{12}, orotic acid and methionine act on nucleotide metabolism, a series of investigations were undertaken on the effects which these three substances have on the enzymes, related to this metabolism

The present communication concerns the experiments on the effect of vitamin B_{12}, orotic acid and methionine on the liver ribo- and deoxyribo-nuclease activity in B_{12}-deficient chicks This work is of particular interest owing to the essential part that these enzymes play on the normal balance between synthesis and degradation of the polynucleotide structures

Our experiments were performed on the liver of New Hampshire × White Leghorn chicks

The animals were divided into 4 groups according to their body-weight One group was kept on the B_{12}-deficient diet[4] and the other three were fed the same diet supplemented, in the case of each group, with 10γ vitamin B_{12}, 10 mgm orotic acid, 150 mgm of DL-methionine per 100 gm respectively

At the end of the experiment (5 weeks), the animals were killed and their livers were homogenized at 0° C. in a glass Potter apparatus with 0 1 *M* sodium acetate buffer *p*H 5 Ribonuclease was assayed at 37° C , 1 ml of homogenate (5 per cent) and 2 mgm of purified RNA in a total volume of 2 ml were incubated The reaction was stopped after 30 min by adding 2 ml of ice-cold 10 per cent perchloric acid containing 0 25 per cent uranile acetate The mixture was centrifuged after 1 hr in the cold and the extinction of supernatant read at 260 mμ in a Beckman *DU* spectrophotometer

Deoxyribonuclease was measured in a similar way; 2 ml of ice-cold 10 per cent perchloric acid were used for stopping the reaction and the mixture was left for 10 min in the cold before centrifuging Appropriate blanks were determined in these assays and subtracted from the observed values[5]

Table 1 LIVER RIBONUCLEASE AND DEOXYRIBONUCLEASE ACTIVITY OF CHICKS

Group No	Supplement/ 100 gm diet	Ribonuclease activity*	Deoxyribonuclease activity*
1	—	4 66 ± 0 43†	1 62 ± 0 14
2	10γ vitamin B_{12}	5 08 ± 0 09	2 92 ± 0 29
		t = 1 06, P > 0 3	t = 13, P < 0 001
3	10 mgm orotic acid	5 13 ± 0 07	2 04 ± 0 10
		t = 1 07, P > 0 3	t = 2 4, P < 0 05
4	150 mgm DL-methionine	5 51 ± 0 42	2 03 ± 0 27
		t = 1 4, P < 0 2	t = 1 3, P > 0 2

* Ribonuclease and deoxyribonuclease activity is expressed as the increase in optical density at 260 mμ of the acid-soluble supernatant produced by 1 gm (wet weight) of tissue in 30 min under the condition of assay. The values are the mean of six animals

† *S E*

The results show that the vitamin B_{12}, the orotic acid and the methionine exert no effects on liver ribonuclease activity, in fact the values for the B_{12}-deficient animals do not show significant variation from those of treated animals On the contrary, vitamin B_{12}, orotic acid and methionine seem to exert a certain action on the activity of deoxyribonuclease In fact the values of this enzymatic activity in the livers of treated animals are significantly higher than those of deficient animals

On the basis of these results we believe that the observed increase of the liver RNA content of treated animals as compared with the deficient animals is due to the effect of vitamin B_{12}, orotic acid and methionine on the biosynthetic process rather than on those of degradation

With regard to DNA, since the content of these structures does not show any variation in the livers of deficient and treated animals, even if in the latter the deoxyribonuclease activity is higher, one is tempted to think that vitamin B_{12}, orotic acid and methionine enhance not only the catabolic processes but also the anabolic processes, so that the DNA content of the cells remains practically the same

C M CALDARERA
M MARCHETTI

Istituto di Chimica Biologica,
Università di Bologna

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Nucleic Acid Content of Inflammatory Cells

IT is generally known that, during the inflammatory process, morphologically different cell-types appear in a certain sequence in the exudate Recently, on the basis of autoradiographic experiments, Speirs *et al* [1] have reported that eosinophil polymorphonuclear cells were phagocytized by mononuclear cells and this phenomenon is probably responsible for the synthesis of antibodies in these cells

In our experiments sterile paraffin-oil was injected intraperitoneally into rabbits and the cells which appeared in the exudate were collected in various periods of inflammation The cells obtained on the first day were, morphologically, mainly polymorphonuclear leucocytes, on the third day a mixture of about equal numbers of polymorphonuclear and mononuclear cells, and ultimately on the fifth day a fairly pure mononuclear cell population was found

The cells were washed with saline at 4° C and their nucleic acids were isolated by the Schmidt–Tannhauser method, the ribonucleic acid phosphorus was determined by the orcinol reaction and the deoxyribonucleic acid phosphorus by the indole reaction In addition, the fractions of acid-soluble nucleotide, the lipid-phosphorus (using Griswold's method) and the protein (using Lowry's method) were determined

Some characteristic results are given in Table 1, which demonstrates that the deoxyribonucleic acid phosphorus content of the cells is essentially constant, but the ribonucleic acid phosphorus content varies in the different cell-series, that is, it increases three- to four-fold in mononuclear cells[2] On the basis of protein content a certain difference appears to exist between the mononuclear series In some series the increase in content of ribonucleic acid is not followed by the increase in content of protein, and in others the content of protein is also increased significantly

G J KOTELES
F ANTONI
E J HIDVÉGI

Institute for Radiobiological Research,
Budapest 22

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Acid-catalysed Reaction of Reduced Pyridine Nucleotides

IN a recent publication[1] it was demonstrated that the catalysed reaction of DPNH and TPNH in weakly acidic buffers follows first order kinetics, and that the rate of the TPNH reaction at constant acidic pH is considerably greater than that of DPNH Almost simultaneously. other authors published similar results[2] The results of a more detailed comparison of the rates for the reduced nucleotides are given in Fig 1 For TPNH the relationship is log K_1 = 3 71–0 46 pH, for DPNH it is log K_1 = 3 67–0 49 pH, where K_1 is the first-order constant (t = sec) The figure represents results from experiments using three different lots of enzymatically reduced nucleotides (TPNH, 96 5 per cent DPNH, 99 per cent, Sigma and Co) For a particular nucleotide no essential difference of rates was noted in the following buffers

Table 1

Day of collecting	No determined	DNA-P / cell pg	RNA-P / DNA-P	Acid-soluble ribose / DNA-P	Lipid-P / DNA-P	Protein / DNA-P	
1	16	0 590* 0 109† 0 473–0 690‡	0 453 0 053 0 377–0 501	1 19 0 131 1 03–1 34	0 523 0 056 0 470–0 602	46 72 7 51 38 9–56 9	Polymorphonuclear cells
3	8	0 655 0 102 0 500–0 875	1 049 0 005 1 045–1 052	2 07 0 547 1 68–2 46	0 357 0 012 0 349–0 366	49 26 3 56 47 98–50 91	Polymorphonuclear + Mononuclear cells
5	8	0 637 0 126 0 531–0 828	1 43 0 088 1 36–1 49	2 32 0 224 2 15–2 48	0 667 0 060 0 624–0 710	38 40 8 31 32 29–44 51	Mononuclear cells
5	16	0 645 0 134 0 517–0 853	1 98 0 144 1 86–2 14	3 45 0 334 3 23–3 95	1 42 0 247 1 11–1 71	93 29 13 29 83 81–112 6	Mononuclear cells

* Mean value † Standard deviation ‡ Range

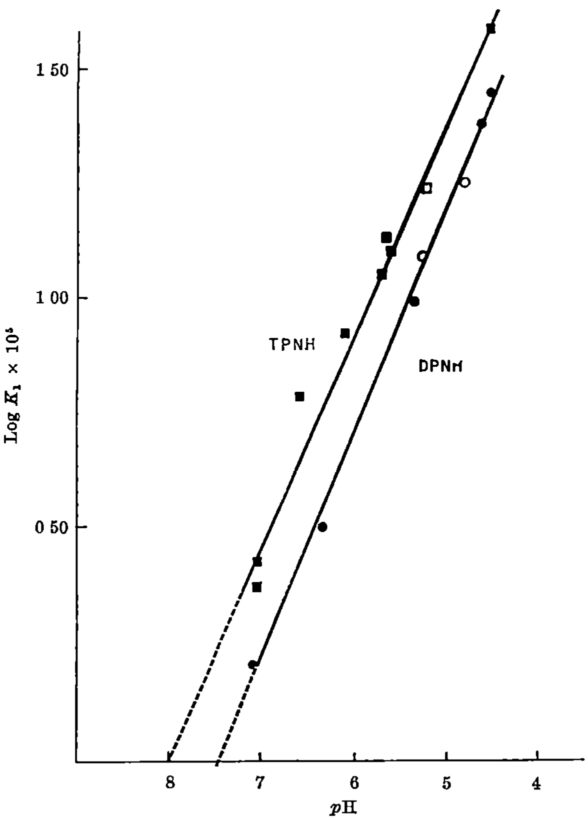

Fig. 1. Effect of pH upon the reaction-rate for TPNH and DPNH. 1 ml. of reduced nucleotide in distilled water at pH 8.0 was added to a 1-cm. silica cuvette, followed by 2 ml. of buffer of designated pH. The solutions were mixed by inversion and readings taken immediately at 340 mμ against a buffer blank. Equimolar concentrations were based on a molar extinction coefficient of 6.22 × 10³ (ref. 8). The pH measurements are final readings. Black squares and circles, phosphate buffer; white squares and circles, citrate buffer.

at equal concentration and pH: phosphate, citrate, *tris*-hydrochloride, and ethylenediamine tetraacetic acid. It is significant to observe that the two lines converge and, by extension, the rate of the reaction of the two nucleotides in 0.1 *N* hydrochloric acid theoretically would be indistinguishable experimentally. It is perhaps this which may account for the fact that the difference between the nucleotides has not been observed previously. By extrapolation, the theoretical pH, where the rate becomes zero for both nucleotides, may be obtained. Actually the rate never becomes zero, but the extrapolation may be useful in determining the constants in the overall rate equation. First-order kinetics were obeyed only at low pH where (H+) is not rate determining, and for small changes in nucleotide concentration.

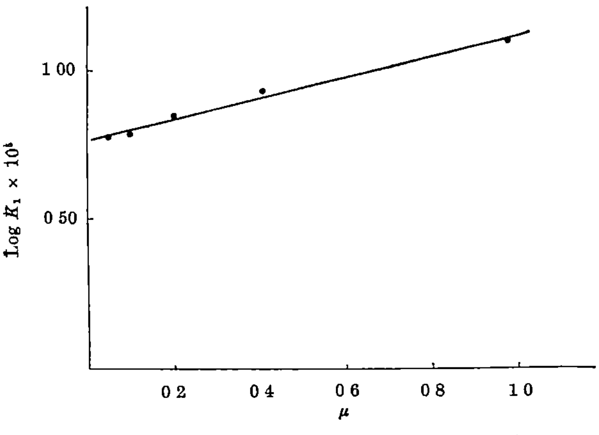

Fig. 2. Effect of ionic strength (μ) on the reaction-rate for DPNH. Citrate buffers of varying concentration prepared with the final pH adjusted to 5.36. Complete ionization for citrate is assumed.

The reaction-rate is influenced by the ionic strength of the medium in the same manner as has been reported for model analogues[3,4] (Fig. 2). It is conceivable that this effect of charge density may have some bearing in explaining the accelerated TPNH reaction-rate as compared with DPNH.

The reaction products of the reaction are at present unknown, although there is evidence that the reaction is sequential[5]. The reaction with a model analogue, 1-benzyl-3 acetyl-1,4-dihydropyridine[4], resulted in the addition of water across the double bond, placing a hydroxyl at position 6. This was believed to be followed by an acid-catalysed ring opening at position 6 forming the tautomeric amino aldehyde.

This sequence of reactions is in harmony with the present kinetic data, if one assumes that the acid-catalysed ring opening is rate limiting, for it could explain why log K_1 is proportional to $\sqrt{\mathrm{H}+}$ instead of (H+) through the formation of the weakly dissociating substituted amine salt. In addition, it explains the reported rise in pH accompanying the reaction[6]. On the other hand, Segal and Stein[7], using 1-benzyl-1-4 dihydronicotinamide as a model analogue, obtained a hydroxyl derivative in the 5 position which rules out the possibility of the tautomeric amine aldehyde and ring opening. It would thus appear that the ring substituents markedly affect the course of the reaction, and extrapolation of results from model analogues to the reduced nucleotides should be done with great caution.

I. G. Fels

Virginia Institute for Scientific Research,
Richmond, Virginia

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One-Dimensional Starch-Gel Electrophoresis of Bovine Skim-Milk

Aschaffenburg and Drewry[1] found by paper electrophoresis that the milk of individual cows may contain either β-lactoglobulin *A* or *B* singly or a mixture of the two proteins. These authors presented evidence that the production of the β-lactoglobulins is controlled by two allelic autosomal genes[2].

By the technique of starch-gel electrophoresis of whole skim-milk, a distinct separation of the whey proteins can be obtained without any preliminary treatment of the skim-milk. Skim-milk samples from individual cows have been submitted to starch-gel electrophoresis for 5 hr. at 12 V./cm length of the gel, using the boric acid buffer of Smithies[3]. The

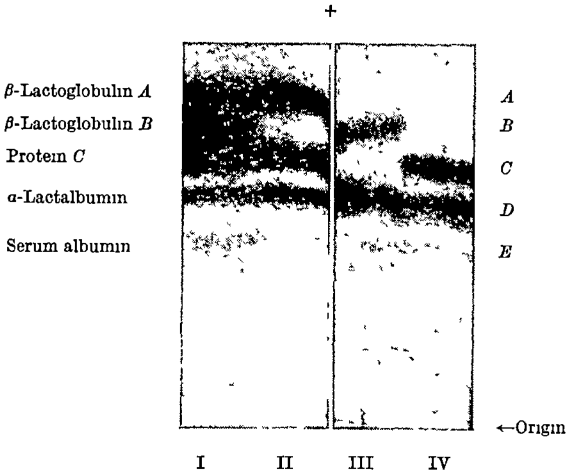

Fig 1 Starch-gel electrophoretic patterns of four skim-milks from individual cows Left to right I, A B C, II, A C, III, B, IV, C protein patterns

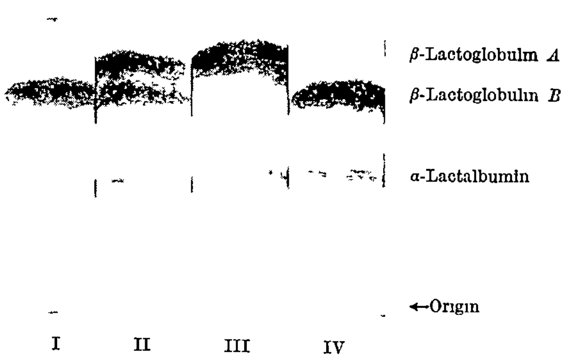

Fig 2 Paper electrophoretic patterns at pH 8 6 of the concentrated 'lactalbumin' solutions of skim-milk samples containing, left to right I, B, II, A C III, A, IV, C proteins on starch-gel electrophoresis

samples were applied on Whatman E 17 filter paper inserts which were removed from the gel 1 hr after the start of the electrophoresis

A comparison of the skim-milk and acid whey starch-gel electrophoretic patterns indicates that all, or at least the major, protein zones in the skim-milk pattern represent whey proteins The colloidal casein of milk does not enter the gel within the time the inserts remain in the gel The major protein zones in the starch-gel pattern of skim-milk are designated by the letters *A*, *B*, *C*, *D*, and *E* (Fig 1) and have been identified by a combination of the starch-gel electrophoresis of skim-milk and paper electrophoresis at pH 8 6 of the concentrated 'lactalbumin' solution Zone *A* is identical with β-lactoglobulin A, whereas zones *B* and *C* migrate at the same rate and in the position of β-lactoglobulin *B* (Fig 2) It can be shown that zones *D* and *E* represent α-lactalbumin and serum albumin respectively

Paper electrophoresis of the concentrated 'lactalbumin' solutions of milks containing *A*, *AB*, *AC*, *B*, *BC*, *C*, and *ABC* proteins on starch-gel electrophoresis was carried out to determine the corresponding β-lactoglobulin types (Table 1) Aschaffenburg and Drewry[4] have demonstrated by paper electrophoresis that all the proteins with the exception of the β-lactoglobulins are precipitated from the 'lactalbumin' filtrate when the pH is reduced to approximately 2 However, protein *C* as well as *A* and *B* is resistant to this low pH, further indicating the similarity of proteins *A*, *B*, and *C* Any of the proteins *A*, *B*, or *C* may be either present or absent in the milk of individual cows, suggesting that they are genetically determined From the genetical point of view, it is of interest that all three proteins have been found to occur together in one milk Two Jersey cows in different herds have been found which lack both *A* and *B* proteins in the milk, but protein *C* was present in each case (Fig 1)

Table 1

Starch gel electrophoretic pattern	β-Lactoglobulin type (ref 2)
A	*A*
A B	*A B*
A C	*A B*
B	*B*
B C	*B*
C	*B*
A B C	*A B*

Some of the milks containing protein *C* have been sampled at monthly intervals throughout the lactation period and the *A B C* patterns remain constant It would appear that protein *B* represents β-lactoglobulin *B*, as it occurs with greater frequency than protein *C* in the herds studied This new protein, *C*, was found in 24 out of 135 Jersey cows, but it was only detected in one of 85 Australian Illawarra Shorthorns From Table 1, it is obvious that the existence of protein *C* in the population may lead to an excess of β-lactoglobulin *B* if determined only by the paper electrophoretic method[2] The mode of inheritance of these proteins is being determined and will be presented later

This work was carried out during the tenure of a United Graziers' Association fellowship and supported by a grant from the Rural Credits Development Fund of the Commonwealth Bank of Australia

K Bell

Sir William McGregor School of Physiology,
University of Queensland,
Brisbane

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Liver Enzymes in Biliary Retention

The toxicity of bilirubin has been demonstrated in animals It is known to inhibit brain respiration *in vitro*[1], and to uncouple oxidative phosphorylation in isolated mitochondrias[2] Its cytotoxic action on almost every cell in the animal has been recently emphasized by Rozdilsky[3]

In man, damage, resulting from biliary retention, on the liver, has been established for a long time and is known to end in biliary cirrhosis, bile appearing thus as a potent hepatotoxine[4]

The actual toxicity of bile for the liver cell in the early stage of biliary retention has been tested by estimation of several liver enzymes in cases of biliary obstruction in man

Liver biopsies were taken from patients undergoing various surgical treatments for calculus of the choledocus or malignant disease of the pancreas or the bile ducts Biopsies were taken at the onset of operation and immediately prepared for enzymes assays Thorough pathological examination was carried out on the same piece of liver which was used for enzymatic investigations Whenever cirrhosis or any specific lesion was present, with the exception of biliary retention itself, the case was discarded and considered inadequate for enzymology Obviously enough, sclerosis, steatosis, malignant or inflammatory infiltration as well as any condition which disturbs liver structure is to be expected to modify the ratio of hepatocytes to other cells and therefore makes the estimation of enzymes meaningless The following enzymes were estimated alcohol dehydrogenase (ADH), glutamic dehydrogenase (GDH), lactic dehydrogenase (LDH), isocitric dehydrogenase (IDH), glutamic pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT), glutaminase I (phosphate activated), and ornithine-carbamyl-transferase (OCT) Activities are referred to 1 gm fresh liver

Table 1

Enzymes	Normal subjects No of cases, 15		Biliary retention No of cases, 10
ADH	Range, 262 9–350 3 Mean value (m v), 306 6 ± 43 7	*P* 0 01	Range, 54 1–92 1 m v , 73 1 ± 19
GDH	280–377 5 m v , 329 1 ± 48 4	*P* 0 01	163–218 m v , 190 6 ± 27 4
LDH	3,065–4,805 6 m v., 3,935 4 ± 48 4	*P* 0 01	1,295–2,129 m v , 1,712 ± 417
IDH	728 8–901 2 m v , 815 ± 86 2	*P* 0 01	425 2–599 6 m v , 512 4 ± 87 2
GOT	154 682–190 746 m v , 172 714 ± 18 032		133 931–267 211 m v , 200 571 ± 66 640
GPT	57 804–83 910 m v., 70 857 ± 13 053		59 985–96 871 m v , 78 428 ± 18 443
Glutaminase	11 2–15 6 m v , 13 4 ± 2 2		9 5–21 9 m v , 15 7 ± 6 2
OCT	22 7–25 1 m v , 23 9 ± 1 2		21 6–24 m v , 22 8 ± 1 2

Results are expressed for the dehydrogenases in μmoles of substrate hydrolysed/hr /gm fresh liver, for the transaminases in Wroblewski spectrophotometric units, for glutaminase and ornithine-carbamyl-transferase in terms of μmoles of N-NH_3 evolved/hr at 37°/gm fresh liver

As shown in Table 1, ADH is reduced in all cases of biliary retention to a quarter of its normal value The latter was calculated on the basis of fifteen estimations made in patients free of biliary retention and operated on for various diseases in which liver was not involved GDH is reduced to almost half its normal value as is also IDH, while LDH activity has to be divided by a factor of 2 5

In contrast with the dehydrogenases, both transaminases show no significant change and GOT is, if anything, increased Glutaminase and OCT are very close to normal and do not appear to be modified by biliary retention

These results suggest that bile *per se*, whatever the cause of the retention, suffices to produce striking enzymatic changes in the liver cell, possibly by altering respiration or permeability to substrates or co-factors

It is noteworthy that toxicity of bile for enzymes is selective transaminases, glutaminase and OCT are not affected while the dehydrogenases are

Whether enzyme inactivation is due to temporary inhibition by bile or to the actual destruction of the enzyme cannot be asserted from our work This point is being further investigated by comparing activities in the course of jaundice and after treatment Yet it is clear that prolonged biliary retention will result in aggravation of the enzymatic lesion and of the cell thereafter

In most cases the severity of damage caused to the enzymes was found to be proportionate to the degree of biliary retention as assessed by the levels of bilirubin in blood, and independent of the cause of the retention, whether malignant or not

The nature of the bile component or components involved has not been revealed by our experiments

We have tested the action of pure bile on all enzymatic reactions reported herein Bile was obtained, aseptically, by puncture of the gall bladder, and diluted 50 times before it was added to the reaction It showed no inhibition whatsoever in the normal, nor in patients with biliary retention

It is concluded that toxicity of bile on enzymes is mediated through the intact liver cell and has no effect on homogenates at the concentrations used

V SCHWARZMANN
C COUINAUD
N BERTHAUX

Laboratoire de Recherches d'Enzymologie,
Clinique et Experimentale
and Centre de Recherches d'Hepatologie,
Institut National d'Hygiène,
Hôpital St Antoine, Paris

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Serotonin Degradation by Homogenates of Tissues from *Heloderma horridum*, the Mexican Beaded Lizard

IN a previous report[1], the finding of serotonin and of amine oxidase activity in the venom of *Heloderma horridum* was recorded The purpose of this communication is to present results of studies which demonstrate that tissue homogenates of selected organs from two animals of this species degrade serotonin readily in an *in vitro* test system In addition, the findings with respect to efforts to inhibit this activity with various compounds will be noted

The beaded lizards were sexually mature females that had been in captivity for more than a year They were killed by bleeding, and various biochemical and hæmatological values of their blood were determined[2] Immediately after bleeding, the respective organs were removed and rinsed with physiological saline solution The organs were then individually homogenized in a Waring blender in 2 vol of physiological saline solution and frozen at − 20° C This material was thawed at the time of testing, and serial 2-fold dilutions of the homogenates were made in physiological saline solution Evidence for serotonin degradation was sought by employing 300 μgm serotonin creatinine-sulphate as substrate for the respective homogenate dilutions and analysing for residual serotonin by the method of Udenfriend *et al* [3] The determinations were made at *p*H 7 4 Homogenates of striated muscle, liver, œsophagus, stomach, intestine and pancreas were tested Marked serotonin degradation occurred with homogenates of each of these tissues This finding was not unexpected in view of the widespread distribution of monoamine oxidase activity in other animal tissues[4] However, it should be noted that striated muscle from *H horridum* was active in this regard in contrast to human striated muscle, which does not exhibit this capability in the same test system[5]

In an effort to characterize further the pathways by which these homogenates metabolize serotonin, a series of tests with various enzyme inhibitors was performed The test procedure was as follows homogenate dilutions which had been found on prior titration to metabolize between 65 and 80 per cent of the substrate were used in the test system One ml of the homogenate dilution was added to a 20-ml beaker with 1 0 ml of 0 5 *M* phosphate buffer (*p*H 7 4) and 0 5 ml of the solution containing the substance to be tested for inhibition of the reaction The beakers were pre-incubated for 15 min on a Dubnoff metabolic shaker Solutions of serotonin creatinine sulphate and the other test substances were freshly prepared and placed in the test system within 10 min of preparation Final concentration in the incubation mixture was 4 9 × 10^{-4} *M* for serotonin Incubation was continued for 1 hr and determination of residual serotonin was made as already described Controls included tests with boiled tissue homogenates, and, for colour interference, recovery of serotonin after incubation with the respective test substance in the absence of tissue homogenate The following substances were tested for reaction inhibition at a final concentration of 1 × 10^{-3} *M* iproniazid (IPZ), isoniazid (INH), hydralazine, procaine amide, mercuhydrin, and D,L-penicillamine Results are given in Table 1

Table 1 EFFECT OF CERTAIN ENZYME INHIBITORS ON SEROTONIN DEGRADATION BY INDICATED HELODERM TISSUE HOMOGENATES

Tissue Homogenate	Inhibition (per cent)					
	IPZ	INH	Hydralazine	Procaine amide	Mercuhydrin	Penicillamine
Striated muscle	35	14	0	8	3	35
Liver	30	15	0	0	67	22
Œsophagus	33	36	0	50	30	32
Stomach	49	16	46	55	48	34
Intestine	28	11	0	24	78	34
Pancreas	73	0	—	0	0	87

Iproniazid, procaine amide, and hydralazine are known monoamine oxidase inhibitors Isoniazid is not an inhibitor of monoamine oxidase activity, but it does impede the ceruloplasmin pathway of serotonin metabolism, as do the foregoing compounds[5] Mercuhydrin is believed to effect enzymatic activity through its metal moiety, while D,L-penicillamine is a chelating agent It is of interest that none of the compounds employed was found to inhibit completely the degradation of serotonin by any of the tissues tested It can be seen that marked inhibition of the enzymatic processes involved in the metabolism of serotonin by pancreatic homogenates was achieved with iproniazid and D,L-penicillamine, and by mercuhydrin in tests with hepatic and intestinal homogenates Lesser inhibition or no effect was detected in the remaining tests These findings suggest that channels of serotonin degradation other than monoamine oxidase are active in several organs of *H horridum*

CHRIS J D ZARAFONETIS
JOHN P KALAS

Department of Medicine,
University of Michigan Medical School,
Simpson Memorial Institute,
Ann Arbor, Michigan,
and
Department of Medicine,
Temple University School of Medicine,
Philadelphia, Pennsylvania

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Urea Synthesis in Rats treated with α-D,L-Methylaspartic Acid

α-D,L-Methylaspartic acid (MA) is a strong inhibitor of hepatic argininosuccinate synthetase, inhibition of this enzyme was first described by Braunstein *et al* [1], on the basis of *in vitro* experiments, in which 200 μmoles of MA were added to samples containing L-citrulline, L-aspartate, adenosine triphosphate (ATP) and a respiratory substrate, in presence of rat liver slices and homogenate

In this communication we report some results on the effect of *in vivo* administration of MA to rats of hepatic argininosuccinate synthetase, and urinary excretion of urea and ammonia Some of these results were objects of short communications at "VI^es^ Journées Biochimiques Latines" (Geneva, May 25–28, 1961)[2], and to the Fifth International Congress of Biochemistry (Moscow, August 10–16, 1961)[3]

MA (500 mg/kg of body-weight), in buffered solution at *p*H 7 4, was injected endoperitoneally into male albino rats, average weight 250 g, the animals were killed at various times after injection, the liver was quickly removed and cooled, and it was homogenized with M/15 phosphate buffer, *p*H = 7 4 (1 g tissue + 2 ml phosphate buffer) Samples were prepared in Warburg vessels, according to the following method enzymatic source, 0 5 ml of the homogenate (= 166 mg fresh weight tissue), $MgSO_4 7H_2O$, 10 μmoles, ATP, 5 μmoles, fumarate, 20 μmoles, L-citrulline, 20 μmoles, L-aspartate, 20 μmoles Final volume 3 ml, *p*H = 7 4 Samples were also prepared in which citrulline or aspartate were omitted A mixture of oxygen (95 per cent) and carbon dioxide (5 per cent) was then allowed to pass through the samples, and incubation was carried out at 37° C for 1 h Reaction was stopped by placing the vessels in iced water, and urea was determined on aliquots of the mixture, according to Krebs and Henseleit[4] At the same time, samples were prepared containing as enzymatic source the homogenate obtained by liver of control rats, which had been injected with an equal volume of phosphate buffer (*p*H = 7 4) in place of MA The results, reported in Table 1, show that endoperitoneal introduction of MA is capable of causing 100 per cent blockage of urea synthesis by citrulline and aspartate Inhibition is maintained totally until about the eighth hour after injection One deduces from the results reported, therefore, that to achieve total and permanent inhibition of hepatic argininosuccinate synthetase, injections must be repeated at intervals of 8 h The enzyme in question would thus be inhibited for a long time, and it would be possible to study the effects of inhibition on urea synthesis *in vivo*

Table 1 INHIBITION OF UREA SYNTHESIS BY CITRULLINE AND ASPARTIC ACID IN HOMOGENATES OF LIVER OF RATS TREATED WITH MA

H after injection	μmoles extra-formed urea in incubation mixture		Inhibition (per cent)
	Control rats	Rats treated with MA	
2	9 1	0	100
3	6 8	0	100
4	10 0	0	100
6	9 6	0	100
8	10 2	0	100
12	7 4	3 5	52

Male albino rats were kept in special metabolism cages, and uniformly fed, water was allowed *ad libitum*, the urine was collected daily, and urinary ammonia was determined according to Seligson and Hirahara[5], Kitamura and Iuchi's method[6] was used to estimate the urinary urea After some days, the rats which were excreting fairly constant quantities of urea and ammonia were isolated, and treated with MA (500 mg/kg body-weight, one injection every 8 h) The urine was collected 24 h after the first injection, and urea and ammonia were determined as before Table 2 shows the results of these experiments It appears that total quantities of urea and ammonia excreted in the urine by rats treated with MA do not differ significantly from the correspondent values in control rats (the same rats before treatment) It should be emphasized that in the livers of the treated animals argininosuccinate synthetase is blocked completely throughout the treatment (Table 1) Then, if the reaction between citrulline and aspartic acid were fundamental to the process of urea biosynthesis, that is, if it were necessarily involved in the path which leads from protein nitrogen to urea nitrogen, a stoppage, or at least a reduction, in urea formation would be expected when this reaction is blocked. Our results establish that this is not the case

Table 2 EXCRETION OF UREA AND AMMONIA IN RATS BEFORE AND AFTER TREATMENT WITH MA

mg urea per kg and per day		mg NH_3 per kg and per day	
Before treatment	After treatment	Before treatment	After treatment
1,119 ± 31	1,125	18 0 ± 0 5	17 9

In addition, preliminary results of Cedrangolo and co-workers[7], show that treatment with MA (500 mg/kg body-weight) is able to inhibit urea synthesis in liver also by carbamylphosphate (CP), ornithine and aspartate These results, if considered in the light of those exposed in this paper, throw doubt also on the role of CP as the donor of the first nitrogen atom of urea

Our experimental data results are in contradistinction to all the assumptions concerning the mechanism of urea synthesis held as valid up to now, and there is need for a comprehensive theoretical interpretation in the light of the problem of terminal nitrogen metabolism in mammals

F Cedrangolo
G Della Pietra
D. Cittadini
S Papa
F De Lorenzo

Institute of Biological Chemistry,
University of Naples.

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Aspartic Transcarbamylase from Lettuce Seedlings : Case of End-Product Inhibition

ASPARTIC transcarbamylase (ATC-ase) an enzyme which catalyses the carbamylation of aspartic acid by carbamylphosphate (CAP) to yield carbamylaspartic acid (CA) is known in micro-organisms and animals[1] Its presence in higher plants has been mentioned[1] but, so far, has not been investigated to any extent

Lettuce seedlings, var Grand Rapids, were grown for 3–5 days in darkness at 26° C The enzyme was extracted by homogenizing the entire seedlings in a mortar and pestle or with an 'Omnimixer' using a buffer-sucrose solution The homogenate was transferred through gauze and centrifuged at 300*g* to remove the cell debris

Table 1 CELLULAR LOCALIZATION OF ATC-ASE ACTIVITY

Enzyme fraction and aspartate addition		CA formed		
		C p m / 0 05 ml	Net c p m *	µmoles CA/gm seedlings
None	+ asp	42		
Whole homogenate	− asp	97		
	+ asp	150	53	0 73
300–20,000*g*	− asp	43		
precipitate	+ asp	93	50	0 22
20,000–100,000*g*	− asp	42		
precipitate	+ asp	43	1	
100,000*g* super-	− asp	85		
natant	+ asp	125	40	0 53

1 gm of 5-day-old seedlings was homogenized in 10 ml phosphate buffer (0 1 *M*, *p*H 7 0) sucrose 0 4 *M*, transferred through gauze and centrifuged at 300*g* for 8 min. The supernatant was made up to 20 0 ml and 15 0 ml were centrifuged at 20,000*g* for 30 min The precipitate was dispersed in 5 0 ml of the same buffer The 20,000*g* supernatant was centrifuged at 100,000*g* for 45 min. and the precipitate dispersed in 2 0 ml buffer

The reaction mixture contained 80 µmoles potassium pyrophosphate buffer at *p*H 9 0, 5 µmoles of aspartic acid where indicated, 5 µmoles of CAP (specific activity 76,000 c p m /µmole), and 0 5 ml of enzyme in a total volume of 0 75 ml The reaction mixture was incubated for 10 min at 37° C when 0 5 ml of 20 per cent TCA was added to stop the reaction The tubes were then heated for 6 min at 80°–90°, and centrifuged in the cold Of the decanted supernatant, 0 05 ml was plated on glass planchets, dried and counted in a thin end window Geiger–Muller tube

* Corrected for counts present in the absence of aspartic acid, and those due to the non-enzymatic reaction

ATC-ase activity was measured by following the increase in acid-stable radioactivity (CA) according to the method of Lowenstein and Cohen[2], where CAP labelled with carbon-14 and unlabelled aspartic acid are substrates Radioactive CAP was synthesized from ^{14}C-urea[3,4] and, except for the use of trichloroacetic acid (TCA) as deproteinizing agent rather than hydrochloric acid, the assay method was not changed By electrophoresis of the deproteinized reaction mixture on paper and autoradiography of the paper, it was found that the radioactive product is indeed CA Controls without aspartic acid as well as controls without enzyme (for following the chemical non-enzymatic reaction) were routinely performed

It was found that about 25–30 per cent of the activity of ATC-ase is located in the 300–20,000*g* particulate fraction and the rest in the 100,000*g* supernatant (Table 1) This partition of activity remained the same when different media were used during homogenation: tris buffer 0 1 *M*, *p*H 8 0–sucrose 0 25 *M*, or phosphate buffer 0 1 *M*, *p*H 7 0–sucrose 0 4 *M* with or without versene 0 01 *M* This particulate fraction activity remained practically constant on washing and reprecipitation Osmotic shock, treatment with digitonin, and freezing and thawing did not change the activity When the 300–20,000*g* particulate fraction was refractionated

Table 2 END-PRODUCT INHIBITION OF ATC-ASE

Compound	Final concentration (*M*)	Percentage of inhibition
Cytidine-5′-phosphate	0 02	30
Cytidine-5′-phosphate	0 04	62
Uridine-5′-phosphate	0 0025	76
Uridine-5′-phosphate	0 005	90
Uridine-5′-diphosphate	0 015	56
Uridine-5′-triphosphate	0 01	8
Uridine	0 04	none
Cytidine	0 04	none
Guanine-5′-phosphate	0 04	none
Adenine-5′-phosphate	0 04	none
Phosphate	0 04	none

The enzyme used was an ammonium sulphate fraction of the 20,000*g* supernatant isolated between 25–60 per cent saturation and dissolved in a volume equal to half the volume fractionated Otherwise the experimental conditions as in Table 1.

by centrifugation, 70 per cent of the activity was found in the 2,000–20,000g precipitate suggesting that the particulate enzyme may be located in the mitochondria[5] About 90 per cent of the mitochondrial enzyme can be rendered soluble by freezing and thawing It will be of interest to see if the mitochondrial and cytoplasmic enzymes have separate physiological roles

Some of the characteristics of the enzyme of the cytoplasmic fraction were studied next Its pH optimum is between 9 and 10, the K_m for aspartic acid was found to be 6×10^{-4} M and for CAP 2×10^{-3} M. The enzyme is markedly inactivated by dialysis (up to 80 per cent) This is probably not due to losing a co-factor because passage of the enzyme over a 'Sephadex G-25' column produces only a small loss of activity (about 15 per cent) Heating the enzyme for 5 min at 80° C causes an inactivation of 90 per cent The enzyme is inhibited by iodoacetic acid and also by maleic and succinic acids resembling the ATC-ase from *Streptococcus faecalis*[6]

ATC-ase is the first enzyme in the biosynthesis of pyrimidines in bacteria and mammals[1] Yates and Pardee[7] first observed, using a crude extract, that *E coli* ATC-ase is inhibited by cytidine-5′-phosphate This inhibition is thought to be a physiologically significant regulator of pyrimidine biosynthesis in bacteria well known as feedback or end-product inhibition[8]

End-product inhibition has not been observed previously in higher plant enzymes[9], but, on the basis of the data presented here, it seems reasonable to suggest that this regulatory mechanism exists here also As can be seen in Table 2, ATC-ase from lettuce seedlings is inhibited strongly by various pyrimidine nucleotides The inhibition appears to be specific for pyrimidine nucleotides since the respective nucleosides and two purine nucleotides as well as orthophosphate do not inhibit Uridylic acid is at least ten times as potent an inhibitor as cytidylic acid, and the inhibitory activity among the uridine compounds decreases with increasing phosphorylation These last two features of the ATC-ase from lettuce seedlings differ markedly from the ATC-ase from *E coli*[7,10], which is sensitive to cytidine rather than uridine nucleotides and where the inhibition increases with increasing phosphorylation Whether the inhibition specificity for different end-products reflects a variance in the amount of free nucleotides present in the cell sap of *E coli* and lettuce seedlings remains to be ascertained

JOSEPH NEUMANN *
MARY ELLEN JONES †

Graduate Department of Biochemistry,
Brandeis University, Waltham, Mass

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* Fellow of the Rockefeller Foundation Permanent address Department of Botany, Hebrew University, Jerusalem

† Scholar of American Cancer Society, National Science Foundation grant (G-14717)

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PHYSIOLOGY

Endocrine Control of the Plasma Albumin/Globulin Ratio

REVERSAL of the albumin/globulin ratio is commonly encountered in Africans in different parts of the continent of Africa This reversal is ascribed in large measure to malnutrition and/or infection Whatever the cause, the mechanism for this reversal still remains obscure

Since we had indicated previously that the fatty liver of kwashiorkor is more than likely dependent on a particular endocrine configuration, induced by whatever is responsible for the acute episode of kwashiorkor[1], the possibility was entertained that the reversed albumin/globulin ratio might also be linked in some way to a particular endocrine physiology Accordingly, this possibility was investigated in baboons

Ten normal and three totally hypophysectomized baboons were used in this work The normal animals were housed under standard conditions in the colony The operated animals remained in a constant-temperature room overnight

A transpalatine approach was used to expose the hypophysis and the gland was removed under direct vision Five and a half months after hypophysectomy, B413 received 20 mgm hydrocortisone daily for seven weeks

Oxalated blood samples for electrophoresis and for estimation of total serum protein, albumin and globulin were always collected at 9 o'clock each morning, 16 hr after the last meal

Paper electrophoresis was carried out on hanging strips of Whatman filter paper No 1 in veronal buffer (0 05 M pH 8·6) for 16 hr The paper strips were stained with bromophenol blue A Zeiss densitometer was used for the photometry of stained strips Total serum protein and albumin concentrations were estimated by the biuret method and the globulin level was obtained by difference

Serum protein analyses were performed on ten normal baboons The mean total protein (6 94 ± 0 47 gm per cent), albumin (3·74 ± 0 31 gm per cent) and globulin (2 71 ± 0 32 gm per cent) concentrations and the albumin/globulin ratio (A/G ratio) were determined together with their standard deviations (Table 1) Similar estimations were performed on three hypophysectomized baboons, from seven to twenty-six weeks after the operation The total serum protein concentrations of B413, B450 and B390 were significantly lower than those prior to hypophysectomy, being 5 28, 6 14 and 5 98 gm per cent respectively The albumin concentrations of all three hypophysectomized animals (1 30–2 04 gm.

Table 1 SERUM PROTEIN CONCENTRATIONS IN NORMAL AND HYPOPHYSECTOMIZED BABOONS

Baboon No	Total protein (gm %)	Albumin (gm %)	Globulin (gm %)	Albumin / Globulin
10 normal baboons*	5 60–7 38	3 20–4 40	2 40–3 22	1 07–1 62
Mean ± S D	6 94±0 47	3 74±0 31	2 71±0 32	1 378±0 152
B 390				
Normal	6 55	3 70	2 85	1 296
Hypophysectomy	5 98	1 30	4 68	0 278
B 413				
Normal	6 74	3 92	2 82	1 394
Hypophysectomy	5 28	1 92	3 36	0 571
B 450				
Normal	6 39	3 66	2 79	1 293
Hypophysectomy	6 14	2 04	4 10	0 503

* The figures presented for ten normal baboons are the range and the mean ± standard deviation

Table 2 ALBUMIN, GLOBULIN AND γ-GLOBULIN EXPRESSED AS PERCENTAGE TOTAL PROTEIN (T P.) IN NORMAL AND HYPOPHYSECTOMIZED BABOONS

Baboon No	Treatment	Albumin (% of T P)	Globulin (% of T P)	γ-Globulin (% of T P)
10 Controls	Nil*	57 48	42 52	—
26 Controls	Nil†	—	—	18 30
B 390	Normal*	56 50	43 50	—
	Hypophysectomized*	19 23	80 77	—
	Hypophysectomized†	18 30	81 70	50 30
B 413	Normal*	58 20	41 80	—
	Hypophysectomized*	36 40	63 60	—
	Hypophysectomized†	41 50	58 50	26 80
B 450	Normal*	57 30	42 70	—
	Hypophysectomized*	33 25	66 75	—
	Hypophysectomized†	30 71	69 29	33 10

* Biuret
† Electrophoresis

per cent) were significantly lower and the globulin levels (3 36–4 68 gm per cent) higher than normal The A/G ratio (1 378 in normal baboons) was markedly depressed to values between 0 278 and 0 571 (Table 1) Furthermore, γ-globulin concentrations in the operated animals, seven or more weeks after hypophysectomy, were significantly elevated above the normal level of 18 3 per cent of total protein[2] γ-Globulin concentration in *B*413 had risen to 26 8 per cent of the total protein, to 33 10 per cent in *B*450 and to 50 3 per cent in *B*390 (Table 2)

*B*413 received 20 mgm hydrocortisone daily for seven weeks Both the total protein and albumin concentrations rose to levels well within the normal range (6 50 and 3 40 gm per cent respectively) Weekly estimations of serum proteins, furthermore, showed the globulin concentration to have decreased by 0 48 gm per cent to a level of 3 10 gm per cent, which may be considered normal The increasing albumin and decreasing globulin concentrations during the period of hormone treatment are thus reflected in the changing A/G ratio, which rose from 0 622 to 1 098 (Table 3)

Table 3 EFFECT OF HYDROCORTISONE THERAPY ON HYPOPHYSECTOMIZED BABOON, *B* 413

Treatment	Total protein (gm %)	Albumin (gm %)	Globulin (gm %)	Albumin / Globulin
Nil	5 80	2 22	3 58	0 622
Hydrocortisone 20 mgm /day				
After 2 weeks	6 04	2 64	3 40	0 741
After 4 weeks	6 26	2 84	3 42	0 829
After 5 weeks and 2 days	6 38	3 28	3 10	1 058
After 6 weeks and 2 days	6 42	3 44	2 98	1 149
After 7 weeks and 2 days	6 50	3 40	3 10	1 098

Long-standing hypophysectomy in three baboons caused a reversal of the albumin/globulin ratio This change is reflected both by a decrease of the serum albumin and an increase of the serum globulin concentration, which in large measure is a reflexion of an increase in γ-globulin, while the total serum protein-level was slightly depressed

Levin and Leathem[3] concluded from their work on both adrenalectomized and hypophysectomized rats that alterations in the serum protein pattern following hypophysectomy could be attributed to an adrenal as well as a thyroid effect They suggested that in the rat the serum albumin-level is maintained under adrenocortical influence while the serum globulin-level is associated with activity of the thyroid gland Following hypophysectomy a change in both fractions occurs because of the decrease of both adrenocortical and thyroid activity

Hartman *et al* [4] demonstrated a drop in serum albumin and a rise in serum globulin following adrenalectomy in rats In one of our hypophysectomized baboons, the elevated serum globulin was reduced to within the normal range after treatment with hydrocortisone Indeed, the albumin concentration was more markedly affected than the globulin by hydrocortisone therapy. Albumin concentration rose by nearly 1 2 gm per 100 ml serum but the globulin-level was decreased by 480 mgm per 100 ml serum.

In view of the observations in baboons cited here, the reversed albumin/globulin ratio in kwashiorkor, like the fatty liver, is most probably dependent on a particular endocrine physiology which emerges in malnutrition and in kwashiorkor

CYRIL BEARDWOOD *
CHRISTINE GILBERT *
JOSEPH GILLMAN *

Joint C S I R /University Nutrition Research Unit,
University of the Witwatersrand,
Johannesburg, South Africa

* Present address National Institute of Health and Medical Research, P O Box M 32, Accra, Ghana

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β-Hydroxybutyrate and Sodium Citrate as Stimuli of the *in vitro* Secretion of Insulin

BRACHMACHARI *et al* [1,2] have shown that certain metabolites like β-hydroxybutyrate, citrate, isocitrate and succinate, when injected into the animal (rabbit), produce hypoglycæmia, and this is interpreted as being due to a stimulus of the secretion of insulin β-Hydroxybutyrate and citrate, according to the aforementioned experiments, seem to act, directly or indirectly, by different mechanisms, in view of the fact that the effect obtained is considerably more with citrate than with β-hydroxybutyrate and because this metabolite seems to increase first the content of active insulin/gm of pancreas

As we have shown that pancreas slices when incubated *in vitro* respond to certain stimuli (with insulin secretion) we thought it would be interesting to find out if β-hydroxybutyrate and citrate, when added to the buffer surrounding pancreas slices, produce an increase in secretion of insulin

Table 1 INSULIN EFFECT OF THE INCUBATION BUFFER PLUS β-HYDROXYBUTYRATE OR SODIUM CITRATE SURROUNDING PANCREAS SLICES (No of experiments in parentheses)

Metabolites	Glucose uptake (mean)		
β-Hydroxybutyrate $4 \times 10^{-4} M$ (30)	0 470	± 0 017	$P > 0 05$
Control	0 436		
Sodium citrate $1 \times 10^{-3} M$ (20)	0 574	± 0 029	$P < 0 01$
Control	0 388		
Sodium citrate $7 \times 10^{-5} M$ (30)	0 331	± 0 013	$P = 0 01$
Control	0 281		

The technique already described[3] was adopted, using concentrations of citrate of 1×10^{-3} and $7 \times 10^{-5} M$ and $4 \times 10^{-4} M$ of β-hydroxybutyrate As can be seen in Table 1, the citrate causes secretion of insulin, but not β-hydroxybutyrate, at the given dosage This fact suggests to us that if both metabolites cause secretion of insulin in the animal, it does so by different mechanisms Possibly by an indirect way in the case of the β-hydroxybutyrate and by a direct mechanism in the case of the citrate It could be by synthesis of adenosine triphosphate

J L R-Candela
R R-Candela
D Martin-Hernández
T Castilla-Cortazar

Instituto 'G Marañón',
Velazquez 138,
Madrid 6

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Correlation of the Effects of Insulin and of Muscular Contraction *in vitro* on Uptake of Glucose by the Frog

Heimreich and Cori[1] and later R-Candela[2] among others have shown that the specificity to sensitive sugars is similar both with insulin and muscular contraction On the other hand, the great resistance of toads to insulin injections is known from the work of Rapela *et al* [3]. We have confirmed this finding in frogs injected with up to 40 U /100 gm without showing any signs of hypoglycæmia

These results inspired us to examine the possible correlation of the effects of insulin *in vitro* and of muscular contraction *in vitro* on uptake of glucose by the frog recti abdominis muscle

Frogs (*Rana esculenta* L) weighing 28–35 gm were used After killing, the muscles were removed and weighed, one of the muscles was always used as control of the other They were incubated in small vessels in a Dubnoff shaker at 37° C and stirred for 2 hr The incubation medium was 2 ml of frog-Ringer containing 3 mgm /ml glucose Contraction was elicited by incorporation of 10^{-5} *M* acetylcoline Insulin was used at a concentration of 0 1 and 0 5 mU /ml

In another series of experiments alternating 15-min periods of contraction and relaxation were induced in the same muscle, by transferring it from the buffer containing acetylcholine to the relaxation buffer and vice versa, after very careful washing and blotting on filter paper

Determinations of glucose were carried out by the Somogyi–Nelso method

The results shown in Table 1 are different from those described for mammals

(*a*) Gemmill *et al* [4,5] showed that uptake of glucose and synthesis of glycogen are stimulated in the rat diaphragm *in vitro* by the addition of insulin Our results, on the other hand, show that insulin does not increase uptake of glucose by frog muscle

(*b*) Helmreich[1] and R-Candela[2] have shown, using different experimental conditions, that muscular contraction increases the uptake of glucose sugars with a specificity similar to that found when using insulin

Table 1

	Without insulin	With insulin (0 5 U /ml)	Alternative contraction and relaxation without insulin
Contraction	0 171 ± 0 041 (20)	0 180 ± 0 047 (20)	Contraction every 15 min 0 158 ± 0 036 (20)
Relaxation	0 170 ± 0 041 (20)	0 201 ± 0 047 (20)	Relaxation every 15 min 0 148 ± 0 036 (20)

No significant differences

In the case of the frog, muscular contraction does not increase uptake of glucose

Though Gemmill[6] and Beloff-Chain[7] state that insulin does not increase oxygen consumption by mammalian muscles, Hall[8], Gourley[9], Fisher[10], Smillie and Manery[11] find that insulin increases significantly the consumption of oxygen by frog skeletal muscle

These experimental findings indicate the existence of a correlation between the effects of insulin and muscular contraction on uptake of glucose by muscle

R R-Candela
M Rodriguez Lopez
T. Castilla-Cortazar
J L R-Candela

Instituto 'G Marañón',
Velazquez 138,
Madrid, 6

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Reversible Blocking of Nerve Conduction by Alternating-Current Excitation

In recent years, the attention of control engineers has been attracted to the communication characteristics of nerves, particularly in regard to their function in the feedback control of muscle Muscles are highly non-linear power amplifiers, which require complex nervous control circuitry to permit smooth and continuous movement of the limbs This control circuitry consists of multiple feedback loops, and the problem is to determine experimentally the function of individual loops One approach is to open circuit specific peripheral neural paths and observe the effect on over-all performance

This can be done, and problems encountered with surgical and pharmacological methods avoided, by subjecting the nerve to alternating-current excitation This method offers the advantage of reversibility, and also enables fibres to be selectively blocked according to their size An investigation has been undertaken on peripheral nerves to study the effect of alternating-current excitation on axonal conduction The sciatic nerves of large green frogs were used and a section of nerve, approximately 8 cm in length, extending from the thigh to the peroneal and tibial extensions, was supported on an array of six platinum electrodes in a bath of mineral oil (see Fig 1*a*) A pulse was applied repetitively to the stimulating electrodes and the response at the recording electrodes was amplified and displayed on an oscilloscope The centrally located blocking electrodes were excited from an audio oscillator Since the signal at the recording electrodes contained, superimposed, both the time-response of the nerve and an alternating-current component due to the ohmic coupling with the oscillator, a subtraction circuit was incorporated

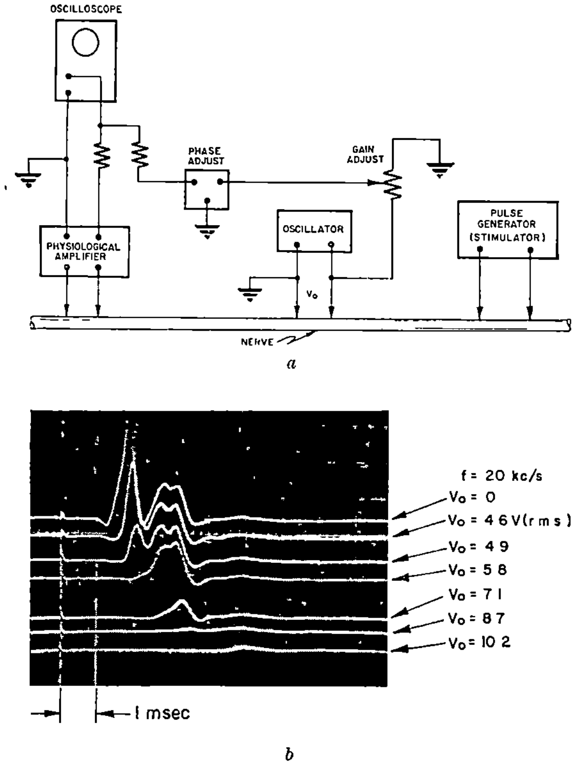

Fig. 1. *a*, Circuit arrangement for investigating reversible block of nerve conduction; *b*, characteristic compound action potential of frog sciatic nerve subjected to various levels of alternating-current excitation at 20 kc./s.

to remove the alternating-current component. A typical record is given in Fig. 1*b*, which shows the effect of various levels of alternating-current excitation at 20 kc./s. on maximal evoked α-, β- and γ-fibre responses. It will be noted that the blocking is selective, the response of the α-fibres showing considerable attenuation before any significant changes appear in the β- and γ-responses. On removal of the alternating-current excitation, the original response (corresponding to $V = 0$) is immediately restored, that is, the effect is reversible.

This experiment has also been conducted with the central portion of the nerve placed between two transversely located electrodes, in a separate bath of saline. The results were similar.

It is suggested that blocking is due to local depolarization of the nerve and that the selectivity in relation to fibre size is consistent with the membrane impedances. As a first approximation the equivalent circuit[1,2] of a membrane can be considered as a parallel combination of resistance and capacitance. The α-fibres, because of their larger surface area, have a large capacitance and a smaller resistance per unit length than the β- and γ-fibres. Therefore at any given level of alternating current excitation, the current through the large fibres would be greater than through the small fibres and the depolarizing effect would be correspondingly greater.

J. A. TANNER

Instruments and Control Systems Laboratory,
Division of Mechanical Engineering,
National Research Council,
Ottawa, 2.

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PHARMACOLOGY

Influence of Actinomycins on the Content of 17-Hydroxycorticosteroids in Guinea Pig Plasma

FOR some time we have been examining effects of the actinomycins in animals. For this purpose we used a pure crystalline preparation, aurantin, which contained fractions A_1, A_2, A_3 identical respectively to C_3, C_2 and C_1 fraction of the actinomycin C [1,2]. The difference between the preparations seems to be only in quantitative proportions of the aforementioned fractions[2,3]. There are indirect indications that the antibiotics of this group stimulate the function of the adrenal cortex, but there are no published results testifying to an increased concentration of corticosteroids in blood caused by the influence of actinomycins. The report here gives the results obtained in an investigation of this problem.

Male guinea pigs weighing 250–300 g have been tested. The blood was taken from the tested animals by heart puncture at a strictly definite time. For each blood-count separate groups of animals were used with six guinea pigs in each group. The free 17-hydroxycorticosteroids in the blood plasma were determined by Yudaev and Pankov's modification of Silber and Porter's method[4].

The results obtained through a single intraperitoneal aurantin injection are represented in Table 1. The *t*-test was used to evaluate the significance of the results. From these data it follows that the concentration of 17-hydroxycorticosteroids increases 1.5–2 times during 2 days after a single injection of 30 μg/kg of antibiotics. The single injection dose 75 μg/kg causes an increased concentration of 17-hydroxycorticosteroids during 7 days. Their maximum content is determined on the second day of the injection of the preparation and exceeds the control-level almost six-fold. The above doses of aurantin did not have any conspicuous toxic effect on guinea pigs at a single injection.

Table 2 shows results of investigation obtained at an intraperitoneal aurantin injection of 30 μg/kg three times a week. The blood was taken from the animals 24 h after the antibiotic injection. It can be seen from Table 2 that this increase in concentration of 17-hydroxycorticosteroids occurs 1 h after, and is observed during the whole period of the injection of the preparation and also 10 days after its termination. It should be noted that a continuous use of the preparation at this dosage caused a reduction in weight and death of some animals. With continuous aurantin injections of a non-toxic dose of 10 μg/kg the increase in concentration of 17-hydroxicorticosteroids does not begin until a fortnight after the start of investigations.

Thus, using aurantin once in a non-toxic dose of 75 μg/kg it is possible to achieve a greater effect than at a 30 μg/kg dose injection 3 times a week, though the latter injected in the way described exerts a toxic influence.

Hence, it may be supposed that reduction in the weight of the lymphoid organs observed during the injection of the antibiotics of the actinomycin group to the laboratory animals is associated with the influence of the latter on the function of adrenal cortex.

It is possible that a similar mechanism of the actinomycin action is of some importance in the therapeutic

Table 1

No	Group	Plasma concentration of 17-hydroxycorticosteroids in µg/100 ml					
		Prior to injection	1 day	2 days	4 days	7 days	12 days
1	Control	21 2±3 7	44 5±6 0	23 3±2 8	27 9±3 9	25 7±4 3	31 2±6 8
2	Aurantin 30 µg/kg	21 2±3 7	64 0±5 8	49 4±10 2	31 9±3 5	26 8±5 0	—
3	Aurantin 75 µg/kg	21 2±3 7	66 5±4 8	137 0±29 6	54 3±7 4	59 7±11 9	48 2±6 7
	P		1–2<0 05 1–3<0 05 2–3>0 05	1–2<0 05 1–3<0 01 2–3<0 05	1–2>0 05 1–3<0 05 2–3<0 05	1–2>0 05 1–3<0 05 2–3<0 05	1–3>0 05

Table 2

No	Groups	Plasma concentration of 17-hydroxycorticosteroids in µg/100 ml								
			1 injection						10 days after the end of injection	25 days after the end of injection
		Prior to injection	1 hour	24 hours	2 injection	3 injection	6 injection	7 injection		
1	Control	36 7±6 2	61 2±7 4	40 0±3 5	32 6±5 8	40 2±4 4	49 5±6 3	44 0±7 1	36 3±9 3	41 2±6 8
2	Aurantin 30µg/kg	36 7±6 2	86 6±2 6	74 4±12 3	61 9±9 3	53 6±3 6	85 2±11 8	73 2±8 1	70 2±11 2	47 5±7 4
	P		<0 01	<0 05	<0 05	<0 05	<0 05	<0 05	<0 05	>0 05

effect of these preparations in treatment lymphogranulomatosis and some forms of leucoses

J PLANELLES
N OZERETSKOVSKY
O DJEKSENBAEV

N F Gamaleya Institute of
Epidemiology and Microbiology,
Academy of Medical Sciences of the U S S R,
Moscow

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Refractoriness (Tachyphylaxis) to 'Sarin'

APART from experiments reported in 1954 by Barnes and Duff[1], the occurrence of tachyphylaxis to poisons which act by inhibiting cholinesterase has been little publicized, although the phenomenon appears to offer possibilities as a means of investigating cholinergic function The following is an account of a method which, in a proportion of experiments, was found to lead to a high degree of refractoriness to 'Sarin' (isopropyl methyl phosphonofluoridate) in the cat, the conditions being those of tachyphylaxis

The anæsthetic was pentobarbital and all drugs were given intravenously (femoral vein) The median lethal dose of 'Sarin' for the cat anæsthetized in this manner is approximately 0 06 mgm /kgm, and the initial phase of the experiment consisted in the injection of about ten times this amount, the animal being protected by a combination of atropine sulphate (2–4 mgm /kgm) and pentamethonium bromide (20–40 mgm /kgm), supplemented on occasion by oxygen or artificial ventilation The antidotal drugs were given fractionally to lessen their vasodepressant action When recovery from the acute effects of the initial injection of 'Sarin' was complete, a further period of 0 5–1 5 hr was allowed to elapse and the cat was then injected with more 'Sarin' The procedure followed was to give repeated injections at 2-min intervals The early ones were extremely small (0 02 mgm /kgm each), and, as a rule, after three or four such injections a crisis developed, with a profound fall in blood pressure and virtual cessation of breathing However, on interrupting the injections rapid recovery took place, and, on resuming the injections it was found, in successful experiments, that larger and larger doses of 'Sarin' had progressively less and less effect Finally, repeated doses as large as 10 mgm / kgm each have been given, and cats have survived amounts totalling 50–200 times a lethal dose of 'Sarin', after allowing for the 10-fold protection to be expected from the antagonist drugs given One animal permitted to regain consciousness was retested one week later and found normally susceptible to 'Sarin', which indicates that the condition is temporary only

The nature of the physiological or biochemical changes in the animal associated with refractoriness has not been examined

C A DECANDOLE

Defence Research Board of Canada,
Suffield Experimental Station,
Ralston, Alberta

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HISTOCHEMISTRY

Histochemical Demonstration of 6-Phosphogluconate Dehydrogenase in Proliferating and Malignant Cells

IN a series of histochemical studies on tissues of the human and of rats and mice, the activity of the enzyme 6-phosphogluconate dehydrogenase was found to be remarkably increased in proliferating cells, whether they were benign or malignant This enzyme is part of the respiratory mechanism known as the pentose pathway, or 'shunt', which may provide an alternative glycolytic pathway from glucose to pyruvate it may also be implicated in the synthesis of the nucleic acids[1] Because of the possible significance of the results obtained in diverse experiments during the past two years, it was considered advisable to communicate these preliminary findings

Pieces of fresh tissue, about 5 mm^3, were chilled by being pressed lightly against the inside of a glass tube (3 in × 1 in) which had been cooled overnight in solid carbon dioxide ('Cardice') in a Dewar flask The tube was recorked and replaced, as quickly as possible, in contact with the solid carbon dioxide in the flask Sections were cut at 8 or 12μ on a refrigerated microtome, maintained at about −25° C, and they were mounted on glass slides The sections were removed from the refrigerated cabinet and were covered with the appropriate warm incubation medium The complete fluid was prepared as follows neotetrazolium (Universal Crop Protection, Ltd) was dissolved in warm 0 05 M phosphate buffer at *p*H 7 8, at a concentration of 1 mg per ml To a few millilitres of the filtered solution was added triphosphopyridine nucleotide (TPN, 5 mg/ml) and this solution was saturated with barium phosphogluconate Serial sections were treated with a similar incubation medium which lacked either the TPN or the phosphogluconate The sections were incubated in a moist chamber at 37° C for 2 h A positive result was recorded only for those areas of the section which showed a significant deposit of the formazan with the complete medium, but a negligible deposition when incubated in the media which lacked either substrate or TPN

Normal rat liver showed only very slight or even no staining by this method This was surprising, since it was known[1] that appreciable activity had been detected, by biochemical procedures, in this tissue Thus it would appear either that the method, as described, is insufficiently sensitive, or that the substrate cannot penetrate to the site of the enzyme in these sections in which the integrity of the cytological structure is well preserved[2] That the latter is the likely explanation is indicated by the finding that the livers of rats treated with ether or chloroform become stained fairly strongly by this procedure very shortly after the animal has been subjected to these anæsthetics[3] Hence in these studies it was necessary to kill the rats and mice with nitrogen This effect of increasing the penetration of the substrate might be rather analogous at the subcellular level to the inability of 6-phosphogluconate or TPN to penetrate the membrane of the human erythrocyte Thus these cells, when tested by a spectrophotometric biochemical method[4], showed no activity in the presence of this substrate and TPN On the other hand, definite activity was observed immediately after the addition of a saponin, which assumably disrupted the membrane and liberated the enzyme

The first studies were concerned with the respiratory changes that occur during carcinogenesis in the liver of rats fed with 4-dimethylaminoazobenzene (DMAB) A marked increase in the phosphogluconate dehydrogenase activity was found in the liver of rats fed with the basal diet which Elson[5] recommended for facilitating the induction of tumours (diet *C*) In all these experiments this diet was reinforced by the thrice weekly addition of about 20 g of cabbage for each rat, rats fed with this did not produce tumours, although a high yield was obtained when it was supplemented with 0 06 per cent DMAB Examination of such livers showed that both regenerating and neoplastic areas gave high levels of enzyme activity In some cases well-differentiated tumours are difficult to distinguish from regenerating livers Hence, as an additional precaution, the enzyme levels of secondary growths in the diaphragm and lung were studied, the activity of this enzyme was found to be very high in these growths On the other hand, negligible staining was observed in those areas which showed abnormal histological architecture and which, but for the apparent paucity of mitoses, might have been considered malignant In other studies on mice, a similar stimulation of the phosphogluconate dehydrogenase activity by this diet was observed in the liver, the bronchial epithelium was also responsive to this reaction, the degree varying apparently directly with the amount of hyperplasia detected In a third investigation, on human lung cancers, marked staining of the growing areas of both squamous and oat cell cancers has been seen in the relatively few cases investigated Normal bronchial epithelium contrasted strongly with adjacent neoplastic tissue and could be easily distinguished on this reaction alone

It is necessary to assess the significance of these results, and thus the limitations as well as the advantages of the histochemical procedure Clearly it is only by such methods that the biochemistry of relatively few active cells, such as may comprise small metastases, can be investigated However, it is invariably more satisfactory if the histochemical technique has been tested against biochemical estimations in such circumstances as this may be feasible, and the validity of the histochemistry so established At present this has not been done, facilities for effecting this have only recently become available On the other hand, these histochemical findings have a certain significance in that it has been shown that for this reaction to occur in the actively proliferating tissue studied it is essential to have both phosphogluconate and TPN present in the incubation medium Thus these results may be of interest in indicating a probable stimulation of phosphogluconate dehydrogenase in cellular proliferation and particularly in neoplastic growth Moreover, the findings from the experiment on the effect of the altered diet suggest that tissues may become predisposed towards an enzymatic situation which is characteristic of proliferation

We thank Dr E Nassau, Mr J Jackson and Mr G F Cummings of Harefield Hospital for their collaboration We also thank Mr H J Cotes, without whose personal generosity this work could not have been completed, and acknowledge other financial assistance from the British Empire Cancer Campaign and from the Prophit Fund (L B)

J CHAYEN
L BITENSKY
E K AVES
G R N JONES
A A SILCOX
G J CUNNINGHAM

Department of Pathology,
Royal College of Surgeons of England,
London, W C 2

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Intracellular Mucus in the Human Bronchial Tree

A FURTHER step in the investigation of the composition of mucus in chronic bronchitis and other pulmonary diseases has been made possible by the use of combined histochemical and enzyme digestion methods to characterize epithelial mucus-secreting cells

An important component is acid mucopolysaccharide which can be detected by its staining blue in a combined alcian blue/periodic acid–Schiff stain (AB/PAS)[1] Two types of acid mucopolysaccharide can be traced If two consecutive sections are stained by AB/PAS, one after digestion with neuraminidase, the site of neuraminidase-susceptible neuraminic acid is indicated by the elimination of blue staining[2] The other accepted source of acid in mucopolysaccharide—sulphate—has been traced by Pelc's method of autoradiography–sulphur-35 as sulphate, given intraperitoneally to mice and rats, the animals being killed up to 2 hr after injections of 8 μc /kgm body-weight in human bronchi, by tissue culture of bronchial biopsy material in medium to which labelled sulphate has been added

In the mouse, surface epithelium and glands show no sulphate uptake, all acid mucopolysaccharide being susceptible In the rat a regional variation not previously described has been found (1) in peripheral airways no uptake of sulphate occurs, any acid mucopolysaccharide being neuraminidase-susceptible, (2) in the trachea most (but not all) cells containing acid mucopolysaccharide take up sulphate[3], none being susceptible to neuraminidase, (3) in the large bronchi of the hilar region both types of acid mucopolysaccharide are found

After 1 hr in the culture medium uptake of sulphate by the human bronchus is found only in acini which are granular, there is uptake of sulphate in an occasional distended cell of the surface epithelium and also in cells which do not stain for mucopolysaccharide after tissue culture, but probably represent granular cells That these sulphated cells stain poorly may be an example of the irregular staining with alcian blue by known sulphated compounds[4]

The finding of acid mucopolysaccharide in human bronchus is thus independent of any uptake of sulphate except for the occasional cell mentioned above Granule-containing ('granular') cells as well as those distended with secretions ('distended' cells) are both found in surface epithelium, duct and gland acini Generally, granules show the same range of staining characteristics as distended cells, and with the AB/PAS method both types include blue-, purple- and red-staining cells, while after sialidase-digestion cells of the same colours are seen but in different proportions Epithelial mucopolysaccharides have been isolated which contain a large proportion of neuraminidase-resistant neuraminic acid[5,6] Sialidase converts some blue cells to purple, some blue to red and some purple to red, that is, some blue and purple cells contain an acid mucopolysaccharide which is completely susceptible to sialidase, but others contain mucopolysaccharide resistant to this enzyme although no uptake of sulphate is seen The significance of purple-staining cytoplasm is not clear, it may represent a lesser concentration of acid mucopolysaccharide than does the blue, but it may also be due to acidic groups other than neuraminic acid or sulphate Evidence can be offered for each of these hypotheses

These resistant blue cells are relatively more numerous on the surface and in ducts than in the glands As the volume of the glands is much greater than that of the goblet cells[7] it may be that these surface cells contribute so small a fraction to total secretion that this has not been detected *in vitro*

One of us (R A G) acknowledges the support of the Population Council Inc , Rockefeller Institute, New York

LYNNE REID
C MCCARTHY
JALE DUVENCI
Institute of Diseases of the Chest,
Brompton Hospital, London S W 3

R A GIBBONS
National Institute for Research in Dairying

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HISTOLOGY

Dermal Elastin in Actinic Elastosis and Pseudoxanthoma Elasticum

FOR sixty-five years the identity of the fibres staining with elastic tissue stains in actinic (senile) elastosis has been the subject of debate[1–5] Recent work has shown that elastotic dermis contains more hexosamine and less hydroxyproline than covered skin and that these changes are localized in the upper dermis[6] This communication describes the estimation of elastin from elastotic skin determined after extraction in hot alkali[7]

Chronically sun-damaged skin (actinic elastosis) was obtained from three human volunteers Skin from one individual with pseudoxanthoma elasticum was obtained at necropsy The presence of increased elastic tissue staining was confirmed histologically in all cases Skin of the abdomen from necropsied adult humans was used as controls, only the upper 0 3 mm elastotic and control skin were used corresponding to the localization of the staining abnormality The entire thickness of defatted pseudoxanthoma elasticum skin was used The epidermis was heat separated and the dry weight of dermis obtained Dermis was minced in a high-speed homogenizer and extracted for 15 min four times in 0 1 *N* sodium hydroxide in a boiling water bath The suspension was centrifuged at 20,000*g* and the residue hydrolysed overnight in sealed tubes at 100° C with 4 *N* hydrochloric acid Hydroxyproline and Kjeldahl nitrogen determinations were performed on aliquots Elastin content was calculated by multiplication of the hydroxyproline by 62 5, the nitrogen by 5 78, based on hydroxyproline content of 1 6 per cent and nitrogen of 17 3 per cent[8,9]

Table 1 gives the results expressed as mgm elastin/100 mgm dry weight dermis, based on hydroxyproline content The mean of the ten controls was 2 41 ± 0 7 In contrast, elastotic dermis contained 8 02, 13 08 and 10 45 mgm elastin and dermis from one individual with pseudoxanthoma elasticum had an elastin content of 6 34 mgm It should be noted that, based on the nitrogen content of the alkali-insoluble residue, the elastin content would be even higher for the elastotic specimens

The method of preparation of these fractions and their hydroxyproline content suggest that the increased elastic tissue staining seen in histological sec-

Table 1 ELASTIN IN HUMAN DERMIS
(mgm /100 mgm dry weight)

Controls	Actinic elastosis	Pseudoxanthoma elasticum
1 55	8 02	6 34
2 71	13 08	
3 12	10 45	
2 66		
2 06		
3 72		
1 80		
2 38		
2 79		
1 28		

Elastin content of the upper 0 3 mm of control and actinic elastosis skin Entire thickness of pseudoxanthoma elasticum skin used Based on hydroxyproline × 62 5

tions of actinic elastosis and pseudoxanthoma elasticum is probably true elastin More vigorous identification of this material as elastin must await complete amino-acid analysis, which is in progress

This work was supported by the Frederic M Hanes Trust Fund and a grant from the National Institute of Arthritis and Metabolic Diseases, *A*, 4315 Actinic elastosis skin specimens were obtained through the co-operation of Dr Charles C Tindall, Kissimmee, Florida

J GRAHAM SMITH, JUN
EUGENE A DAVIDSON
RICHARD D CLARK

Division of Dermatology, Department of Medicine,
Department of Biochemistry, and
Center for the Study of Aging,
Duke University Medical Center,
Durham, North Carolina

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IMMUNOLOGY

Recovery of Thyroid Antibodies from Human Thyroid Preparations sensitized *in vitro*

THE development of circulating autoantibodies to thyroglobulin in some forms of experimentally produced animal thyroiditis or in chronic human thyroiditis has been described[1]

A method for isolating the thyroid antibodies in relatively pure form is of value for serological and physicochemical studies A convenient method for recovery of blood group antibodies from the powder of erythrocyte stroma was previously described[2] A somewhat similar procedure was used in the present work to obtain 'pure' thyroid autoantibodies from sera of patients suffering from chronic thyroiditis, and thyroid heteroantibodies from sera of rabbits injected with human thyroid extract

Human thyroid gland was ground in a Waring blender, spread in a thin layer in Petri dishes, and dried at 37° C The preparation was gently scraped off with a razor blade, and the fine thyroid powder was obtained

Table 1 REACTIONS OF ANTI-THYROID SERA AND ELUATES OBTAINED FROM SENSITIZED HUMAN THYROID POWDER

		Tanned cell hæmagglutination, highest dilution of the specimen tested giving marked reaction with human red blood cells coated by human thyroid extract		Complement fixation test, highest dilution of 50 per cent human thyroid extract giving marked reaction with the specimen tested	
		Whole serum	Eluate	Whole serum	Eluate
Rabbit anti-human thyroid serum	No 775	2,187	243	65,610	21,870
Rabbit anti-human thyroglobulin serum	No 767	6,561	729	65,610	21,870
	No 766	59,049	6,561		
Human thyroiditis serum	War	2,187	243	< 90	< 90
	Swi	2,187	243	< 90	< 90

As expected, the suspension of thyroid powder reacted strongly in complement fixation tests with rabbit antisera against human thyroid and human thyroglobulin On washing, the thyroid powder released a potent soluble antigenic material, presumably thyroglobulin, which could be demonstrated in supernatants from at least six consecutive washings

When the suspension of thyroid powder was heated for 30 min at 100° C its antigenic activity was not influenced at all, a finding which was not surprising in view of the thermostability of thyroglobulin[3] However, washing fluids of heated powder were apparently devoid of any *in vitro* serological activity It seemed conceivable that heat coagulation of the surrounding protein material might have bound thyroglobulin to the thyroid particles

A fine suspension of thyroid powder was prepared by vigorous shaking of 0 2 gm of the powder in 10 ml of saline Some experiments were performed with unaltered preparations, whereas for others, the suspension was heated for 30 min at 100° C The powder was washed at least three times with saline To 0 4 ml of washed sediment was added 1 ml of undiluted human serum from patients suffering from chronic thyroiditis or rabbit antiserum against human thyroid The tubes were shaken for 2 hr on an electric shaker Parallel experiments were performed at 4°, 23° and 37° C The sensitized powder was washed at the temperature of sensitization for at least four times and resuspended in 0 5 ml of saline In the fourth washing fluid of sensitized thyroid powder no thyroid antibodies, or only traces of them, could be detected Then the tubes were shaken for 5 min in a water bath at 56° C , and centrifuged for 5 min at 3,000 r p m in a heated centrifuge at a constant temperature of 56° C After centrifugation, the eluates were withdrawn as quickly as possible

Eluates from unheated thyroid powder did not react in tanned cell haemagglutination and complement fixation tests with human thyroid extract This finding may be attributed to the release from the unheated powder of active antigenic material which probably neutralized most of the antibodies Anticomplementariness found in eluates of thyroid antibody of rabbit origin might have indicated the presence of soluble antigen–antibody complexes

As is to be seen in Table 1, eluates from heated thyroid powder contained thyroid antibodies, rabbit antibodies were active in tanned cell hæmagglutination and complement fixation tests, human antibodies were active in tanned cell hæmagglutination tests only (as were the original thyroiditis sera) The activity of eluates apparently did not depend upon the temperature of sensitization, preparations obtained from thyroid powder sensitized at 4°, 23°, and 37° C were equally potent Eluates from similarly treated powder of human organs other than thyroid did not contain thyroid antibodies Serological reactions with eluates of thyroid antibodies were weaker than the reactions with the corresponding original sera

The first elution was never complete and two to three consecutive eluates still showed marked antibody activity Antibodies against human serum proteins present in rabbit anti-human thyroid sera were reduced to traces in eluates

The eluates of human thyroid autoantibody effectively inhibited the activity of rabbit anti-human γ-globulin serum in bringing about the agglutination of Rh^+ red blood cells sensitized by incomplete Rh antibody In addition, sera of two rabbits immunized intravenously with these eluates acted as potent Coombs reagents These experiments established the fact that eluates contained γ-globulin

If desired, the eluates of thyroid antibodies might be concentrated by freeze-drying or evaporation and further properties of the tissue-reactive antibodies studied

This work was supported by grant C–2357 from the National Institutes of Health

F Milgrom
N R Rose
E Witebsky

Department of Bacteriology and Immunology,
School of Medicine,
University of Buffalo

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Transfer of Nephrotoxic Glomerulonephritis in Rats by means of White Blood Cells

Transfer of experimental glomerulonephritis on originally healthy rats has been accomplished in this and other laboratories by means of parabiosis[1,2] Transfer was observed in random-bred as well as in inbred rats, either in short-term experiments or after intervals up to 71 days between institution of nephrotoxic serum glomerulonephritis in the primarily nephritic animal, and parabiotic conjunction with the originally healthy partner[3–5] Fluorescein-labelled antisera were used to exclude the involvement of the primarily injected heterologous (rabbit) antibodies in the transfer phenomenon[6] From these experiments it was derived that a secondary nephritis-producing factor, appearing as a consequence of the nephrotoxic serum glomerulonephritis in the circulation of the primarily nephritic rat, was transferred to the primarily healthy animal capable of attacking the corresponding organs of the latter

This conclusion was supported by recent experiments which were an attempt to localize the transfer factor in question First, the parabiosis was substituted by continuous cross-transfusion (that is, bilateral carotid artery-jugular vein anastomosis), and glomerulonephritis was transferred on 5 out of 15 healthy rats following cross-circulation of 2 5–4 days duration[7] Secondly, plasma and white blood cells from nephritic rats were injected (for 4–15 days) or infused (by using a modification of the technique of Armin *et al* [8] for continuous infusion of solutions in unanæsthetized animals) into healthy recipients As in the former experiments, a highly inbred strain of Sprague–Dawley rats was used consistently Development of experimental glomerulonephritis was observed in 5 out of 31 recipients following transfer of 210–450 million white blood cells from 4–10 nephritic donors, only in 1 out of 32 animals receiving 10–35 ml of plasma from the same donors, the controls, 52 recipients of plasma and white blood cells from healthy donors, being completely negative

Since in rats 75–80 per cent of the white blood cells are lymphocytes and transfer of experimental 'autoimmune-nephrosis' on immunologically tolerant rats recently was achieved by injecting lymph node cells[9], the results reported herewith are in favour of a lymphocyte-bound nature of the secondary nephritis-producing factor in nephrotoxic serum glomerulonephritis likewise in rats In contrast to the transfer of an autoimmune-nephrosis, however, Freund's adjuvants was not used in our experiments They suggest, on the other hand, the induction of an autologous hypersensitivity reaction of the delayed type following a passive heterologous anaphylaxis in the glomeruli Possibly this secondary mechanism is responsible for the progression of the disease, once it has started Further work is required for elucidating the immunological nature of the secondary nephritis-producing factor in question

This work was supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg, Germany

E F Pfeiffer
W Müller-Ruchholtz
K Federlin

I Medizinische Universitatsklinik,
Ludwig-Rehnstr, 14,
Frankfurt/Main

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BIOLOGY

Significance of Mitotic Duration in evaluating Kinetics of Cellular Proliferation

Mitotic rate or 'rate of cell renewal' can be defined as the number of cells which complete their mitosis per unit time

$$\text{Mitotic rate} = \frac{\text{mitotic count}}{\text{mitotic duration}}.$$

As can be seen from this equation, and as also emphasized by Leblond and Walker[1], mitotic count can be used to estimate mitotic rate only if mitotic duration is not significantly influenced by the experimental conditions

The literature on kinetics of cellular proliferation seems to display an agreement concerning the time for synthesizing DNA (S) and the mitotic duration (M) which are assumed to remain relatively constant even during periods with changing rates of cell renewal The intermitotic 'resting period' (G_1) is, on the other hand, supposed to change parallel to the changes in growth-rate of the tissues under examination This has led to the assumption that mitotic count and number of cell nuclei which are labelled with a radioactive DNA precursor can be used synonymously with mitotic rate and rate of DNA synthesis The correctness of this assumption may be doubted after the following investigations, which demonstrate experimentally induced changes of mitotic duration

By the colcemid technique the mitotic duration of the epidermal cells of 'normal' hairless mice has been estimated to about 1 hr , and the average mitotic count has been found to be 0 57 per cent The animals were killed between 9 and 10 a m

The estimation of the DNA synthesizing time was made by using repeated injections of tritiated thymidine with subsequent autoradiography, and the rate of DNA synthesis was analogously calculated from the number of labelled cell nuclei and the DNA synthesizing time The DNA synthesizing time is thus found to be about 5 3 hr and the rate of DNA synthesis to 0 55 per cent per hour between 9 and 10 a m

In a previous paper[2] it has been shown that the mitotic count and the number of DNA synthesizing cells in the epidermis of hairless mice decreased during the first two days after application of 0 005 ml of 1 per cent 3 methylcholanthrene in benzene A very short initial increase in the mitotic count has been shown to be due to blocking of the mitoses[3] After the first two days an increase both in mitotic count and in number of DNA synthesizing cells was observed The values reached a peak on the 11th day after the application of methylcholanthrene, and then fell slowly for the next four weeks

After application of different carcinogens to the epidermis of these animals, it was observed, in subsequent experiments, that the mitotic duration and the DNA synthesizing time as well as the mitotic rate and the rate of DNA synthesis vary considerably The experiments show that the rate of mitoses and of DNA synthesis roughly parallel each other, and are increased about three times 24 hr after the application of methylcholanthrene The mitotic duration and the DNA synthesizing time are at the same time decreased to a quarter of normal values The mitotic count is markedly increased eleven days after the application of methylcholanthrene The mitotic rate, however, is only slightly above the rate in untreated epidermis, and the mitotic duration is increased to about 2 hr

It has thus been demonstrated that the application of certain skin carcinogens to mouse epidermis heavily affects the mitotic duration and the DNA synthesizing time Previous calculations of the rate of cell renewal directly by mitotic counts after application of carcinogens are thus open for re-evaluation

ARNE EVENSEN

Institutt for generell og eksperimentell patologi,
Universitetet i Oslo

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Leptospira Infection in the Gold Fish (*Carassius auratus*)

THE important role of waterholes, rice fields, swamps slow-moving streams and sewers in the epidemiology of human and animal leptospirosis has been established[1-3] Wild animals and migratory birds, present in surrounding areas, have been found to be infected and have been implicated in the dissemination of the disease[4-7] On the other hand, little is known about the role of strictly aquatic forms such as fish, or of partially aquatic forms such as amphibians Negative results have been obtained in isolation attempts from minnows[8] and amphibians[5], equivocal findings have been reported in frogs[9,10], leptospiral antibodies have been found in water turtles[11,12]

The ability of *Leptospira* to penetrate the bodies of fish living in contaminated water has been determined in the experiments reported here, the course of the infection in the host's tissues has been followed

Goldfish (*Carassius auratus*) 5 cm long, free from infection, were used They were maintained in tap water which was contaminated with 7-day-old cultures of *L pomona* and *L canicola* The concentration of micro-organisms was determined by subculturing serial ten-fold dilutions in semi-solid Fletcher's medium containing 10 per cent rabbit serum The water was aerated and the temperature regulated at 18°–20° C The fish were fed twice daily The original *p*H of the water was 5, but before contamination it was raised to neutrality by addition of 10 per cent sodium bicarbonate solution

Dark field microscopy and fluorescent antibody procedure were utilized in the detection of micro-organisms in the sediment obtained by high-speed centrifugation of water samples Cultures of water in semi-solid Fletcher's medium were made in the first experiment only

The search for *Leptospira* in fish was made by cultural means (the seeding of about 0 05 ml of brain, heart, kidney and muscle tissues collected and shredded aseptically by means of a capillary pipette) and by means of fluorescent antibody procedure applied both to smears and to frozen tissue sections Cultures were submitted every second week for six weeks to dark-field microscopy and in case of contamination or of negative results, a drop was smeared on a slide and 'stained' with the fluorescent antibody technique

Methods used in the preparation of immune sera, fluorescent conjugates, sorption, collection and sectioning of tissues, and microscopic observation have been reported earlier[13] Anti-*L pomona* and *L canicola* conjugates were used in the first and second experiment, respectively They intensely stained smears made from cultures of *L icterohaemorragiae*, *L canicola* and *L pomona*, and did not stain *L biflexa* Sorption of the conjugates with dog liver, chicken embryo, and trout muscle extracts gave satisfactory results The smears and sections were fixed for 5 min in acetone They were then dried and washed in buffered saline for 10 min before applying the conjugate This was left in contact with the experimental material for 1 hr at room temperature before washing and mounting with buffered glycerin

Specificity of the fluorescent antibody procedure was tested on materials not containing *Leptospira*, materials containing *Leptospira*, and by inhibitions of staining by application of anti-*Leptospira* immune, non-labelled globulin before use of the fluorescein-labelled globulin.

In a preliminary experiment carried out for orientation purposes fish were introduced into a tank of contaminated water of *p*H 7.2 containing 50 *L. pomona* per ml. At 1 hr., 4 hr. and 1, 2 and 8 days, 20 ml. samples of water and two fish were collected and examined for the presence of *Leptospira*.

The micro-organisms were demonstrable in the water by means of dark-field microscopy for 24 hr., and by means of the fluorescent antibody procedure for 8 days following contamination. *Leptospira* were present in the gills, liver and muscle of the fish from 1 hr. after contamination to 8 days, the end of the experiment. The gills appeared to be the site of highest concentration of *Leptospira*, liver and muscle were next highest in that order. In the muscle, limited numbers of *Leptospira* were present in the blood vessels, while in the liver, on the eighth day, they were also present in the parenchyma.

A second experiment was extended over a longer period of time (17 days) and a higher number of *Leptospira* were present in the water (800,000 *L. canicola* per ml.). After being kept in the contaminated water for 24 hr., fish were transferred to clean water of *p*H 5. This passage was frequently repeated during the course of the experiment in order to eliminate the possibility of a super-infection.

A water sample and one fish were collected at intervals for a period of 17 days. Cultures were made from brain, heart, liver, kidneys and abdominal muscles of the fish. *Leptospira* were visible in the water by means of dark-field microscopy and fluorescent antibody 'staining' for 24 hr. after contamination, but never in the samples collected at a later time. Cultures obtained from brain, heart and muscle of the fish were positive from 1 hr. to the tenth day, while cultures from the kidneys were positive from the second to the seventeenth day. Fluorescent antibody 'staining' demonstrated the presence of the infection in the gills and the liver at 1 hr., in the muscle at 24 hr., in the kidney at 48 hr., and persistence of the infection in all tissues to the seventeenth day. The highest concentration of *Leptospira* was found on the surface and the tissues of the gills. High concentrations were also present in the hepatic parenchyma from the third to the eighth day, and in the kidney from the eighth to the seventeenth day.

It seems to be obvious that *Leptospira* can penetrate into goldfish through the gills because only sections of this tissue and not of intestine and of skin showed the presence of micro-organisms at the beginning of the experiment. A large volume of water is constantly circulating through the gills, and fine particles can be mechanically detained there, for example, *Leptospira*, yeasts, bacteria and amorphous debris. *Leptospira* can then apparently find their way into the blood-stream and spread throughout the animal's tissues.

Results obtained through the application of cultural and fluorescent antibody procedures were similar. In a large number of contaminated cultures, the fluorescent antibody procedure detected the presence of the *Leptospira* where dark-field microscopy had given negative results.

Goldfish are susceptible to *Leptospira* infection when living in contaminated water and also appear to harbour these organisms for at least 17 days even when living in water with a *p*H which is lethal to the organism. No serological or histopathological work was performed, and thus no evaluation made on the immunological and tissue responses of the host to the infection. The gills and kidneys seem to be the major site of localization of the *Leptospira* after the septicæmic stage, suggesting the possibility of shedding micro-organisms into the surrounding water.

The aforementioned results, although obtained from the study of few individuals, strongly support the possibility of fish occupying an important role in the epidemiology of *Leptospira* infection in certain environments. The results also indicate that an investigation of aquatic animals in endemic areas might yield useful information.

This work was supported in part by the National Institutes of Health grant *E*-2181.

G. MAESTRONE*
M. A. BENJAMINSON†

Animal Medical Center,
New York.

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* Present address: Squibb Institute for Medical Research, New Brunswick, New Jersey.

† Present address: Bronx Hospital, New York.

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Sexual Differentiation of Merozoites of *Barrouxia schneideri* (Butschli)

SCHELLACK[1] in a study of Coccidia from *Lithobius* and *Scolopendra* recognized the presence of more than one type of merozoite in *Barrouxia schneideri*. He nevertheless concluded they did not represent sexually dimorphic forms. In recent cytochemical investigations of the same parasite two types of merozoite were recognized, which differed in the amount of carbohydrate reserve stored in the cytoplasm and in nuclear structure. Using the periodic acid–Schiff test, some bundles of merozoites were shown to have no carbohydrate reserves other than a few fine granules. These correspond to the microgametocytes which have sparse reserves of fine, discrete granules occupying the central cytoplasm. Other merozoites, often shorter and broader in form, have considerable reserves already in the form of large granules merging together. There is no difficulty in tracing a series of developmental stages between these and the macrogametocytes with their abundant carbohydrate reserves. Furthermore nuclear differences existing between the two types of merozoite can be traced to the gametocytes. The conspicuous polar nucleolus of the 'female' merozoite persists throughout the development of the macrogametocyte. A nucleolus is absent from the nuclei of the 'male' merozoite and the micro-gametocyte.

Such a demonstration of what is highly probably sexual dimorphism in the merozoites of *Barrouxia*, again raises the question if two sexes exist throughout the entire life-cycle as a general rule in the Coccidia. We know from the work of Dobell and Jameson[2] on *Aggregata eberthi* that the reduction division occurs, not during gametogenesis but in the first division of the zygote, so that the parasite is haploid. This is now accepted as universal in the Coccidia. Furthermore, from Tyzzer's demonstration[3] that a patent infection with *Eimeria maxima* can be achieved with a single sporulated oocyst we know that the zygote carries the characters of both sexes. This is now a routine method of producing pure-line infections of Coccidia. Sexual differentiation occurs at some point between the diploid, bisexual zygote and the haploid macro- and micro-gametocytes.

If sex determination is genotypic the male and female characters must be separated at nuclear division either during schizogony or sporogony. If separation of the sex characters occurred at zygotic meiosis the resulting sporocysts would be unisexual, male or female, and it would not be possible to produce a patent infection with a single sporocyst. If separation occurred at nuclear division within the sporocyst both male and female sporozoites would be enclosed within the sporocyst, which would then be sufficient to establish an infection resulting in oocyst production. If separation occurs only at schizogony the sporozoites must be bisexual and a single sporozoite would be sufficient to establish a patent infection.

The existence of two sexually differentiated types of merozoite and the inability of a single sporocyst to produce both sexes would indicate zygotic meiosis as the point of sex separation. Reyer[4] was unable to obtain a patent infection of *Barrouxia schneideri* using a single sporocyst but could do so with a single oocyst. Though he unfortunately did not establish if the sporocyst resulted in the production of one type of gametocyte his results, together with the present demonstration of the existence of two types of merozoite, is strongly indicative that the reduction division is the point of sexual differentiation.

Single sporocyst and single sporozoite infections are being attempted with *Eimeria tenella* to ascertain the sex of the bodies resulting. If either all male or all female infections can result from inoculation of a single sporozoite, phenotypic sex determination is ruled out.

ELIZABETH U. CANNING

Department of Zoology,
Imperial College of Science and Technology,
London, S.W.7.

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Discharge of Marrow Eosinophils following Re-injection of Foreign Protein

In an earlier investigation[1], using the quantitative methods developed in this laboratory[2], it was shown that very large reserves of mature and nearly mature eosinophils are present in the bone marrow of normal guinea pigs. The question arose as to the circumstances in which these cells might be discharged, and for this reason it seemed important to carry out a quantitative investigation during the circulatory eosinophilia which follows the re-injection of foreign protein in sensitized animals. Percentage changes in bone marrow eosinophils have been noted in these conditions[3].

Preliminary experiments showed that a high blood eosinophil-level was present in guinea pigs, 24–30 hr. after the last of 4 subcutaneous injections of horse serum at 5-day intervals; when the fourth injection was replaced by saline or egg albumen, a much lower level was found. In the main series, three groups, each containing 10 healthy male guinea pigs of the Mill Hill strain, were examined; the final body-weight of each animal was approximately 400 gm. Four sterile subcutaneous injections of 1 ml. volume were administered at 5-day intervals. Group *A* received saline on each occasion. Group *B* received horse serum for the first 3 and saline for the fourth injection. In Group *C*, all 4 injections were of horse serum. To make the groups comparable with others still under investigation, each animal also received subcutaneous injections of saline at 1, 9 and 24 hr. after the fourth injection; at 29 hr. the animal was killed and the numbers of eosinophils per $mm.^3$ of blood and bone marrow determined by methods essentially similar to those reported earlier[1].

The average counts of eosinophils per $mm.^3$ of blood ($\pm$ *S.D.*) were: group *A*, 71 ± 65; *B*, 150 ± 68; *C*, 976 ± 443. In group *A*, the average number of eosinophils per $mm.^3$ of marrow (76,500 ± 15,500) was similar to normal[1]. The average number in group *B* (128,000 ± 39,200) was about 70 per cent higher and this increase was largely the result of an increase in the marrow reserves of band and segmented eosinophils; this can be interpreted as being the result of a proliferation of the eosinophil series following the last foreign protein injection some 6 days before. The average number of eosinophils in group *C* (74,300 ± 36,000 per $mm.^3$) was 42 per cent less than in *B*, and again, mature and nearly mature forms were largely responsible for the difference. As there was no evidence of increased destruction or phagocytosis of eosinophils in the marrow, this difference may give a measure of the number of extra eosinophils discharged into the circulation of group *C* in the 29 hr. following the re-injection of specific antigen. (All the differences mentioned were significant at the 0·01-level of probability.)

By using quantitative data already obtained for bone marrow volume[4] and blood volume in the 400-gm. guinea pig, one can make very approximate calculations of the total numbers of eosinophils involved[1]. It would appear that on average about 340×10^6 band and segmented eosinophils had been discharged from the marrow reserves of group *C* but that less than 10 per cent of this total was present in the blood-stream. One may presume that more than 90 per cent had already left the circulation.

It would seem that quantitative studies of similar design might be utilized to throw light on other factors influencing the release of eosinophil leucocytes from the bone marrow.

G. HUDSON

Department of Anatomy,
University of Bristol.

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Triops granarius (Lucas) (Crustacea) as a Natural Enemy of Mosquito Larvæ

LITERATURE on the natural enemies of mosquitoes reviewed by Hinman[1-3], contains little to suggest that crustaceans are of much significance as biological control agents. Therefore, and in view of the fact that there is renewed interest in biotic factors in vector control[4], this communication may stimulate others to pursue the matter further than I can.

El Bur (4° 41′ N., 46° 36′ E.) in the Mudugh plain of central-northern Somalia is a village of about 300 huts placed on a small elevation above a depression in which some shallow wells have been dug. The climate is typical of the region with a total of about 10 in. rain falling during April–May (*gu* rains) and September–November (*der* rains).

In 1956 El Bur was visited by the Somalia National Malaria Service during the *gu* rains when 15–20 adult *Anopheles gambiae* per room were caught by hand. An attempt to find larvæ either in the wells or in a large shallow depression which had filled with rain water, however, was unsuccessful. This was the more puzzling since no other water suitable as breeding places was anywhere to be found.

During this search it was noted that the water along the whole fringe of the depression was swarming with a large crustacean later kindly identified for me by Mr. Longhurst of the British Museum as *Triops granarius* (Lucas) (Fig. 1). It seemed that the presence of this crustacean corresponded with the absence of larvæ and this was afterwards confirmed by numerous other observations in Mudugh and Mijustinia during 1956–59, as, for example, at the villages of Adado, 1956–57, Sinadogo 1957, El Bur 1957–59, Mataban 1957, Salahdadab 1957, Goffafo 1957, Mirrich 1957–59 (Maffi 1960)[5]. In Sinadogo, Mirrich and Adado it was particularly noticeable that those wells which had mosquito larvæ contained no *Triops*. Such wells were separated by only a few metres from wells with *Triops* and no mosquito larvæ.

At no time were *Triops* actually caught in the act of catching or devouring mosquito larvæ, but the possibility that they do so or in some way make the water unattractive or uninhabitable for mosquito larvæ cannot be dismissed.

Longhurst[6] indicates that *Triops* usually occur in temporary pools and that the eggs show great powers of resisting desiccation. In Somalia, *Triops* are mostly found in exposed shallow collections of still and muddy water without vertical vegetation and in shallow wells, its habitats thus presenting conditions highly favourable for *A. gambiae*.

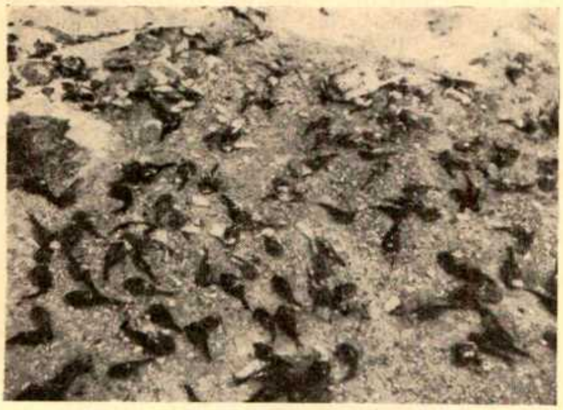

Fig. 1. *Triops granarius* along the margin of a shallow pool at El Bur, Somalia, 1956

Though the evidence is far from conclusive it is obvious that the matter merits further study. The particular characteristics of *Triops* which are of interest to the sanitarian are, of course, the ability of the eggs to withstand desiccation and the preferential breeding places which coincide so nicely with those of *Anopheles gambiae*.

MARIO MAFFI *

P.O. Box 8067,
Causeway, Salisbury,
Southern Rhodesia.

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* Formerly, Somalia National Malaria Service, Mogadishiu, Somalia.

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Maturation of Schistosome Eggs *in vitro*

EGG-LAYING *in vitro* by *Schistosoma japonicum* and *S. mansoni* in serum media was not described by early investigators[1-4], and presumably did not occur. Later it was reported that a few eggs were laid when flukes were kept for up to two months in a glass apparatus with provision for daily change of medium (horse serum)[5]. A method of maintaining *S. mansoni* flukes so that a regular output of 30–40 eggs a day was obtained has recently been published[6-8]. Some cell division was seen, but the eggs did not mature in serum media, saline or Tyrode's solution.

During experiments in which *S. mansoni* pairs were kept in whole blood continuously dialysed against serum, some mature eggs were found after 15 days, and a few hatched out apparently normal miracidia. After further investigation, it became clear that the presence of blood cells assisted maturation and that eggs were laid and would mature in medium consisting of one part of serum, two parts of Tyrode's solution and a small addition of red and white cells; dialysis of the medium was not necessary. Attempts were then made to obtain enough miracidia to infect snails.

Paired *S. japonicum* and *S. mansoni* flukes were removed from portal veins of infected hamsters and placed in Carrel flasks containing 3 ml. of a guinea pig serum/Tyrode medium (1 : 2) with added glucose, streptomycin and penicillin. This had proved to be the best medium for regular egg production[8]. Five to nine days later, when eggs had been laid, the medium was changed to human serum/Tyrode with a few drops of buffy coat. Full sterile precautions were observed. Although many eggs were produced and cell-division began, none reached maturity, and the medium was clearly unsatisfactory.

In spite of the pre-eminence of guinea pig serum, it was decided to keep *japonicum* flukes in human serum/Tyrode (3 : 10) and *mansoni* flukes in baboon serum/Tyrode (3 : 10) with added glucose (2 mgm./ml.) and penicillin and streptomycin (100 units/ml.). One pair was incubated at 37° C. in each 6 in. × ¾ in. tube containing 5 ml. of medium. Tubes were opened after 14–17 days and the eggs recovered, washed well in saline and hatched in tap water in a watch glass.

Twenty to thirty miracidia were obtained from each *S. japonicum* tube and at least 30 from each *S. mansoni* tube These came from the layings during the first few days of incubation, and many more live immature eggs at all stages of development were seen Snails (*Australorbis glabratus* and *Oncomelania hupensis*) were exposed for 2 hr to approximately 10 freshly hatched miracidia per snail and penetration was seen to take place They were checked for the production of cercariæ between 4 and 11 weeks after infection and when all were found to be negative, were squashed and examined No sign of infection could be found Although the method has been successful in maturing the eggs of these two species of schistosome *in vitro*, the miracidia, the behaviour of which appeared normal in every way, were unable to develop after penetrating the snail hosts

S mansoni flukes have been cultivated *in vitro* from 7 days old to near maturity[9-10] Now that egg-laying by adults and maturation of the eggs have been achieved, only two stages of the mammalian cycle *in vitro* remain, that from cercaria to 7 day larva and from sexually immature to mature adult

J NEWSOME

Medical Research Council
Bilharzia Research Unit,
Winches Farm,
Hatfield Road,
St Albans, Herts

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Multiple Medullæ in Wool Fibres

IN the course of investigations on the fleece of Blackface sheep[1], we encountered several of the multi-medullated fibres described by Jones[2] We made no permanent record of these fibres, but they certainly occurred in samples from more than one sheep Since this time we have been examining samples from different strains of the same breed in a different locality and again have found similar fibres Out of 50 sheep in one experiment, multi-medullated fibres were found in samples from five individuals In each case the fibres occurred in samples taken in two separate years We estimate that between 1 and 5 per cent of fibres in these samples showed this characteristic at some stage in their growth A few fragments were found in which the medulla could be seen to change from single to double

Multiple medullæ are most commonly found among the fine wool fibres, derived from secondary follicles, of which about 70 per cent in this breed are medullated The type of medullation in the fine fibres varies from the uninterrupted to the fragmental grades described by Wildman[3] Whereas the multiple medulla have been commonly found in fine wool fibres of between 36 and 48μ diameter we have recently found some to occur in the coarse hair fibres associated with the primary follicles Invariably these were in the finest of the coarse fibres, ranging from 60–74μ, and almost always occurred in a region in which the normal lattice type medulla was absent We have previously regarded such fibres as developmental aberrations of unknown origin although it seems that they are more or less permanent features of their parent follicles

J M DONEY
W F SMITH

Hill Farming Research Organization,
48 Palmerston Place,
Edinburgh

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Occurrence of *Schoenus nigricans* L. in the Blanket Bogs of Western Ireland and North-West Scotland

THE anomalous position of the fen plant *Schoenus nigricans* in the blanket bogs of western Ireland[1,2] and north-west Scotland[2] can be explained to some degree by the extreme sensitivity of this species to aluminium ions

Growth chamber experiments have shown that concentrations of aluminium ions as low as 1 p p m will severely inhibit root elongation in *Schoenus*

The experiments described here were carried out in water culture, at 21° C , 16 hr day-length and 90 per cent relative humidity The culture solutions were replaced three times in a week The seed was collected from Lough Carra, Co Mayo The seedlings were germinated on filter paper in distilled water and used when one month old

The effect of aluminium ions on the root elongation of *Schoenus* is shown in Fig 1

The results of an experiment showing root elongation of *Schoenus* in natural blanket bog water collected

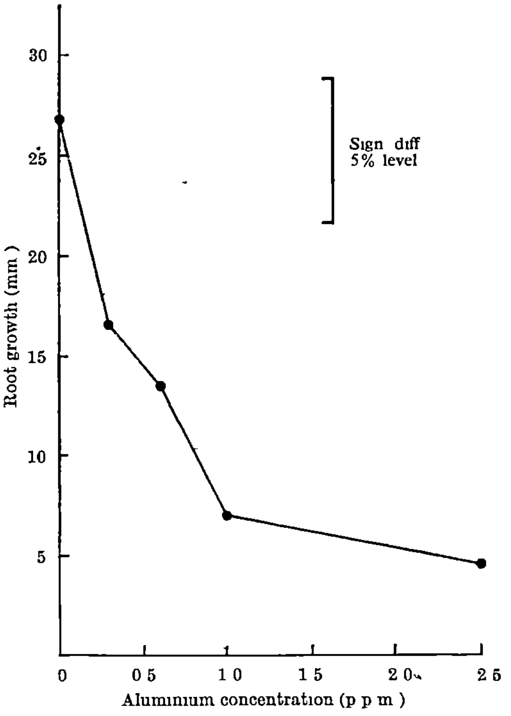

Fig 1. Root elongation of *Schoenus* seedlings at various aluminium concentrations. The ionic concentrations of other cations in solution were calcium, 15 p p m , magnesium, 9 p p m , sodium, 12 p p m , potassium, 3 p p m , iron, 1 p p m , *p*H 4 5 Duration of experiment, 42 days

Table 1

	Root elongation (mm.)	*S.E.*
Natural blanket bog water	51.1	± 7.1
With 1 p.p.m. aluminium added	12.2	± 3.7
'Synthetic' blanket bog water	48.1	± 7.9
With 1 p.p.m. aluminium added	7.6	± 2.9

Duration of experiment, 42 days

from western Ireland and that of a culture solution made up to resemble this in all major inorganic ions, are given in Table 1.

Aluminium would adversely affect the establishment and growth of the plant due to its inhibition of root growth.

The results of analyses of ombrotrophic bog waters are presented in Table 2.

Table 2. CALCIUM AND ALUMINIUM CONCENTRATIONS OF SEVERAL BOG WATERS IN THE BRITISH ISLES

Bogs with *Schoenus*	Aluminium (p.p.m.)	Calcium (p.p.m.)
Blanket bog drain, Skye	0.31	3.00
Blanket bog, Glenamoy, Co. Mayo	0.03	1.26
Blanket bog, Sheskin Lodge, Co. Mayo	0.51	0.87
Blanket bog, Owennaglogh R., Co. Mayo	0.38	0.50
Bog near Cromaglan Br., Co. Kerry	0.40	0.63
Blanket bog, Owenmore R., Co. Kerry	0.28	0.80
Bogs without *Schoenus*		
Coom Rigg Moss, North Pennines	1.4	0.50
	2.8	0.50
Rusland Moss, Lancs	1.1	0.80
Blanket bog, near Tongue, Sutherland	1.2	0.73
Woodford raised bog, Co. Galway	1.1	0.78
Raised bog, near Ballinasloe, Co. Roscommon	1.03	1.20

I suggest that *S. nigricans* is excluded from other ombrotrophic bogs in the British Isles by the presence of high concentrations of aluminium ions.

The main source of aluminium in ombrotrophic bogs is probably from atmospheric dust particles. The west coast of Ireland, with its higher humidity and prevailing westerly winds, receives less dust than the rest of the British Isles.

I thank Dr. P. J. Newbould for his help. This work was carried out during the tenure of a Department of Scientific and Industrial Research studentship.

J. SPARLING

Botany Department,
University College, London.

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Isolation of *Microsporum gypseum* from Cow-dung

Microsporum gypseum, known as a soil saprophyte of worldwide occurrence, has recently been traced in the soil in cucumber greenhouses[1,2].

Contrary to the findings reported by Alsop and Prior[1], in my survey of Dutch greenhouses no keratinous fertilizers had been added to the soil[2]. In a search for the origin of this soil infection various soil improvers introduced into the greenhouses were tested mycologically using the hair-bait method. Of all materials examined only 'pot-soil', commonly marketed in Holland as a soil improver for the cultivation of cucumbers, yielded a growth of *M. gypseum*.

According to the firm selling 'pot-soil', this preparation consisted of seven different components, that is, various kinds of peat-moor and peat-bog, dune-sand and cow-dung rich in straw content. All component parts were mycologically tested, but only from one of these, namely, cow-dung, was *M. gypseum* isolated.

This finding, together with the fact that nowhere outside the greenhouse could *M. gypseum* be isolated from the soil, gave me an indication that this fungus might have been introduced from outside into the greenhouse. Gentles[1,3] has also directed the attention to this possibility.

Later on, independently of this finding, *M. gypseum* could be traced to a heap of cow-dung on a farm where the labourer had contracted suppurative ringworm caused by *M. gypseum*. For several months the patient's chief duty had been the carrying and spreading of cow-dung into the fields. The heap of cow-dung from which the isolations were made had been lying outside the cow-shed for about a month. Wheat straw was the only other substance apart from cow-dung, both being directly derived from the housed cattle.

These two different cases in which *M. gypseum* could be traced to cow-dung indicate that *M. gypseum* infection in cucumber greenhouses is likely to be a 'man-made infection', this fungus being introduced into the greenhouse during efforts to improve the soil. Moreover, this finding might provide a clue to the ultimate habitat of *M. gypseum*, which accounts for its patchy global occurrence.

A. H. KLOKKE

Institute of Dermatology,
Department of Mycology, Rotterdam.

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N(6)-benzyladenine as a Senescence Inhibitor for Selected Horticultural Crops

A STUDY was undertaken on the post-harvest application of N(6)-benzyladenine (manufactured and patented as '*SD* 4901' by the Shell Development Co., Modesto, California) to certain fruits and vegetables in an attempt to delay the onset of senescence. This is a continuation of investigations into the extension of the storage life of fruits, vegetables, and other products with ionizing radiations[1-3], modified atmospheres[4], antibiotics and antifungal chemicals[5-7], and growth regulators[8].

It has been reported[9] that after a crop is harvested, general degradation sets in. This results in the destruction of the soluble ribonucleic acid. Thus, protein synthesis slows down, and the subsequent disintegration into amino-acids provides a medium for the growth of micro-organisms. As the mechanism of protein formation is disturbed, the pigments and other constituents disintegrate and unedible products become evident. The primary step in the degradation of the soluble ribonucleic acid is thought to involve the loss of the end group adenine. A treatment with N(6)-benzyladenine therefore should provide the necessary adenine and restore the soluble ribonucleic acid molecule. Protein synthesis thus would be maintained and the treated produce would stay fresh for a longer time.

Sweet cherries, cauliflower, endive, parsley, snap beans, lettuce (head, leaf, and romaine), radishes with leaves, bunching onions, and cabbage of prime quality were selected for the experiments. Before treatment they were stored at 5 ± 1° C. for a week in an attempt to accelerate enzymatic activity. Such acceleration seemed desirable to convert complex carbohydrates into sugars which would then be available in larger amounts over a longer time for the process of respiration. The produce was then dipped for a minute in 5, 10, and 20 p.p.m. aqueous

Table 1 INCREASE IN STORAGE-LIFE OF HORTICULTURAL CROPS BY THE TREATMENT WITH N(6)-BENZYLADENINE (20 P P M) OVER THE NON-TREATED SAMPLES WHEN STORED AT 5° C AND 95 PER CENT RELATIVE HUMIDITY

Crop	Increase storage-life (days)	Crop	Increase storage-life (days)
Sweet cherries	0	Snap beans	4
Cauliflower	2	Parsley	5
Radishes	2	Lettuce—leaf	3
Celery	3	romaine	5
Endive	3	head	7
Bunching onions	3	Cabbage	8

solutions of N(6)-benzyladenine 'Triton *B* 1956' (a product of Rohm and Haas, Philadelphia, Pennsylvania) at 0 1 per cent was used as a wetting agent The treated samples, along with controls (a dip in distilled water containing the wetting agent), were stored at 5 ± 1° C and at 95 ± 2 per cent relative humidity The produce received two additional applications separated by weekly intervals in the early part of the experiments in an attempt to simulate water treatments given by wholesale dealers and grocers Replicates of some of the crops were also used for studying the effects of the chemical and its concentrations on the respiratory behaviour of the produce A respiratory rate based on evolution of carbon dioxide was measured according to the method of Claypool and Keefer[10]

The respiratory rate varies with the crop In general, it is inversely proportional to the storage life and subsequent marketability of the fresh produce For some of the treated vegetables the respiratory rates (to be published in detail elsewhere) decreased over time This was attributable to the application of the N(6)-benzyladenine which prolonged the storage life and marketable quality by delaying the onset of senescence of the several crops treated (Table 1)

Detailed investigations along the lines discussed are contemplated in co-operation with local wholesale dealers, grocers, and the Shell Development Company Effects of pre-harvest treatments with N(6)-benzyladenine on the post-harvest storage of fruits and vegetables may also be studied

D K SALUNKHE
A S DHALIWAL
A A BOE

Food Technology Section,
Department of Horticulture,
Utah State University, Logan, Utah

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Response of Plants to Root-absorbed Chemical Growth Substances applied in Culture Solutions

A CONTINUOUSLY available supply of biologically active chemicals from a large external pool during the course of experimentation with higher plants is often desirable Foliage sprays or μl solution applications of growth substances to the vegetative tips of plants result in the imposition of heroic dosages to restricted parts Such treatments constitute a crude approach in duplicating an endogenous synthesis and in maintaining physiologically tolerable levels in the plant

Table 1 PHYSIOLOGICALLY EFFECTIVE DOSAGES IN NEGATIVE LOG MOLAR CONCENTRATIONS OF SEVERAL PLANT-GROWTH SUBSTANCES ADDED TO AERATED CULTURE SOLUTIONS USED AS THE ROOT-GROWTH MEDIUM

Plant growth substances*	*Cucumis sativus* L	*Lycopersicon esculentum* Mill	*Pisum sativum* L
	(Concentration in -log molar)		
Indolyl-3-acetic acid	3–5	4–6	4–5
Gibberellin A_3	3–6	4–6	5–7
Kinetin (3-furfurylaminopurine)	5–6	5–8	5–6
2-Chloroethyl trimethylammonium chloride and related compounds	3–5	4–7	3–5
2,3,5-Triiodobenzoic acid	5–6	4–6	—
Maleic hydrazide	4–5	4–5	—
2,4-Dichlorobenzyltributylphosphonium chloride	4–5	—	—
α-Naphthaleneacetic acid	5–8	—	—

* Gibberellin A_3 supplied by Merck and Co , Rahway, New Jersey, 2-chloroethyl trimethylammonium chloride supplied by Dr N E Tolbert, Michigan State University and the American Cyanamid Co , Stanford, Connecticut, maleic hydrazide by the Naugatuck Chemical Co , Naugatuck, Connecticut, 2,4-dichlorobenzyl tributylphosphonium chloride supplied by the Virginia-Carolino Chemical Corp , Richmond, Virginia

A suggested approach is to add the test chemical to the root medium commonly used in growing plants in aerated culture solutions[1] The method is reproducible and offers control of the duration of exposure to the chemical stimulus Absorption-rates for isotopically labelled compounds may be determined, and changes in root growth as well as in aerial parts observed

Recently, several plant-growth inhibitors have been applied to the soil in which the roots are growing[2-4] Results of the biological activity are often unreliable and difficult to interpret because of many soil-imposed variables cation fixation, micro-organism destruction, leaching, and unequal distribution of the applied chemical For evaluating 'steady state'[5] growth responses of plants it should be advantageous to apply chemical stimuli separately or in combination to the culture solution The solutions can be renewed at will

Addition of plant-growth substances to aerated culture solution also provides a means of recording responses to chemicals such as kinetin, which are often ineffective when applied by other methods[7] Compounds, such as 2-chloroethyl trimethylammonium chloride and 2,4-dichlorobenzyl tributylphosphonium chloride are often toxic as foliar sprays, but give a marked response when added to culture solution

The culture solution technique, however, is not without some of the limitations encountered in other methods of applying biologically active compounds to intact plants Micro-organism destruction may be minimized, but not eliminated Oxidation *p*H inactivation, and chelation by heavy metals may occur Also, the marked sensitivity of roots to some chemicals may result in morphological changes which may produce indirect as well as direct effects on other plant parts

The response-range of higher plants to several plant-growth substances added to aerated culture solution are summarized in Table 1 The negative log molar values are in close agreement with concentrations used in examining growth responses of detached tissues, and for seed germination

Attention has recently been directed to the culture of isolated apical buds in solutions of various chemical stimuli[8,9] The culture solution technique for exposing plants to biologically active substances is, in many respects, comparable and has the advantage of retaining the identity of the intact plant

W D MITCHELL
S H WITTWER

Department of Horticulture,
Michigan State University,
East Lansing, Michigan

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Isolation of Esculetin from Sweet Potato Roots with Black Rot

UMBELLIFERONE and scopoletin have been isolated from the sweet potato roots injured by the black rot disease due to the infection of *Ceratocystis fimbriata*[1] Formation of esculetin (6,7-dihydroxycoumarin) was also indicated on the basis of paper chromatography, but the actual characterization has remained open This communication presents the evidence for the production of esculetin in the pathogenically degenerated sweet potato root tissue

Coumarin containing fractions extracted from the fungus-degenerated root tissue (48 hr after the black rot infection) by ethanol was transferred to 5 per cent sodium carbonate solution, which was continuously extracted with ethyl ether for about a week after adjusting the *p*H to 6 5–6 8 by dilute sulphuric acid and adding sodium phosphate at the final concentration of 1 *M* (*p*H 6 5) After concentration of the extract, condensed coumarin materials were applied to a column embedded by silica gel, eluted first with chloroform to obtain umbelliferone, scopoletin and other unknown fluorescent substances, and secondly with ethyl acetate Concentrate of the latter eluate was again applied to the silica gel column and was continuously eluted with 30–70 per cent ethyl acetate in *n*-hexane The two main fluorescent substances were found, and the initially moving component was proved to be caffeic acid by the isolation in a crystalline form It did not show the depression of the melting point when mixed with the authentic sample Isolation of caffeic acid from the same source had been reported already by one of us[2] The later moving fluorescent component was devoid of caffeic acid chromatographically, and evaporation of the eluate gave a crude crystal By the vacuum sublimation (3 5 mm , 170°–180°), a light yellowish crystal was obtained and was further recrystallized from dil-methanol Its melting point was 269 5° (decomp corrected) and did not show the depression on mixing with the authentic sample of esculetin Homogeneity of the esculetin fraction at the final purification step was conveniently verified by the silica-gel chromatostrip technique[3] (solvent: water-saturated *n*-butanol) and by paper chromatography (solvent ethanol/conc ammonia/water, 80 · 5 15, or water-saturated *n*-butanol) The detection was carried out by spray of ferric chloride reagent or Haphner's reagent, as well as by exposing to ultra-violet light, under which esculetin gave white greenish-blue fluorescence and was strongly intensified by ammonia gas Ultra-violet spectra of the isolated esculetin were identical with those of the authentic esculetin λ_{max} (ethanol) 230 mμ (log ε 1 35 (sample), 1 34 (ref), 258 mμ (log ε 3 23 (sample), 3 77 (ref), 300 mμ (log ε 3 62 (sample)), 3 58 (ref), 351 mμ (log ε 10 3 (sample)), 9 9 (ref) They were in conformity with the data of Goodwin and Pollock[4] Infra-red spectra of isolated esculetin was also perfectly in agreement with those of the authentic substance

Whereas the production of coumarin compounds in the diseased plant tissue is a rather common event[5–8], so far as we are aware, it is the first finding on the synthesis of this specific coumarin derivative The magnitude of the esculetin synthesis in the fungus-infected root tissue was found to be far below that of umbelliferone and scopoletin, but the present observation would be of value for the further investigation on the biosynthetic mechanism of coumarins in general as well as for the metabolic change of the diseased plants associated with the physiological role of esculetin in the fungus infection

TAKAO MINAMIKAWA
TAKASHI AKAZAWA
IKUZO URITANI

Laboratory of Biochemistry,
Faculty of Agriculture,
Nagoya University,
Anjo, Aichi, Japan

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Production of Monocaryons in Basidiomycete Cultures by the Action of Toxic Chemicals

MOST basidiomycete cultures, whether isolated from decay in wood, from portions of basidiocarps, or from spore casts, are dicaryotic Monocaryotic mycelia needed for special investigations, such as interfertility testing, are usually obtained from single basidiospores Monocaryons may, however, arise directly from the dicaryotic mycelium of some species during culture on artificial media, and in most species they can be produced from dicaryons by microsurgery[1,2], by high-speed blending[3,4], or by the action of cholic acid derivatives[5]

During extensive laboratory use of *Lenzites trabea* (Pers) Fr as a test fungus, we observed several instances of reversion to the monocaryotic condition, both on normal malt agar media and, more frequently, on media containing high concentrations of arsenate. Since these changes affected our tests, a detailed study of the causes of reversion was made *Lenzites trabea* is very suitable for this work as the dicaryons have abundant clamp connexions and a yellowish silky culture, while the monocaryons have abundant oidia and often a distinctive white powdery culture

In these investigations, all strains of *L trabea* studied tended to revert to the monocaryotic condition when grown on nutrient agar containing sodium arsenate in concentrations sufficient strongly to

Table 1 Toxic Agents responsible for Monocaryon Production

Species	Isolate	Toxic agent	Concentration* (M)
Lenzites trabea Fr	*DFP*2439	Na_2HAsO_4	0 064
	*DFP*2473	Na_2HAsO_4	0 064
	*DFP*6988	Na_2HAsO_4	0 032
	*DFP*7520	Na_2HAsO_4	0 512
	*DFP*7520	$CuSO_4$	0 005
	*DFP*7520	$ZnSO_4$	0 010
Daedalea incompta Berk	*DFP*5065	Na_2HAsO_4	0 002
Coriolus versicolor (Fr) Quel	*DFP*2666	$ZnSO_4$	0 050
	*DFP*7521	$CuSO_4$	0 005
Coriolus sanguineus (Fr) G H Cunn	*DFP*1096	$ZnSO_4$	0 010
Poria pirongia G H Cunn	*DFP*4036	$CuSO_4$	0 005
Poria hyposclera (Berk) Cke	*DFP*6406	$CuSO_4$	0 025

* Lowest concentration of toxic agent in nutrient medium (glucose, 2 0 per cent, peptone, 0 4 per cent, KH_2PO_4, 0 6 per cent, $MgSO_4$-$7H_2O$, 0 3 per cent, thiamine, 0 003 per cent, agar, 1 5 per cent) at which monocaryons were produced

retard, but not to inhibit growth Moreover, we have found that the change can be induced by copper sulphate or by zinc sulphate as well as by arsenate, and that it occurs with other basidiomycetes Examples are given in Table 1

These results suggest that reversion to the monocaryotic condition may be a widespread response of basidiomycete cultures to growth-retarding chemicals, although all species are not changed by the same conditions Some species did not produce monocaryons at all under our test treatments, and further tests of other concentrations and of other toxic agents are being conducted with these

The monocaryons produced can be mated with other monocaryons, produced either in the same way or by monospore culture, to give normal dicaryotic mycelium This enables interfertility testing of cultures for conspecificity to be done without the usual necessity for the production and isolation of basidiospores, a task which is always time-consuming and which, for many species, is very difficult or impossible We have already used this technique in the interfertility testing of a number of basidiomycete cultures and think that, with further improvements, it will prove a valuable procedure in the experimental taxonomy of basidiomycetes

The mechanism of monocaryon production is still obscure By cross-mating the monocaryons produced by *L trabea* (a bipolar heterothallic species) and using the mating factor genes as markers, we have found that all monocaryons produced from the one dicaryon by the one toxic agent tend to carry the same nucleus of the original pair, suggesting that monocaryon production involves a very high degree of nuclear selection This could be of importance in genetic studies of basidiomycetes

The ready production of monocaryons by the addition of toxic agents to the nutrient agar substrate suggested that they might also occur in the treated wood blocks used in standard laboratory evaluation tests of preservatives against wood-destroying basidiomycetes Proof of this was harder to obtain since both microscopic examination of mycelium in the block and culturing of monocaryons from the block presented difficulties, but we have demonstrated the production of monocaryons by *L trabea* in test blocks treated with copper chromate (0 1 lb /ft ^{3}), or with copper chrome arsenate (0 2–1 0 lb /ft ^{3})

These findings suggest that many of the published measurements of 'total inhibition point' (for agar toxicity studies) or of 'threshold value' (for 'soil block' or 'agar block' decay tests) refer, not to the dicaryotic cultures used as inoculum, but to derived monocaryons which could differ appreciably in growth-rate, wood-destroying ability and tolerance of preservatives Though we do not consider that this would, in practice, have any great effect on the evaluation or comparison of preservatives, it may influence some investigations, especially where the test fungus is re-isolated from a treated substrate for future use

Investigations on the range of conditions responsible for monocaryotization, on the role of nuclear selection and other postulated mechanisms, and on the proportion of wood-destroying fungi affected are proceeding and will be reported in detail elsewhere

E W B Da Costa
Ruth M Kerruish

Division of Forest Products,
C S I R O, Melbourne

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RADIOBIOLOGY

Prolonged Survival of Skin Homografts in Adult Mice following Sub-lethal Irradiation, Injection of Donor-Strain Spleen Cells and Administration of A-Methopterin

It has been reported previously from this laboratory[1] that a high proportion of adult *A*-strain mice exposed to 500 r whole-body irradiation followed by the injection (divided between the intraperitoneal and intravenous routes) of 800 million–1,200 million $(CBA \times A)F_1$ hybrid spleen cells, become specifically tolerant of homografts of $(CBA \times A)F_1$ skin On the other hand, four of five *A*-strain mice which received 500 r whole-body irradiation and 700–800 million *CBA* spleen cells died after intervals ranging from 6 to 25 days with clinical and pathological signs of graft-versus-host disease, and the solitary survivor showed no tolerance to *CBA* skin

The observation of Russell[2] that the development of fatal graft-versus-host disease in new-born *C57BL* mice which received 5–15 million spleen cells from adult *DBA*/1 donors could be prevented by administration of *A*-methopterin, and that many of the surviving animals did not reject subsequent homografts of *DBA*/1 skin, suggested that it would be of interest to study the effect of *A*-methopterin in sub-lethally irradiated adult mice which received a potentially fatal dose of allogeneic spleen cells

Sixteen *A*-strain male mice were given 500 r whole-body irradiation by the technique previously described, followed by the intravenous injection of 150 million nucleated spleen cells from male *CBA* mice twice daily for two days, starting on the day of the irradiation (that is, a total of 600 million nucleated cells) Four mice received no further treatment, the remainder were given *A*-methopterin in a dosage of 1 5 μgm /gm body-weight every 2 days for six doses, starting 2, 6 or 10 days after the irradiation Four weeks after irradiation all surviving mice received a homograft of skin from a *CBA* male donor

The length of survival of the recipients and the behaviour of the skin grafts is shown in Table 1

It will be seen that the incidence of fatal graft-versus-host disease was greatly reduced by admin-

Table 1

Time of starting *A*-methopterin	Survival of animal from day of irradiation (days)	Appearance of skin grafts 6 weeks after transplantation
Not given	9, 12, 19, 27	
10 days after irradiation	9*, 10*, 12, 13	
6 days after irradiation	7, 14, 28†, >70	Graft on surviving animal shows good hair growth, and no contraction or scabbing
2 days after irradiation	56, >70, >70, >70	Two animals have grafts showing no contraction or scabbing, but with only sparse hair growth The other two rejected their grafts in less than 10 days

* These animals actually died before the first dose of the drug had been given

† Accidentally killed at time of grafting

>70 implies animal alive and well 70 days after irradiation

istration of *A*-methopterin starting on day 2 (reckoning the day on which the irradiation and first spleen cell injection was given as day 0), and reduced to a lesser extent by administration of the drug starting on day 6 Moreover, 3 of the 5 surviving animals have so far shown no evidence of reaction against skin homografts from mice of the strain which provided the spleen cells

It seems likely that the animals with long-surviving skin grafts are lymphoid chimæras and that the non-reactivity to the skin grafts is a manifestation of specific immunological tolerance This is being investigated (*a*) by experiments with donors carrying the *T*6 chromosomal marker, (*b*) by using the Simonsen discriminant assay as described in the earlier study[1] The results will be reported fully elsewhere.

I thank Mr J R Wilson, of Lederle Laboratories, for a supply of *A*-methopterin ('Methotrexate')

M F A WOODRUFF

Department of Surgical Science
and the Medical Research Council
Research Group on Experimental and
Clinical Problems of Transplantation,
University of Edinburgh

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ENTOMOLOGY

Environmental Influences in Sex Determination in the Hymenoptera

MOST Hymenoptera reproduce by arrenotoky (unfertilized eggs giving rise to males and fertilized eggs to females), but some species reproduce by thelytoky (unfertilized eggs producing females, males being generally absent), and other species by deuterotoky (unfertilized eggs producing both males and females) With rare exceptions, males are haploid and females diploid irrespective of the mode of reproduction, though haploidy and diploidy are not necessarily sex determining in themselves, as it is known in a few species that sex determination depends on multiple sex alleles

The demarcation between thelytoky and deuterotoky is obscured by the occasional production of males in many thelytokous species In the uniparental *Ooencyrtus submetallicus*, which is usually thelytokous, males and sex intergrades (regarded as gynandromorphs) are also produced, and it has been shown that sex is determined by the temperatures to which the female is exposed during development and oviposition[1,2] In this species, environmental temperature determines whether reproduction is by thelytoky or arrenotoky (though females are not produced arrenotokously as the males are not reproductively functional), and deuterotoky consists of the occurrence of arrenotoky and thelytoky in the same female, and is not a distinct mode of reproduction

In a culture of *Ooencyrtus submetallicus*, there appeared an aberrant strain that produced male, female and gynandromorphic progeny at temperatures at which the normal strain produced almost entirely female progeny (A paper dealing with this matter will be published soon) In the normal strain the cytological processes on which sex determination depends are evidently temperature-sensitive, so that parentally experienced temperatures determine whether an egg develops into a male, female, or gynandromorph It appears that in the aberrant strain this temperature-sensitive mechanism has been disrupted by genetic change, so that male, female, and gynandromorphic progeny are all produced in an uncontrolled manner, irrespective of environmental temperature The evidence in uniparental Hymenoptera for association of thelytoky with low temperature and of arrenotoky with high temperature[3] is sufficient to suggest that temperature-sensitive mechanisms controlling sex determination may not be uncommon in uniparental species

Thelytoky sometimes occurs in arrenotokous species (as in the production of queens and workers from unfertilized eggs in the honey-bee[4]), and it is possible that many, if not all, hymenopterous species can produce both sexes uniparentally[3] It is therefore possible that the cytological processes underlying sex determination may be subject to environmental influence in arrenotokous species as well as in thelytokous species, and that many or all hymenopterous species are potentially both arrenotokous and thelytokous, but appear to be only one or the other because of their specific cytological reactions to the temperatures or other environmental factors to which they are usually exposed

A related matter is the occurrence of sex intergrades It is reported in *Habrobracon juglandis* that more male mosaics and gynandromorphs are produced at high temperatures, and in the honey-bee that eggs exposed to low temperatures produce more gynandromorphs, and that a lowering of hive temperature is thought to result in more mosaics for sex[5] In the normal strain of *Ooencyrtus submetallicus* gynandromorphs are produced under particular temperature conditions These facts indicate that environmental temperatures may be important in the production of sex intergrades in both arrenotokous and thelytokous species Also, strains of honey-bee are known that have a hereditary tendency to produce large numbers of gynandromorphs[6] These strains are reminiscent of the aberrant strain of *Ooencyrtus submetallicus*, which regularly produces gynandromorphs irrespective of temperature This suggests that in arrenotokous species with an hereditary tendency to produce gynandromorphs genetic change may have disrupted some mechanism controlling sex determination, as appears to have occurred in the aberrant strain of *Ooencyrtus submetallicus*

Sex determination and the production of sex intergrades in the Hymenoptera undoubtedly have a cytological basis, and it is appropriate that most research on them should be cytological in character. However, it is becoming very clear that proper understanding of the cytological and hereditary aspects of these problems cannot be achieved without paying adequate attention to the influence of environmental factors on the processes concerned

F WILSON

Division of Entomology,
Commonwealth Scientific and Industrial
Research Organization, Canberra

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Absence of Natural Volatile Solvents in Cockroach Grease

THE wax layer generally present in the epicuticle of insects is important because it reduces loss of water by evaporation[1-3] Except possibly for the cricket[4], there has not been sufficient knowledge of the chemical composition of the cuticular wax of any insect to formulate with certainty a physico-chemical basis for the physiological action of the wax[5] A detailed chemical analysis will be published (with Miss M E Cox) of the soft grease extracted from the cast skins of cockroaches Since the preparation of the grease involves recovery from organic solvents, any natural volatile solvents still present in cast skins would not be detected by our techniques Accordingly, attempts reported here were made to distil volatile compounds from the surface of freshly killed cockroaches

Tests for the presence of volatile substances were made directly on specimens of *Periplaneta americana* (L) The glass apparatus was similar to that described[6] for the transfer of gas chromatographic fractions boiling over 60° A specimen container and a capillary for collection of samples were connected by a tube which could contain solid desiccants Since desiccants such as magnesium perchlorate and calcium sulphate also trap alcohols, duplicate runs without desiccant were made and, if an aqueous distillate was obtained, this was extracted with hexane In use, the specimen container was held for at least 1 hr in liquid air before the whole apparatus was evacuated to a pressure of less than 0 01 torr The source of vacuum was removed and the liquid air bath transferred to the capillary collector Distillation under vacuum was allowed to proceed for 2–3 hr after the specimen container had reached room temperature Any distillate or its hexane extract was examined by gas chromatography at a number of temperatures to 200° with a 4-ft column of 5 per cent 'Apiezon *L*' on 'Celite' and by infra-red spectroscopy

When cockroaches, usually 4 in each experiment, were subjected to distillation without desiccant, water and other unidentified volatile constituents were collected If the mouthparts and anal regions of the insects were sealed with paraffin wax, no volatile materials other than water were condensed Even warming of the insects with an infra-red lamp failed to drive off volatile organic compounds Negative results were obtained also with freshly moulted cockroaches tested while still white

The absence of volatile solvents in the surface grease of the cockroach has possible implications in the mechanism of wax secretion[7] and was unexpected since Beament[5] has suggested that volatile paraffins and alcohols of chain-length C_8–C_{12} are present in the soft grease Such solvents provide a plausible explanation of changes observed such as the hardening with time of extracted grease In the present experiments, when 5-µl samples of solvents such as the primary *n*-alcohols, butanol, octanol and decanol, octan-2-ol, and the *n*-hydrocarbons, hexane, octane, decane, dodecane, tridecane and hexadecane were spread on the cuticles of cockroaches, quantitative recoveries and identifications were made Further evidence that particular solvents are absent is the observation that 1 µl of octanol run on to cockroach cuticles caused an immediate loss by the insects of the power of co-ordinated movement followed by death in a few hours Similarly, amounts of 20 µl of dodecane killed one-third of cockroaches tested, while 40 µl killed two-thirds of test samples With the collection techniques used, it was possible to detect volatile solvents, if present, in amounts less than 0 1 per cent by weight of the 0 5 mgm of cuticular grease present per cockroach

The current chemical analysis mentioned earlier has already shown that, besides saturated hydrocarbons and fatty acids, the grease from cockroach cast skins contains unsaturated hydrocarbons in large amount, unsaturated fatty acids and a number of aldehydes The presence of these more reactive types of compound suggests the possibility that changes in the properties of cockroach grease during ageing[5] may have a chemical basis

A R GILBY

Division of Entomology,
Commonwealth Scientific and
Industrial Research Organization,
Canberra

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Steroid Hormone in an Insect, *Bombyx mori*

IT is well known that the metamorphosis of insects is brought about by two hormones, one secreted by the brain and the other by the prothoracic gland The brain hormone activates the prothoracic gland and directly induces metamorphosis with ecdysone[1]

The extraction of the hormone from brains of insects was first reported by Kobayashi and Kirimura[2] Until recently, only a few workers[3,4] had carried out extraction of active principle from brain or central nerve system in insects At present we have succeeded in crystallization of the brain hormone in the silkworm, *Bombyx mori*[5]

This communication deals with the further progress conducted on the chemical character of the brain hormone and with the results obtained on bioassay of the hormone and of its related compounds

The melting point of the crystal (142°–143° C) which was reported previously[5] remained unchanged by recrystallization and no ultra-violet spectrum of the crystal was found The infra-red spectrum of the crystal in potassium bromide showed that it has a $RR'C = CR''H$ type band which was characteristic in the region 750–850 cm^{-1}, and a hydroxyl band which was shown at 3,480 cm^{-1} Furthermore, the characteristic absorption spectrum of crystal was very similar to that of pure cholesterol in all regions The crystal was also analysed by means of gas chromatography with a Barber Colemann model 10 with argon ionization detector. This result showed that there was one main peak with trace amounts of three peaks and that the retention time of the main peak coincided favourably with that of pure cholesterol. Therefore, we are inclined to believe that the crystal was cholesterol accompanied with trace amounts of three impurities.

15 mg of crude sterol mixture was collected from several active fractions of brain hormone extract by the counter-current distribution method and then acetylated in pyridine by a common method. The elute was collected carefully after the acetylated sterol mixture was adsorbed to 5 g of aluminium oxide (according to Brockmann) column and eluted with *n*-hexane. White needle-like crystals of acetylated sterol were formed on recrystallization with acetone and methanol after the solvent was evaporated from the elute. Following further purification by recrystallization the melting point of the crystal became constant at 115°–116° C.

After the purified acetylated sterol was saponified with alcoholic potash solution, 2 mg of crystal was obtained by recrystallization with acetone and methanol. The crystal appeared as pearly leaflets or plated forms when crystallized with dilute alcohol. The melting point of the crystal was 148° C, and also the mixed melting point of the crystal and pure cholesterol mixture was determined to be the same as that of the crystal alone.

The chemical character of purified brain hormone is summarized as follows: no ultra-violet spectrum was found; the infra-red spectrum was identified with that of pure cholesterol; the Liebermann–Burchard reaction was positive; a gas chromatogram exhibited only one peak; m.p. was 148° C.

From the above-mentioned results, it was determined that purified brain hormone was identical with pure cholesterol.

Table 1. BRAIN HORMONE ACTIVITY OF ISOLATED PURE CHOLESTEROL

Dose of cholesterol (μg)	No. of 'Dauer-pupæ' used for bioassay	No. of moths emerged*	Days from injection to emergence	No. of dead individuals
10	6	4	15–23	0
2	6	5	18–23	0
0.2	6	4	17–23	1
0.02	6	3	18–27	3
0	24	0	—	0

* The results obtained for 30 days at 25° C following injection.

The results of bioassay are as follows. The diapausing pupæ of *Bombyx mori* following extirpation of the brain ('Dauer-pupæ') were used as test objects for the assay of brain hormone activity. They were then observed for about 6 weeks at 25° C prior to use. 0.01 ml. of sterol dissolved in 9 per cent ethanol was injected into each test animal. In the first experiment, when the aforementioned isolated pure cholesterol was injected into the 'Dauer-pupæ', the test animals emerged as moths. Positive results of the brain hormone activity were obtained for dosages up to 0.02 μg in pure cholesterol injections as shown in Table 1.

In the second experiment, two kinds of sterols related to cholesterol, cholestanol and 7-dehydrocholesterol were used as dosages for bioassay of brain hormone activity. When each of the sterols, 2 μg of cholestanol or 10 μg of 7-dehydrocholesterol, was injected into three 'Dauer-pupæ' respectively, the following results were observed. In the case of cholestanol, two out of three 'Dauer-pupæ' injected became moths in 16–19 days following injection but the other animal remained in the pupal form without any differentiation for 30 days following injection. In the case of 7-dehydrocholesterol, two out of three 'Dauer-pupæ' became moths in 19 days, while the other animal remained in the pupal form for 30 days. From the aforementioned experiment it seems that these two chemicals also have brain hormone activity, but to a lesser degree than cholesterol.

In the third experiment, 100 μg of each of the following chemicals, β-sitosterol, stigmasterol and ergosterol, respectively, was injected into three groups of six 'Dauer-pupæ'. Two of six animals tested with each chemical, in every group, emerged as moths from 14 to 29 days following injection. A total of six individuals in this experiment died, but no other animals emerged for 30 days. However, no moth was obtained in 30 days for twenty control animals following non-injection and/or mock injection by 0.01 ml. of 9 per cent ethanol alone. From these results, it appears that these three chemicals do display brain hormone activity, even though the results of this bioassay were inconclusive. It is concluded that cholesterol is an active principle of the brain hormone, and its related compounds, such as cholestanol and 7-dehydrocholesterol, also have a certain degree of hormone activity.

Generally speaking, cholesterol is the main sterol of most insects and also phytosterols derived from diet are found in phytophagous insects. Most insects, however, cannot synthesize cholesterol from substances which are known to be cholesterol precursors in both mammals and micro-organisms. Although information on the hormone activity of sterols in insects was previously not forthcoming, it has been shown here that sterol is an active hormone in insects.

Considering the importance of sterols and cholesterol precursors as insect hormone, we are currently investigating hormone activity of these compounds in insects.

We thank Prof. G. Tamura, Prof. S. Hara, Dr. T. Iwashige, Dr. G. Chihara, Dr. S. Okabe, and Dr. H. Endo for their invaluable assistance.

JIRO KIRIMURA
MIEKO SAITO
MASATOSHI KOBAYASHI

Sericultural Experiment Station,
Suginami-ku, Tokyo

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MICROBIOLOGY

Identification of a New Type of Colicine (Colicine L)

THE strain *Escherichia coli* 398 of the collection in this Institute produces a colicine, which cannot be identified as one of the known types[1–3]. This new type is related to the colicines of the group *E*, and was at first considered to be a complex colicine, containing colicines *E* and *I*. A more detailed analysis of some biological characteristics, however, showed that this colicine can be considered as a new type. The letter *L* was given as an index to this new type, since the other colicines were identified in a similar manner[2]. In this communication are summarized experiments showing how colicine *L* can be distinguished from the other types.

(1) *Activity.* As other colicines, colicine *L* is active against strains of Enterobacteriaceae, particularly against some strains of *Escherichia coli*, but inactive against *Micrococcus*, *Pseudomonas* and *Mycobacterium* (Table 1). Tests of sensitivity were made by the method of perpendicular streaks[4].

Tests of several hundred wild strains of *E. coli* isolated from human or animal fæces showed that the activity of colicine *L* is quite different from that of 14 other colicines (colicines $V, B, E, I, K, S_3 + I, D, S_4, F, J + I, A, C, H$ and X). These results suggest that colicine *L* cannot be considered identical with any of the others. Nearly 20 per cent of *E. coli* isolated from human fæces are sensitive to colicine *L*, while the percentage of the same strains sensitive to the other 14 colicines varies from 0 to 60 per cent. This is in favour of testing sensitivity of enteropathogenic *E. coli* for epidemiological purposes, as serotypes and phage types of these micro-organisms can be subdivided into several colicinotypes[5].

(2) *Specificity of resistant 'mutants'.* Mutants resistant to colicine *L* obtained from the strain *E. coli* Row[6] are not always specific, as, for example, mutants resistant to colicine *X*[3]. This is the reason it has been difficult at the beginning to identify this type of colicine as a new type. Colicine *L* was not found to be related to colicines V, K, D, A and X, because no cross-resistances to these five colicines were observed. However, cross-resistances were found between colicine *L* and colicines $B, E, I, S_3 + I, S_4, F, J + I$ and C. It is interesting that 5 out of these 8 colicines ($E, F, J + I, S_3 + I$ and S_4) belong to the group of colicine *E*. Cross-resistances of mutants derived from several strains of *E. coli* concerning colicines which are not identical have been already reported by Fredericq[2] and one of us[7].

It was therefore necessary to extend this part of the work in several mutants resistant to different colicines. In experiments done with 20 mutants of four different lines of the strain *E. coli K*12 resistant to colicine *L* we found that cross-resistance between colicine *L* and colicines $B, E, I, S_3 + I$ and $J + I$ do not exist. It was therefore concluded that colicine *L* is not identical with any of these 5 colicines.

Further experiments were designed for the examination of cross-resistances between colicine *L* and colicines S_4, F and C, showing still cross-resistances when lines of *E. coli K* 12 were used. The examination of the other 20 mutants derived from *E. coli* Row, resistant to one of the colicines S_4, F and C, showed that these mutants are not resistant to colicine *L* and proved that also these colicines are not identical with colicine *L*.

This series of experiments shows that colicine *L* is probably related to the group *E* of colicines; however, it is not identical with any of the colicines of this group.

(3) *Morphology of the inhibition zones in agar.* For the examination of the morphology of inhibition zones, the colicinogenic strain is stabbed in nutrient agar and incubated at 37° for 48 hr. The stab cultures are sterilized by chloroform vapour and the indicator strain is seeded over the surface of the agar plate by the double-layer method[4,6]. As for other colicines, the zones of inhibition are different when different sensitive indicator strains are used. For the strain *E. coli* Row the diameter of the zone of inhibition in Difco nutrient agar plates is about 22 mm. Colonies resistant to colicine *L* are rarely found within the zone of inhibition, when *E. coli* Row is used as indicator. However, several small colonies, which are not resistant to colicine *L*, are seen near the edges of the zone of inhibition. The edges of zones of inhibition are well defined.

Table 1. SPECTRUM OF ACTIVITY OF COLICINE *L*

Genus or species	No. of strains examined	No. of strains sensitive
Enterobacteriaceae		
Escherichia coli	100	22
Citrobacter	15	1
Klebsiella	25	0
Salmonella	25	0
Proteus	25	0
Serratia	13	0
Other micro-organisms		
Micrococcus	40	0
Pseudomonas	30	0
*Mycobacterium**	25	0

* Strains able to grow in ordinary media within 48 hr.

When lines of *E. coli K* 12 are used as indicators the zones of inhibition are also about 22 mm., but a greater number of colonies resistant to colicine *L* are observed within the zone of inhibition.

(4) *Diffusibility in agar.* Colicine *L* diffuses relatively slowly in Difco nutrient agar plates. It diffuses more slowly than colicine *V*, but more rapidly than colicines *E* and *I*. In one experiment performed with *E. coli* Row as sensitive indicator, the zone of inhibition measured daily for five consecutive days was as follows: 1 day, 13 mm.; 2nd day, 22 mm.; 3rd day, 26 mm.; 4th day, 30 mm.; 5th day, 34 mm.

(5) *Heat resistance.* Heat resistance tests were made in a suspension of a culture of the strain *E. coli* 398 sterilized with streptomycin and containing 128 arbitrary units of colicine *L* per ml. of suspension. The medium used for the culture of *E. coli* 398 contained 30 gm. of Difco casamino-acids and 10 gm. of Difco yeast extract per 1,000 ml. of water. The method of titration in arbitrary units will be published elsewhere.

Results of heat resistance appear in Table 2. Colicine *L* is rapidly destroyed at 100° C., but resists a temperature of 72° C. for 30 min., though the titre falls considerably.

(6) *Sensitivity to proteolytic enzymes.* A suspension of colicine *L*, produced as already described and containing 32 arbitrary units per ml., became inactive after 15 min. action of trypsin at 37° C. In the same suspension, it resisted papaine for 24 hr. at 37° C., but the titre fell from 32 to 4 arbitrary units at the end of the experiment.

Bacterial proteases do not destroy (at least completely) colicine *L*, which resisted the proteases of 4 strains of *Proteus* and of 4 strains of *Pseudomonas*. The method used for the action of bacterial proteases has been described by Gratia and Betz-Bareau[8] and by Fredericq[2,4].

(7) *Filtration.* Colicine *L* is not able to pass through Chamberland *L*5, Seitz *EK* and Hemmings[9] filters.

(8) *Production in different media.* The concentration of colicine *L*, measured in arbitrary units, is variable in different culture media. A good titre was obtained in the following media: Difco nutrient broth additioned with 10 per cent bovine serum,

Table 2. HEAT RESISTANCE OF COLICINE *L* (IN A MEDIUM CONTAINING 128 ARBITRARY UNITS PER ML.)

Time (min.)	Arbitrary units per ml. after heating at 100° C.	Time (min.)	Arbitrary units per ml. after heating at 72° C.
2	4	5	32
3	2	10	8
4	2	20	4
5	0	30	4
10	0		

Difco tryptose, Difco tryptone, Difco Todd and Hewitt broth, Difco casamino-acids 3 per cent additioned with 10 per cent bovine serum or 1 per cent yeast extract, undiluted bovine serum.

(9) *Lyophilization and conservation in suspensions.* Lyophilization of colicine suspensions, prepared as described here, does not diminish the titre of colicine. The titre is also well maintained in suspensions at 22° C. or 4° C. for two months. It is possible that colicine *L* keeps well in suspensions for a longer time at 22° C. or at 4° C., without being lyophilized.

P. HAUDUROY
J. PAPAVASSILIOU

Institute of Hygiene,
University of Lausanne,
Switzerland.

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Adaptation of *Escherichia coli* to Selenate

SELENIUM compounds are usually considered harmful, but evidence is mounting that the element may have other biological effects. The fact that a few higher plants can accumulate relatively large amounts of selenium has been known since the 1930's, several species of *Astragalus* (vetch) have actually been proved to require traces of selenium for normal development[1]. More recently, animals deficient in vitamin E as well as grazing animals suffering from white muscle disease were found to improve after treatment with selenium in quantities 0·1–0·01 less than the usual toxic amounts[2,3]. It has been suggested that selenium at these concentrations may well be an essential element for animals[3].

Micro-organisms respond to selenium compounds in a variety of ways. Inhibition of growth by selenium and antagonism to selenium by sulphur compounds are well documented and have been reviewed elsewhere[1,4]. Complete replacement of required sulphur nutrients has also been demonstrated, selenopantethine for pantethine[5] and selenomethionine for methionine[6]. An organism capable of oxidizing elemental selenium to selenic acid, much as *Thiobacillus thiooxidans* oxidizes sulphur to sulphate, was isolated in 1923[7]; but whether this organism interchanged selenium for sulphur or whether it needed selenium was not determined.

Several micro-organisms show a tolerance to high concentrations of selenite[8–10], a characteristic that has been used as a basis for the isolation and separation of *Salmonella* species from *Escherichia coli*[8]. An adaptation to a selenium compound is seen from work with *Chlorella vulgaris*, all or most of the cells of which can adapt to withstand the toxicity of selenomethionine[11–12]. Adaptation of a micro-organism to inorganic selenate, however, has not been reported. We describe here experimental conditions under which *Escherichia coli* was found to adapt and grow in the presence of otherwise toxic levels of potassium selenate.

Escherichia coli K_{12} was maintained on nutrient agar slants (Difco). For inoculation, cells were first grown overnight in a liquid medium based on that of Anderson[13]. Because sulphate and selenate act as competitive antagonists, the medium was modified by replacement of magnesium sulphate with magnesium chloride and potassium sulphate to allow control of the sulphate concentration. The composition of the medium was NH_4Cl, 0·0187 *M*, $Na_2HPO_4 \cdot 7H_2O$, 0·0422 *M*; KH_2PO_4, 0·0220 *M*, $MgCl_2 \cdot 6H_2O$, 0·0017 *M*, K_2SO_4, 0·00031 *M*, glucose, 0·0220 *M*. Ammonium and phosphate salts were prepared as one stock solution. Aliquots of the stock solutions were pipetted into 'Pyrex' tubes, 15 mm × 125 mm, matched to fit the Klett–Summerson colorimeter, and enough distilled water was added to give a final volume of 5·0 ml. Potassium selenate was added before sterilization. It was prepared according to the method described by Trelease and Beath[1] in order to ensure the absence of selenite and circumvent the formation of elemental selenium during growth

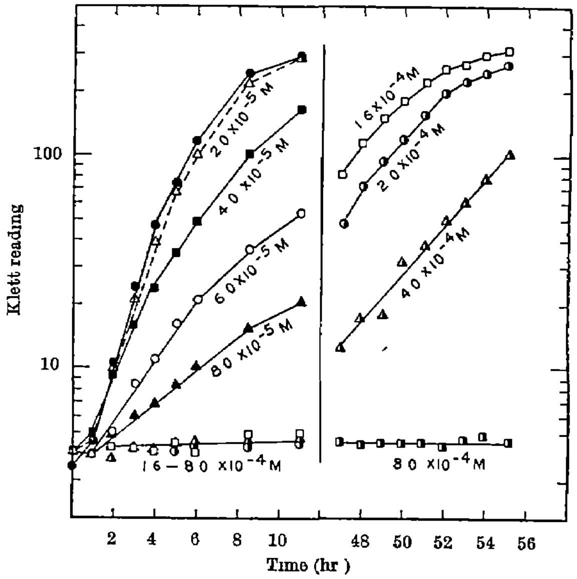

Fig. 1. Influence of potassium selenate on growth of *Escherichia coli* K_{12} in the presence of 2·0 × 10^{-4} *M* potassium sulphate. Numbers refer to molarities of potassium selenate.

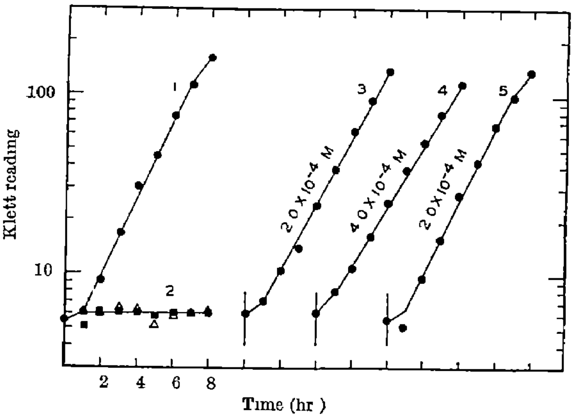

Fig. 2. Growth of selenate-adapted *Escherichia coli* K_{12} with potassium selenate. (1) Unadapted control, no selenate, (2) unadapted control, ■ 2·0 × 10^{-4} *M* potassium selenate, △ 4·0 × 10^{-4} *M* potassium selenate, (3 and 4) adapted cells grown with same level of potassium selenate in which they initially grew, (5) adapted cells previously subcultured on nutrient agar slants free of added selenium. All cultures contained 2·0 × 10^{-4} *M* potassium sulphate. Time scales moved to right for curves 3, 4 and 5.

Glucose and magnesium chloride were autoclaved separately and added after sterilization Autoclaving was at 15 lb for 20 min

Inoculum cells were taken from 14–16-hr old cultures, centrifuged and washed three times with sterile, sulphurless, glucose-free medium The washed cells were diluted until a reading as close to 200 on the Klett as practicable was obtained A 0 1-ml aliquot, when added to the 5 ml of complete medium, gave an initial Klett reading of about 5 Exponential growth began after a short lag of approximately 1 hr To check the adequacy of washing, sulphurless controls were included in each experiment All tubes were prepared in duplicate and were incubated at 37° on a roller tube wheel turning at 5 r p m Readings were made at hourly intervals during the exponential phase of growth and less frequently thereafter in order to observe the effect of the inhibitor on the entire growth curve Filter No 42 (blue) was used to take the Klett readings

With selenate toxicity a function of the sulphate-level[1,4], it was necessary to determine the lowest sulphate concentration that would give an optimum growth response Up to approximately $8\ 0 \times 10^{-5}\ M$, the amount of growth after 24 hr was proportional to the sulphate concentration At higher levels, through $8\ 0 \times 10^{-2}\ M$, there were no differences in the growth curves A sulphate-level of $2\ 0 \times 10^{-4}\ M$ was selected for the experiments described here

The inhibitory effect of serially increased concentrations of potassium selenate is illustrated in Fig 1 A progressive decrease in the exponential growth-rate began at $4\ 0 \times 10^{-5}\ M$, but the final amount of growth equalled that of the uninhibited control At several of the higher selenate-levels, where inhibition seemed to be complete, growth consistently resumed after a lag of 24–48 hr and eventually attained the same maximum as the control Complete inhibition always occurred at a concentration of $8\ 0 \times 10^{-4}\ M$ selenate The effects on growth-rates are similar to those observed in earlier experiments with *Chlorella vulgaris*[14] Adaptation of the alga to selenate, however, was not detected at that time, possibly because maximally inhibited cultures had not been incubated long enough With several other micro-organisms, including *Saccharomyces cerevisiae*[15,16], *Desulphovibrio desulphuricans*[17], and *Aspergillus niger*[18], inhibition of growth is also a function of selenate concentration In those experiments, however, growth was measured at an arbitrarily chosen time interval Rates of growth and lag periods were not measured, therefore, any adaptation would have been overlooked

To determine whether cultures that had resumed growth were now resistant to selenate, cells were prepared for inoculation as described here and inoculated into fresh medium identical with that from which they had been withdrawn Controls with unadapted cells were run simultaneously Fig 2 (curves 3 and 4) shows that the prolonged lag typical of adapting cultures was eliminated The cells were now able to grow exponentially at a rate almost equal to that of the unadapted control and reached the same maximum densities Growth of adapted cells in sulphate alone was no different from that of the control

Adapted cells, when re-exposed to selenate after nine transfers on nutrient agar slants free from added selenium over a twelve-day period, nevertheless remained adapted (Fig 2, curve 5) The adaptation under these conditions therefore appears to be a stable one

The work was supported by a grant, *RG*-9086, from the U S National Institutes of Health

A SHRIFT
ELLEN KELLY

Laboratory of Comparative Biology,
Kaiser Foundation Research Institute,
Richmond, California

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CYTOLOGY

Ring Chromosomes in Human Beings

RING chromosomes have been found in some species, the most intensively studied being maize[1] and *Drosophila*[2] They have also been induced by irradiating human cells *in vitro*[3] and are known to occur in human tumours[4] We have recently found a ring chromosome in two children, a different chromosome being involved in each case There are no apparent signs of neoplastic disease in either patient

In Winnipeg, Manitoba, the chromosomes of a male infant with multiple congenital anomalies have been studied by the blood culture technique[5] The child resembles somewhat the 18 trisomy syndrome[6], with rather odd-looking facies, large abnormal ears, small mandible, epicanthic folds, right inguinal and umbilical hernias, limited hip abduction, high arched palate and a simian crease on the right palm He has had several minor motor seizures, is failing to thrive and at 5 months of age gives little evidence of intellectual progress The mother, age twenty-four years, has a history of pelvic X-ray exposures prior to conception She had X-ray pelvimetry during a previous pregnancy complicated by an iniencephalic fœtus She later had diagnostic X-rays for fractured lumbar vertebræ

In all of more than 100 cells examined one of the large acrocentric chromosomes in group 13–15 is missing, being replaced by a ring-shaped chromosome The size of the ring appears to be constant with the exception of a few large ones (Fig 1) The total number of chromosomes in every cell analysed is 46 One member of chromosome 3 has a submedian centromere and is larger than its homologue Since this infant has many of the anomalies found in 18 trisomics we suspect that a translocation of a piece of the 18th chromosome is present

In London, Ontario, chromosome studies were carried out on a patient during investigation of mental retardation The mother was twenty-three years old at the time of the patient's birth, having had two normal children and a miscarriage previously Following the patient's birth there was a second miscarriage The mother never had an X-ray before conception of the patient nor during pregnancy The patient has a speech defect, deafness due to complete atresia of both ear canals, hypertelorism, bilateral epicanthic folds and bilateral syndactyly of second and third toes He has a history of minor motor tonic seizures (without a clonic phase) since the age of 5 years, but the electroencephalogram is normal His intelligence quotient is 53 on the basis of performance only (owing to his deafness) using the Wechsler intelligence scale for children

The majority of cells contain 46 chromosomes In every cell, one of the chromosomes in the 16–18 group is missing (probably number 18) and a ring-shaped chromosome is found in its place In most metaphase plates this chromosome appears as a monocentric ring, each ring being smaller in diameter than the length of the chromosomes in the 21–22 group However, in about 15 per cent of the cells the ring is twice the size seen in the majority of cells and appears to have two constrictions The ring structure is most clearly seen in prometaphase (Fig 2)

In both patients, all cells analysed have a ring chromosome This suggests that in man, as in *Drosophila*, the ring may be persistent Since these children do not appear to be too severely affected, especially the second case, any deletions involved in ring formation must be relatively small

The work in Winnipeg is supported by funds from the U S Public Health Service grant *RG*–7950, the U S National Foundation and a Dominion-Provincial Health grant The London (Ontario) work is supported by Mental Health grant No 605–5–287 of the National Health Grants programme of Canada

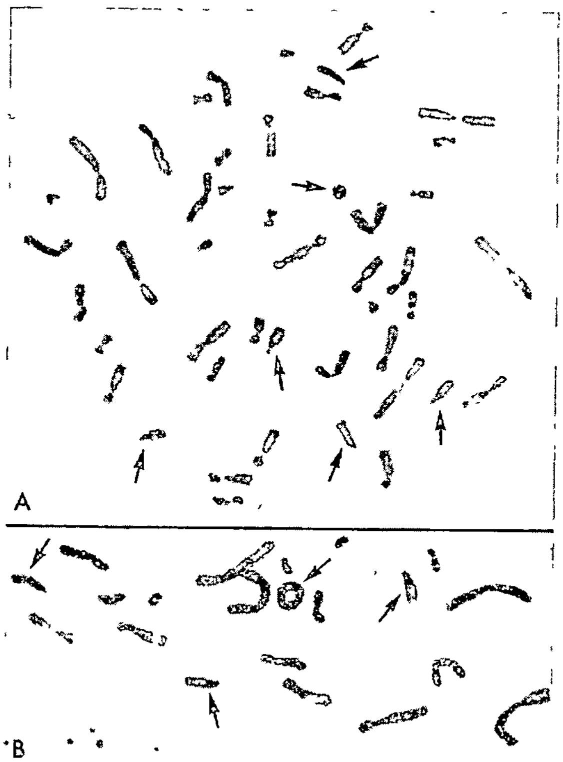

Fig 1 *A*, Complete cell of first patient showing 46 chromosomes, arrows indicate five chromosomes in group 13–15 and small ring, *B*, part of another cell of the same patient showing large ring

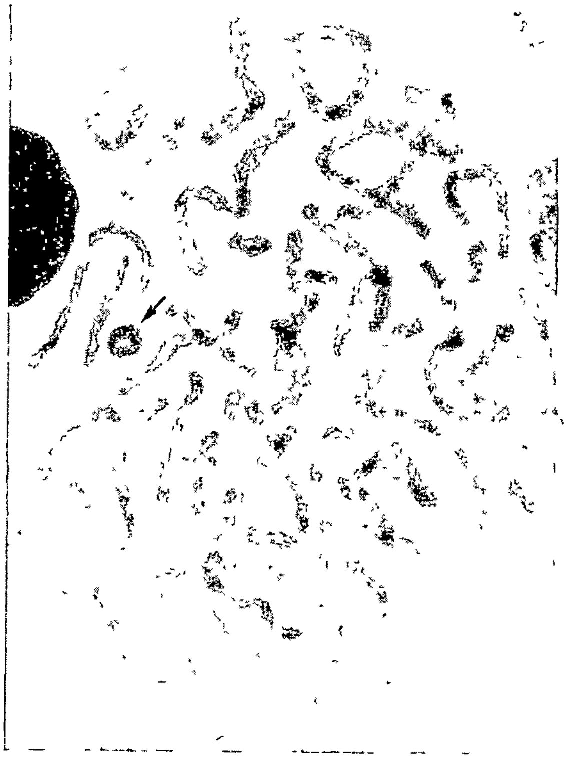

Fig 2 Ring chromosome in cell of second patient at prometaphase

Hsi-Chang Wang
John Melnyk
Lyle T McDonald
Irene A Uchida
Department of Medical Genetics,
Clinical Investigation and Research Unit,
Children's Hospital, Winnipeg, and
Department of Paediatrics,
University of Manitoba
David H Carr
Benjamin Goldberg
Department of Microscopic Anatomy,
Faculty of Medicine,
University of Western Ontario, and
Children's Psychiatric Research Institute,
London, Ontario

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Variable Height of Intestinal Absorptive Cells

The height of absorptive cells of the small intestine is known to vary in different parts of the gut and also according to their position on the villi In paraffin sections it is seen that the cells at the tip of the villus are shorter than those along its sides However, it is not generally appreciated, particularly in paraffin sections, that even adjacent cells may vary considerably in height This becomes obvious when cells

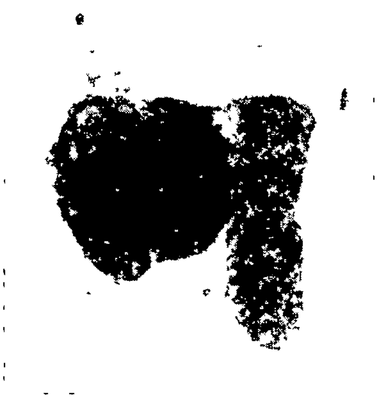

Fig 1

are studied in scrapings from fresh gut suspended in isotonic saline, Fig 1 represents a not infrequent appearance of cells of the albino rat (×1,500) The cells have been lightly stained with toluidine blue Their striated borders, terminal webs and terminal bars are in alignment The bases of all three cells reach different levels, one projecting far below the others A projecting base has also been seen in a cell in the middle of a row on other occasions The occasional bi- and tri-nucleate absorptive cells of giant size reported by me (*Acta Anat*, Basle, in the press) would also probably stand with the striated border aligned with their neighbours by having their bases sunk into pockets of basement membrane and lamina propria

Palay and Karlin[1] have shown that the cytoplasm of absorptive cells is stiffer in the microvilli and the terminal web, and adhesion of the cell membrane is firmest at the terminal bars (a rather misleading term in view of the bars seen in sections being only parts of a hoop encircling the cell) The rest of the cytoplasm has been called by these authors "a moving stream of semisolid material, which lies upon a contractile substratum", the latter comprising the lamina propria of the villus They have shown that the lateral parts of the cell membrane below the terminal bars are merely apposed and not adherent

We assume that the basement membrane and the immediately subjacent part of the lamina propria behave like a fluid cushion during life, and local deformations to accommodate cells of different heights occur as required Experimental work with cholinergic substances now in progress leads us to believe that in the living state the height of absorptive cells may show marked changes during the span of an hour But in spite of such fluctuations, the cells retain alignment in the apical region by accommodating each other along their lateral faces below the terminal bars and through the resilience of the substratum The fact that sudden variations in heights of cells are not noticeable in paraffin sections may perhaps be explained by the shrinkage of cells during preparation, and accommodation along their lateral faces while the basement membrane takes in the slack as it shrinks along with the lamina propria

A Mohiuddin

Department of Anatomy,
University of Singapore

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VIROLOGY

A Sensitive Polarographic Test for the Detection of Protein Impurities in Viral Nucleic Acid

Recently it was shown that the polarographic method is suitable for the determination of small amounts (0 2 per cent and more) of non-virus protein in tobacco mosaic virus (TMV) preparations[1] In further experiments we have found this method equally suitable for the detection of traces of protein contaminants in the infective ribonucleic acid (RNA) preparations, isolated from TMV by the phenol method according to Gierer and Schramm[2]

A few years ago Berg[3] recommended the polarographic method for determination of protein impurities in preparations of RNA and DNA of various origin He also showed that RNA gives no polarographic wave in standard supporting electrolyte, first used by Brdička[4] for detection of proteins In trying to apply the proposed method it became evident that the polarographic method for determination of protein impurities has many restrictions (1) The method can be used only when a pure preparation of protein which is identical with the contaminating protein is available as a standard, (2) Neither the contaminating protein nor the standard must be denatured, or if so, then to the same degree, (3) When the RNA preparation is contaminated by more than one kind of protein the quantitative evaluation of the protein content is possible only in special cases In the case of infectious RNA preparations isolated from TMV by the phenol method[2] all the above requirements listed in the restrictions are satisfied The TMV particle is composed of an RNA filament of mol weight 2 million embedded in an orderly arranged aggregate of about 2,300 identical protein sub-units[5] In the course of the isolation of infectious RNA by phenol extraction, the viral protein remains in the phenol layer whence it can be easily isolated by methanol precipitation[6] This protein can serve as a suitable standard for determination of protein contamination in infectious RNA After appropriate washing the protein can be maintained in a very stable, solid form and conveniently redissolved in decimolar sodium hydroxide before use In this strong alkaline solution the protein remains in the form of its monomer (mol wt 18,000) Decimolar sodium hydroxide does not change the essential properties of the virus sub-units, when the latter are mixed in favourable conditions with infectious RNA, they reconstitute the original infective particles[6].

In our experiments the viral protein, isolated as described here, has given a very strong polarographic

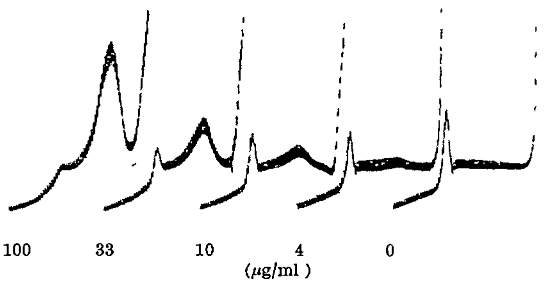

Fig 1 Polarographic records of isolated TMV protein at 0° C The final concentrations of the protein in the standard supporting electrolyte are given below the curves

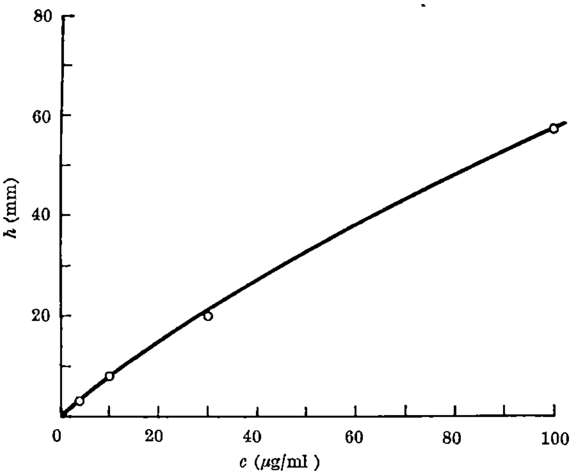

Fig 2 Height (h) of the TMV protein wave versus its concentration (c) in the standard supporting electrolyte at 0° C $h=f(c)$

reaction in ammoniacal solutions of trivalent cobalt, in contrast to RNA, which is inactive Fig 1 shows the polarographic curves of viral protein recorded at 0° C in the supporting electrolyte of the following standard composition 0 001 M $Co(NH_3)_6Cl_3$—0 1 M NH_4OH—0 1 M NH_4Cl The protein concentration decreases from left to right from 100 to 0 μg/ml , the last curve representing the pure supporting electrolyte solution As seen in Fig 1, the smallest amount of protein still detectable in the conditions used is 4 μg/ml Moreover, the sensitivity of the polarographic reaction can be appreciably increased by increasing the ammonia and cobalt concentrations in the supporting electrolyte The approximately linear relation between the height of the protein wave and protein concentration remains up to about 100 μg/ml (Fig 2)

As the groups responsible for the typical polarographic reaction of proteins are absent in RNA, the presence of the contaminating viral protein in infectious RNA preparation can be easily detected by the polarographic method The great sensitivity of this method enables the determination of as little as 0 02 per cent protein contaminant in RNA The demand for analysis is 0 1 ml of 1–2 per cent RNA solution One can use much lower concentrated RNA solutions, but then the sensitivity of the method decreases correspondingly The 0 1 ml volume is, however, not the lowest limit, with a special device it is possible to analyse even smaller amounts

For determination of traces of protein contaminant in the viral RNA preparation the following method is recommended The sample of RNA is incubated in decimolar sodium hydroxide for 1 h and then polarographed at 0° C (ref 1) in the supporting electrolyte of the following composition 0 003 M $Co(NH_3)_6Cl_3$—0 5 M NH_4OH—0 15 M NH_4Cl—0 05 M NaOH The protein content of the sample is determined by the method of standard addition The virus protein isolated from the phenol layer, as already mentioned, and dissolved in decimolar sodium hydroxide serves as a standard The linear relation between the height of the protein wave and protein concentration is valid with good approximation up to 50 μg/ml The last value safely exceeds those encountered in infectious RNA preparations isolated by phenol extraction

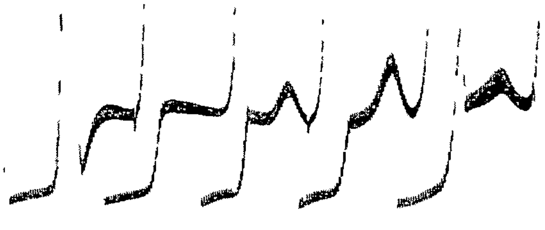

Fig 3 Determination of protein contamination in the infectious RNA preparation Concentration of RNA, 10 mg/ml , the amount of protein standard added, 0 005 mg/ml

Fig 3 shows the results of polarographic determination of protein contaminant in an infectious RNA preparation isolated from TMV by the phenol method according to Gierer and Schramm[2] The infectivity of this RNA was equal to 0 5 per cent infectivity of the intact TMV containing the same amount of RNA The evaluation of the protein content in the RNA sample was performed by the method of standard addition The first curve on Fig 3 represents the polarographic record of the supporting electrolyte alone, the second, the same but with an addition of a small amount of gelatin for suppression of cobalt maximum, the third curve shows the record of RNA preparation under test at 1 per cent concentration The polarographic wave present on this curve is due to the protein contaminant The fourth curve shows the result of the addition of 5 μg/ml TMV protein into the RNA sample The last curve represents the polarographic record of pure TMV protein in 5 μg/ml concentration The increase of the polarographic wave caused by the addition of 5 μg/ml protein standard corresponds exactly to the height due to the same amount of pure standard

Evaluation of the polarographic curves revealed 4 5 μg/ml protein in the 10 mg/ml RNA sample tested This corresponds to 0 045 per cent protein in the infectious RNA preparation Such a small amount of protein can scarcely play any part in the process of infection by the RNA preparation For maintaining the infectivity in the case of TMV RNA the integrity of the whole 2 million mol RNA macromolecule is necessary[7] Association of one 18,000 mol wt protein sub-unit with each RNA molecule would give a protein contamination of about 1 per cent The contamination actually found in our experiments was more than 20 times less As in repeated phenol extraction the infectivity of the RNA preparation does not drop, and as there is no reason for assuming the existence of a certain portion of phenol-resistant particles in the virus population, the role of a whole protein sub-unit in the process of infection must be excluded The method described cannot help us to exclude a similar role of polypeptide fragments of lower mol wt , although such form of their activity is very improbable

G RUTTKAY-NEDECKÝ
V ŠPÁNIK

Institute of Virology,
Czechoslovak Academy of Sciences,
Bratislava, Czechoslovakia

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APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Experimental Officer (with experience of general laboratory techniques and equipment, and preferably a knowledge of gas chromatography and an interest in instrumentation and electronics) in the Biochemistry Department—The Secretary, Institute of Animal Physiology, Agricultural Research Council, Babraham, Cambridge (August 25)

Temporary Lecturer in the Department of Medicine—The Registrar, The University, Liverpool, quoting Ref No CV/154/N (August 25)

Senior Technical Officer (with a degree with botany or agricultural botany as a major subject) in the Official Seed Testing Station—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (August 27)

Assistant Lecturer (graduate) in Dairying—The Registrar, University College of Wales, Aberystwyth (August 31)

Assistant Lecturer (graduate in physical metallurgy or solid state physics, and preferably with an interest or research experience in the field of deformation studies or magnetic properties) in the Department of Metallurgy—The Registrar, The University Manchester 13 (August 31)

Leverhulme Student (with a postgraduate diploma in chemical engineering or equivalent qualifications) in Chemical Engineering—The Registrar, King's College (University of London) Strand, London, W C 2 (August 31)

Research Assistant in Sociology in the Department of Social Studies—The Registrar, The University, Hull (August 31)

Tutorial Student (graduate or about to graduate) in Zoology—The Registrar, King's College (University of London), Strand, London, W C 2 (August 31)

Assistant Lecturer (preferably with experience of research and teaching) in the Department of Pharmacology—Clerk to the Council, School of Pharmacy, 29/39 Brunswick Square, London, W C 1 (September 1)

Research Assistant in Dermatology—The Secretary of University Court, The University, Glasgow (September 1)

Senior Lecturer (preferably with a Ph D degree and suitable experience in electronics) in Electronic Physics, and a Lecturer in Electrical Engineering—The Secretary, University of St Andrews, Queen's College, Dundee (September 1)

Assistant Lecturer (with research interests in the mode of action of hormones at the cellular level, and qualifications and/or experience should include biochemistry or biophysics) in Zoology—The Registrar, The University, Sheffield (September 3)

Research Demonstrator in the Department of Metallurgy—The Registrar, The University College of South Wales and Monmouthshire, Cathays Park, Cardiff (September 6)

Research Fellow in the Nuclear Physics Group of the Department of Physics, to prepare and participate in experiments on the 7 GeV proton synchroton at the Rutherford High Energy Laboratory of the National Institute for Research in Nuclear Science—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1 (September 7)

Senior Lecturer or Lecturer (veterinary surgeon) in Veterinary Medicine, for duty at the field station near Potters Bar, Middlesex, and at Camden Town—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N W 1 (September 7)

Assistant Librarian (with a good honours degree)—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1 (September 8)

Demonstrator (medically qualified, and/or an honours degree in physiology) in the Department of Physiology—The Registrar, The University, Leeds 2 (September 10)

Entomologist (with a good honours degree), to study the effects of insect infestations on the growth and yield of crop plants—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 10)

Lecturer in Anthropology at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (London and New Zealand, September 15)

Staff Tutor (with a special interest in field work) in the Biological Sciences in the Department of Extra-Mural Studies—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (September 15)

Technical Officer (with a good honours degree or equivalent qualifications in electrical, electronic or radio engineering or another approved subject) in the Department of Biochemistry—The Secretary of the Faculty Board of Biology B, Department of Anatomy, The University, Downing Street, Cambridge (September 15)

Chair of Physiology in the University of The West Indies—The Academic Registrar, University of London, Senate House, London, W C 1 (September 17)

Senior Lecturer or Lecturer (preferably with experience in teaching physical geography) in Geography at the Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, September 24)

Professor of Biochemistry—The Dean, Faculty of Science, Hebrew University of Jerusalem, Israel (September 25)

Chair of Chemistry (inorganic or physical) in the University of Queensland, Brisbane, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Brisbane September 30)

Chair of Zoology—The Secretary, The University, Edinburgh (September 30)

Lecturer in Geology at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London September 30)

Lecturer (preferably with experience in invertebrate teaching to B Sc honours level) in Zoology at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall London, S W 1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (September 30)

Regius Chair of Forensic Medicine in the University of Glasgow—The Private Secretary, Secretary of State for Scotland, St Andrew's House, Edinburgh 1 (September 30)

Lecturers and Senior Lecturers in Electrical Engineering at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office) Marlborough House, Pall Mall, London, S W 1 (Australia and London October 8)

Chair of Pathology in the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall London, S W 1 (New Zealand and London, October 15)

Chair of Biology in the School of Biological Sciences—The Registrar, University of East Anglia, 4 Cathedral Close, Norwich NOR 16P (October 31)

Assistant Experimental Officer/Experimental Officer (unestablished) (with G C E "A" level in two scientific subjects (one biological) or H.N.C., or pass degree or equivalent or Fellow of the Institute of Medical Laboratory Technology, and preferably an elementary knowledge of physics) at the Microbiological Research Establishment (War Dept), Porton Down, near Salisbury, for work in the Biophysics Section of factors influencing survival of air-borne micro-organisms—C13A(ii), War Office, London, S W 1

D S I R Research Fellow (with a Ph D or equivalent qualification in applied mathematics or theoretical physics, and experience in the use of digital computers) in Theoretical Seismology in the Department of Astronomy—The Secretary, The University, Edinburgh

Senior Lecturer or Lecturer (with a first- or second-class honours degree or equivalent, together with at least two years experience in teaching, industry or in research establishments, and preferably special experience in the field of the theory of mechanics or machines) in Mechanical Engineering—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire

Senior Scientific Officer (physical chemist, with a first- or second-class honours degree in chemistry and at least three years postgraduate experience, and experienced in handling materials at very low concentrations, for example, a few microgrammes per cubic metre) to investigate the reactions of atmospheric pollutions and their effects on the formation of mists—The Director, Warren Spring Laboratory, D S I R , Stevenage, Herts, quoting Ref WSL 22Z

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Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO , LTD
ST MARTIN'S STREET, LONDON, W C 2
Telephone Number Whitehall 8831 Telegrams Phusis Lesquare London

Advertisements only should be addressed to
T G Scott & Son, Ltd , 1 Clement's Inn, London, W C 2
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THE SPACE GAME

FOUR Russians are now known to have travelled in pre-determined orbits around the Earth. Gagarin was the first to complete one orbit, Titov covered seventeen. Now, on August 15, the next two astronauts have returned safely to Earth, one (Major Andrian Nikolayev) having completed sixty-four orbits, and the other (Lieut.-Colonel Pavel Popovich), forty-eight.

The Prime Minister of Great Britain and the President of the United States of America have already sent messages of congratulation to the Prime Minister of the U.S.S.R. No doubt political leaders of other countries have sent, or will be sending, similar messages. Such congratulations are very well merited, and *Nature* now wishes, on behalf of the whole world of science, to extend its congratulations to the Soviet scientists and technologists who made possible this most recent epic flight. We also express our admiration of both Major Nikolayev and Lieut.-Colonel Popovich for their bravery and devotion to duty.

It is a pity, therefore, that *Nature*, which publishes important research contributions on all scientific subjects (from space science and astrophysics to virology and macromolecular biology) submitted from every country in the world, and which is also read in every part of the world where scientific research, advancement and teaching are going on, is able at this stage to publish only two short paragraphs (p. 755 of this issue) which refer to the latest Russian achievement and which, though written by one of Britain's leading authorities on the subject, can be of special interest to scientists. It is significant that all the rest of the news concerning this event has already appeared through the media of newspapers, television or radio—no doubt, not before severe scrutiny and censorship.

The Soviet scientists (with the practical collaboration of Nikolayev and Popovich) have proved that they have made great strides recently in scientific and technical research. Nevertheless, the first immediate announcement that the two astronauts are to receive the highest Soviet awards as *sportsmen* gives the keynote to the world's attitude and reaction to this great feat—for the world looks on it as a game: a game in space. Sportsmen play games, and games involve competition. Players compete against their opponents.

This latest achievement in space gives some points and honours to the Soviet scientists and technicians. They have, for example, been the first to prove that man can live, work and sleep in apparent weightlessness for days on end, they have been the first to present live television pictures of a man while in orbit, they have been the first to establish radio communication between two men while both are in space, they have been the first to produce a double descent from orbit. But the Russians were not the first to adopt the policy of keeping the world informed of their space activities—failures as well as successes. The credit, and a very important credit, for that must be given to the United States.

Yet, what has world reaction been? We have so far heard or read little about this feat as a *scientific* achievement—yet, fundamentally, it is just that. On the other hand, it is being looked on as a game in which, for the time being, some points go in favour of the U.S.S.R. But that is as far as the analogy goes: for there is no referee, and even the world of onlookers can see only a very small part of this vast arena—and even this is decided by the players or their sponsors of one side only. So politics are already in the picture: those in favour of the communist way of life are meanwhile in various ways expressing their joy, whereas some (though not all) others, in the democratic countries (especially those who have had no training in science and cannot understand its objectives), have betrayed a feeling of jealousy or what is known colloquially as 'sour grapes'.

We cannot afford to ignore this world reaction: we laugh at it at our peril. It all takes us back to the many occasions when *Nature* has warned that it will be an evil day if science ever becomes the tool of diplomatic or political intrigue. Yet that day has now come. Too often do we hear of the need for space research for reasons of prestige—that is, *political* prestige, of course, and among most of us, this means priority—to be the first: yet priority does not bring prestige in the long run. Moreover, it is already accepted by the layman that the most scientifically advanced country is the one which will win the next world war. Thanks to science, another all-out world war was rendered unthinkable long before the first human astronaut went into orbit: hydrogen bombs, research with rockets, etc., have ensured this. More than one country, and not just two, are already in a position practically to destroy the world as we know it, or would wish it to be.

Therefore, although we agree with Sir Bernard Lovell, director of the Jodrell Bank Experimental Station, that it now appears that the Russians are at present masters of space in the military sense, and although we must reiterate our admiration for the Russian scientists in this, their latest, achievement, we cannot accept the idea that the Russians have necessarily taken the lead in this space game. There are far too many facets, hundreds of ways in which space research can be, and undoubtedly is being, carried out, and, therefore, many, many ways in which points can be gained or lost in this competition. There is every reason for believing that the Americans have the lead in certain aspects of space research, as was emphasized in our comments on the great achievement of Colonel John Glenn (*Nature*, 193, 807, 1962). Unfortunately, in our opinion, international politics have intervened in science and, in certain respects, seem to be in control The result is that scientists in one country are left guessing at what their colleagues in another country are doing, mainly because their political superiors maintain the right to decide what shall be published and what shall be considered secret or, to use the modern phrase, 'classified' Readers of *Nature* would probably be amazed at the delay in publication of certain communications (mainly from the Government departments of some countries) in practically all branches of science, merely because a Government official (usually non-scientific) must have the last word, and often takes a long time in saying it In the long run, this cannot be to the advantage of science, so eventually there lies the threat to world peace, because no one can effectively control that which he cannot understand

These views have been expressed many times in the columns of *Nature* Competition in the search for knowledge cannot be healthy, collaboration can This belief has now been stated more explicitly by General Eisenhower in his comments on the latest scientific achievement of Soviet Russia He does not believe that the Russians have the lead in space He quite rightly objects to the word 'gap' in this connexion He believes that the two countries—the U S S R and the U S A—have been working along different lines We entirely support General Eisenhower, and especially when he said "Co-operation in space research between Russia and America would be a fine thing" Sir Bernard Lovell was even more forthright, when he said that "More than ever, one is appalled by the foolishness of these two countries attempting a common problem in competition"

Indeed, it should not be left at that Collaboration between the U S S R and the U S A would be excellent, but better still would be universal collaboration among the scientists of the world, for there are first-class men of science in many countries Throughout her history, for example, Great Britain has had, and still has, every cause for pride in her scientific achievements, and this applies to some other countries as well So spectacular has space research now become, however, that the general public seem to be looking on it as the prerogative of the U S S R and the U S A It is about time that this view was challenged, for it is not true

Referring to the magnificent results recently obtained by the Soviet scientists, through the agency of Major Nikolayev and Lieut -Colonel Popovich, Prof Alexander Topchiev, vice-president of the Soviet Academy of Sciences, said "The Earth's environment up to 300 kilometres from its surface has been turned from a belt of the unknown into a huge laboratory" So be it—a research laboratory No scientist, no matter what his political views, could possibly imagine a research laboratory without team work A laboratory is certainly not a games arena where sportsmen vie with each other it is a place where men and women foregather in a search for knowledge, a seeking after the truth, and this by experiment and above all, by free discussion of methods and results without interference from those who cannot be expected to know much, if anything, about science or its objectives This is not an argument in favour of complete freedom for scientists to do just as they like without reference to other members of the community in which they live, especially the elected leaders of such a community: but it is a plea for scientists to be allowed much more freedom to communicate and collaborate with each other (recognizing especially that the discipline of science, like the arts, music and so forth, cannot acknowledge political frontiers) If this could be conceded, then, at least, two stumbling-blocks to world advancement would be removed—the astronomical waste of State funds because one country must often be repeating what another has already done, and the very grave prostitution of some of the sciences (not only space research) to political aims and objectives

BRITISH PROSOBRANCHS

British Prosobranch Molluscs

Their Functional Anatomy and Ecology By Vera Fretter and Alastair Graham (Publication No 144) Pp xvi+755 (London Ray Society, 1962 Sold by Bernard Quaritch, Ltd , 11 Grafton Street, London, W 1) 168*s*

THE key to the understanding of the Mollusca resides in the prosobranch Gastropoda If *Neopilina* represents primitive molluscan structure, as it probably does, then great secondary simplification preceded the spectacular evolutionary radiation which gave rise to the major molluscan classes The nearest approach to these simplified molluscs are the more primitive archæogastropod prosobranchs, separated from them only by torsion and possibly asymmetrical coiling

In their account of the British Prosobranchia, Dr Vera Fretter and Prof A Graham have given by far the best general account ever written of this most informative of all molluscan groups, in which basic simplicity leads on to a range of adaptive radiation unsurpassed in any corresponding group in the entire animal kingdom The authors are little concerned with taxonomy and only to a minor degree with the shell, indeed, the majority of their species are figured without the shell and with the 'soft parts', it must appear to the conchologist, almost indecently exposed As stated in its sub-title, this book is concerned with "Functional Morphology and Ecology". To this extent it represents something of a new and welcome departure in publications by the Ray Society and one which, in the hands of its uniquely qualified authors, is an overwhelming success

We are carried in the course of some 700 pages, with more than 300 admirable figures, from an introductory account of the Mollusca followed by a general description of *Littorina littorea* as a typical, as well as probably the commonest, British prosobranch, through sixteen chapters dealing with functional morphology, from shell and mantle cavity to reproductive system and the formation of spawn—in which the mesogastropods show such fascinating diversity—followed by descriptions of development and of larval forms All is covered with a precision of wording only equalled by the elegance of the accompanying figures One is initially surprised that so much is known about these prosobranchs, but realization follows that although every source of published knowledge, including the major contributions of the two authors and their pupils, has been utilized, yet a notable proportion of this book, including very many of the illustrations, consists of hitherto unpublished observations

The third section of the book deals with ecology and is concerned with the limpets, whelks and tingle and other prosobranchs of rocky shores, with those of other marine habitats, with prosobranch inhabitants of brackish and fresh-waters and of the land, and finally with the parasites of these animals It is our good fortune that the very occasional appearance of the heteropod *Carinaria* and more frequent appearance of *Ianthina* brings both these pelagic molluscs within the British fauna and so within the scope and detailed description of these authors We are also fortunate in possessing an interesting range of estuarine and fresh-water prosobranchs and even two terrestrial species, one of which the apple shell, *Pomatias*, receives particular and most welcome attention

It is only in the Neritacea that the British fauna, with its solitary fresh-water representative, *Theodoxus*, is seriously lacking The presence of some of the marine and terrestrial representatives so abundant in warmer latitudes would have been welcome because of the treatment they would here have received with perhaps the conclusion that these animals should be removed from the Archæogastropoda (or Diotocardia, as here preferred) to constitute an order of similar standing to the Mesogastropoda (Monotocardia)

The final section deals with relationships of the Prosobranchia with an appendix on classification and another containing a habitat and distribution list This is followed by 37 pages of references

No comparable book on the Mollusca has been published in our time (Thiele's great hand-book is in a different category) This is functional morphology at its best Every malacologist in every country—because its scope far transcends the British fauna—should possess this book It is infinitely revealing in its descriptions of the elaborate intricacy, above all in the buccal and deeper alimentary systems and in the reproductive system, with their remarkable evolutionary developments

I have read carefully through this very long book and find no cause for significant criticism and only one mistake—a reference on page 10 to cuttlefish where octopods are clearly meant C M YONGE

THE COMPANY OF MINERAL AND BATTERY WORKS

Elizabethan Monopolies

The History of the Company of Mineral and Battery Works from 1565 to 1604 By Prof M B Donald Pp xv+256+10 plates (Edinburgh and London Oliver and Boyd, Ltd , 1961) 50*s* net

THE introduction of a new source of power has always resulted in technical advances, but the changeover inevitably involves the design and manufacture of the necessary machinery the cost of which implies the raising of adequate capital The sixteenth century was such a time of marked development in the utilization of water-power, particularly in the mechanisms necessary for its conversion to industrial use in, for example, providing the air blast for furnaces, operating stamp mills for the crushing of ore or in pumping water from the mines themselves

The capital required ultimately reached proportions so high as no longer to be within the scope of the individual, and associations of a number of persons became essential Two such partnership companies were formed in Elizabethan England, both concerned with the manufacture of metals and the products produced from them Both the Company of Mines Royal and the Company of Mineral and Battery Works received their royal charters in 1568, and it is with the second, and smaller, of these that the present work is mainly concerned Unlike the Mines Royal, the capital was raised entirely in Great Britain, and whereas that body was concerned chiefly with the production of copper in Cumberland, the Mineral and Battery Works had three fields of interest—lead in north Derbyshire, iron, largely for the production of wire, near Tintern Abbey, and brass at Isleworth in Middlesex This range of activity proved too extensive to be controlled by any one of those concerned, and this, together with the technical failure at first to produce brass of sufficient ductility to be 'battered' into the required shapes, rendered it easier to run the partnership as a licensing company drawing its income from royalties paid by licensees operating the processes for which the Company held patents

Since the formation and development of these two Companies are the roots of all future company law and procedure—and not only in Great Britain—the main emphasis of Prof Donald's book is on the legal and commercial sides, though there are inevitably excursions into the technology of the time which add to our knowledge of the history of metallurgy in England and Wales at a critical period in its development

The story is one of continual quarrels and law-suits which later played no small part in restraining the Royal prerogative to grant monopolies and patents It is a story well worth telling, and it is well told, though one would like the evidence for the statements on page 96 that at that date "The phosphorus content" of iron "could be controlled by using limestone to give a basic slag to remove it as phosphate" and that "The sulphur content was controlled by selecting ores containing manganese " F C THOMPSON

HIPPED-PLATE AND THIN SHELL STRUCTURES IN ENGINEERING

Hipped-Plate (Folded Plate) Structures

Their Theory and Analysis By Joachim Born Translated by C V Amerongen Pp ix+250 (London Crosby Lockwood and Son, Ltd , 1962) 63*s* net

Theory of Elastic Thin Shells

By A L Gol'denveizer Translated from the Russian and edited by G Herrmann (International Series of Monographs on Aeronautics and Astronautics Division I Solid and Structural Mechanics, Vol 2) Pp xxi+658 (London and New York Pergamon Press, 1961) 105*s* net

THE literature on hipped-plate or folded-plate structures, as such, is not very extensive although, in recent years, quite a number of papers have appeared in the technical press The book by Born is, therefore, welcome in that it presents a thorough study of what is a specialized branch of structural engineering

The first part of the book deals with the analysis of prismatic hipped-plate structures Initially, the analysis is based on the assumption that the junctions of the plates are laterally restrained which leads to the so-called equation of three shears or stresses This equation is then expressed in differential form to enable the deformations at the junctions to be taken into account where warranted These analyses are then applied to continuous structures, to study the effect of transverse diaphragms and edge beams and, in fact, to the practical problems arising in hipped-plate structures

The second part of the book discusses the various flexural theories that have been put forward and shows how these may be applied to practical designs to determine both the 'membrane' and the bending stresses

Both these parts are illustrated by numerous examples which emphasize the essential features of each method of analysis considered and, further, show how the calculations should be performed in the design office in the most rational and economical manner

The final part of the book deals with pyramidal hipped-plate structures of various types, the fundamental structural action of the various types is broken down into the relevant slab components and analyses developed appropriate to a wide range of structures Also in this part of the book, some interesting structural problems are discussed in the light of hipped-plate theory, among these are plate girder bridges, spiral staircases and various torsion problems

The presentation of the material in the book is in the form of a large number of short chapters, each dealing with a specific aspect, this makes for easy reference In general, the figures are clear, but on some the reduction in size has been such as to make reading of numerical data and some of the symbols difficult

The book is written from the point of view of a structural designer rather than that of the mathematical analyst, the examples chosen are admirably suited to emphasize the design aspects of each problem and the most suitable method of dealing with these The author is a consulting engineer and his book can certainly be recommended to others in this profession and to students seeking a fundamental appreciation of hipped-plate structures

The book by Gol'denveizer is a mathematical treatise on the elastic theory of thin shells which, to a certain extent, is complementary to those already published by Vlasov and Novozhilov The first part of the book presents in detail the derivation of the complete fundamental equations for shells using vector notation This section is based on the assumption that plane sections remain plane, which Gol'denveizer states as "the preservation of the normal element", this assumption is discussed in some detail and the implications of it are illustrated with reference to the equations derived earlier

Part 2 of the book considers the membrane theory for shells as an approximation to the equations derived in Part 1 resulting from the assumption that the transverse shear forces and the twisting moments are zero Thus, the membrane theory is treated as an approximation to the more general bending theory and its range of applicability as such is considered

Part 3 deals with the problem of circular cylindrical shells using Fourier series, the main purpose of this study is to emphasize those aspects of approximate analytical methods of interest in more complex shell structures and to enable the implications of the various assumptions in the analyses to be considered

Part 4 is largely a mathematical justification of the various approximate methods using what is called asymptotic integration In this section the higher derivatives of a parameter representing the half thickness of the shell are retained and attempts made to carry out the integration with complete mathematical rigour The final part of the book deals with the derivation of suitable approximate methods for various types of shell structure

This book is reproduced by a photolithographic process which is quite acceptable to the reader, despite its lack of uniformity It is to be regarded as a treatise on shell theory and its usefulness will be primarily to the research worker in this specific field

R E Rowe

MAGNETIC RESONANCE SPECTROSCOPY

NMR and EPR Spectroscopy

Papers presented at Varian's Third Annual Workshop on Nuclear Magnetic Resonance and Electron Paramagnetic Resonance, held at Palo Alto, California By the NMR–EPR Staff of Varian Associates Pp viii+288 (London and New York Pergamon Press, 1960) 80*s* net

It has become an annual practice of Varian Associates to hold a 'workshop' at Palo Alto, California, and a similar meeting is to be held at Zurich later this year The 'workshop' is designed to introduce new workers to the characteristics of nuclear magnetic resonance and electron paramagnetic resonance phenomena, and to keep experienced ones abreast of developments in both fields The 1959 meeting was divided into three parts an introductory session, two days of lectures and laboratory sessions devoted to high-resolution nuclear magnetic resonance spectroscopy, and similarly, two days to electron paramagnetic resonance spectroscopy

The book is essentially a record of these lectures and some of the laboratory sessions, its aim and general arrangement follow the pattern set by the 'workshop' Part 1 contains five chapters which provide an initial survey, outlines of the basis and range of application of nuclear magnetic resonance and electron paramagnetic resonance spectrometers respectively, and then descriptions of their operating fundamentals Part 2 describes the theory and instrumentation involved in high-resolution nuclear magnetic resonance spectroscopy, and some structural and chemical applications, followed by brief accounts of more specialized topics including the determination of relaxation times, the measurement of the radio-frequency field, and the use of magnetic field modulation in integrating systems Part 3 similarly gives a more detailed account of electron resonance spectroscopy, its chemical and biological applications, and its use for photochemical studies, together with short discussions of colour centres and double resonance phenomena

The book therefore, gives a survey of many aspects of nuclear and electron resonance spectroscopy, although there are some important omissions, for example, there is no discussion of the use of nuclear magnetic resonance for the structural analysis of solid samples Considerable space is devoted to the influence of various instrumental parameters on the output signal, and while this is largely described around Varian instruments, most of it will be relevant to other spectrometers The main defect is an apparent lack of editing and co-ordination in producing the book from the material presented at the 'workshop', the repetition which is acceptable and desirable in a lecture series is less welcome in a book Basic topics are described with increasing detail but with considerable overlap on passing through the various chapters of Part 1 to Parts 2 and 3, and, for example, the same photograph of a high-resolution nuclear magnetic resonance spectrometer appears three times in Part 1, and similar drawings of the spectral shape produced by 'dished' and 'domed' magnetic fields occur on pp 60 and 95

In short, the book contains good material but is badly edited It can be recommended to all who need a general introduction to the field, and should be particularly valuable to those who want some insight into the experimental problems involved in the operation of spectrometers R K WEBSTER

DEEP-SEA MICROBIOLOGY

Meeres-Mikrobiologie

Tiefseeforschungen Von Prof Dr A E Kriss Pp ix + 570 (Jena Gustav Fischer Verlag, 1961) 98 10 D M

It has long been recognized that Russian investigators have played an important part in the development of the science of marine microbiology It is unfortunate, however, that owing to language difficulties and the relative inaccessibility of many of their journals and monographs, their results have remained comparatively unknown to most workers outside the U S S R The appearance of another book on marine, and in particular on deep-sea, microbiology where so few already exist would in itself be an event, but where it also summarizes the tremendous volume of recent Russian work in this field it is doubly welcome The present volume is a revised German edition of the 1959 Russian one which gained for its author a Stalin prize A more thoroughly revised English version is due to appear shortly

In 1947 the Institute of Microbiology of the Academy of Sciences of the U S S R organized a series of investigations into the microbiology of the deep layers of the various seas and oceans This was in contradistinction to most of the previous work, both inside and outside the U S S R , which dealt mainly with surface waters and the continental shelf, and which was so ably summarized by ZoBell in his *Marine Microbiology* (1947) Commencing with the Black Sea, these Russian investigations have virtually covered every major sea and ocean from the Arctic to the Antarctic, allowing comparison to be made between various types of marine basins—the Black Sea, an intra-continental deep-sea basin, the Caspian, the largest sea-lake, the Pacific, in regions containing the deepest trenches in the world, the Arctic, with its perennial covering of pack ice and the results to date have now been ably summarized by Prof Kriss

Descriptions are given of the methods, both quantitative and qualitative, used for investigating water masses in depth including two pioneered in this sphere by Kriss and his colleagues, namely, the submerged-slide and membrane-filter techniques As a result, we have for once a composite picture of the vertical and horizontal distribution of bacterial populations throughout the world's seas and oceans, including the effects thereon of various seasonal, geographical and other factors Two long chapters are devoted to descriptions of the various species of bacteria, yeasts and actinomycetes encountered and their morphological variations, both vertical and horizontal Some of these organisms are new to science, for example, the class of Krassilnikoviae, of which some excellent microphotographs are provided

Having described both qualitatively and quantitatively the microflora, and also their general biochemical propensities in transforming a great variety of substances in sea and oceans, Kriss then attempts to give some idea of the microbial biomass in these water basins, utilizing to the full the direct-count and membrane-filter techniques, although some may doubt the reliance placed on these by Russian workers Data indicate that the greatest microbial concentrations occur in certain layers in the Caspian Sea (approximately 250,000/ml or a biomass of 45 mgm /m ^{3}) followed by the Black Sea and the Pacific in the area of the Kuril Islands In the North Polar Sea, even at the season of maximal activity the counts and biomass are approximately only 1/10–1/20 of those of the Caspian

To many readers, the final chapters on micro-organisms in relation to the biological productivity of the ocean, and as indicators of hydrological phenomena, will be of most interest Kriss maintains that insufficient account has been taken of the importance of micro-organisms for investigating problems of biological activity in aquatic environments, as, for example, in their role in providing, in themselves, food for young fish and in producing vitamins and other biologically active substances With regard to the use of micro-organisms as hydrological indicators, Kriss obviously believes that they can be of immense value for the study of the dynamics and origin of water masses and for indicating deep currents at present poorly defined by other methods By the use of micro-organisms, Kriss has been able to substantiate and extend the more widely accepted views concerning the general hydrography of world oceans as expounded by Sverdrup, Johnson and Fleming (*The Oceans, Their Physics, Chemistry and General Biology* New York, 1942), although on occasions he has seemed to differ considerably from these experts

In a volume such as this, full of interesting and often arresting facts and information in almost every page, it is difficult to be critical The major fault appears to be the almost superabundance of data, so that one cannot see the wood for the trees There appears to be a reiteration of material in different chapters The long, detailed Chapter 3, describing the biochemical, morphological and other properties of the various species isolated, seems out of place in this type of book, and could well have been combined with Chapter 6, also dealing with the morphological characteristics of micro-organisms Moreover, it is unfortunate that Krassilnikov's *Determinative Key for Bacteria and Actinomycetes* (Moscow, 1949), known to few outside the U S S R , should have been used for purposes of classification

Despite these criticisms, the book should be read by all interested in marine science, because none by previous authors contains anything approaching the amount of data collected here It is well produced and fully documented, and lavishly illustrated with figures, diagrams and photographs, and deserves to become widely known J M SHEWAN

INTERATOMIC FORCES: MAXWELL TO SCHRODINGER*

By PROF C A COULSON, F.R S.

Mathematical Institute, University of Oxford

(1) Introduction

CLERK MAXWELL played a notable part in the early stages of our theory of interatomic forces It is not true, however, that he initiated the kinetic theory of gases, in which interatomic forces play an essential part What he did was to extend and consolidate this field So this account is divided into two halves In the first half I will try to describe the way in which, a hundred years ago, people were thinking about the kinetic theory of gases—the kind of progress that Maxwell and others were able to make, the difficulties that they had to face, and the problems which seemed to them insuperable This will naturally lead to the second half, in which I shall show all the major problems which could not then be solved, now have been solved We shall find that although there was one direction in which, if he had been able to improve on current accuracy of measurement, further progress could have been made, in the other directions no progress was indeed possible until other new and fundamental concepts and techniques had been developed Maxwell had almost got to the limit of what he could have done in the discussion of interatomic forces We needed the quantum theory, with its many ramifications, before one could go any farther

(2) Early Ideas on the Kinetic Theory

It is hard for us, now, to realize the difficulties in developing the kinetic theory A hundred years ago even the idea of an atom was not universally recognized Indeed, as the celebrated debate involving Ostwald, Boltzmann and others showed, distinguished men of science continued for several more years to deny its existence Maxwell himself, in his famous article on the atom in the *Encyclopœdia Britannica*, begins with the definition "The atom is indivisible", and justifies this with reference to its etymological origin in the Greek, and the comment that although space and time appear to be infinitely divisible, the same is not true of the elementary units of matter But he makes little attempt, at first, to say more precisely what these indivisible units actually are

We all know that the idea of an atomic theory has a very long history, going back to Lucretius, Leucippus and Democritus But although Democritus, in a moment of vision, could say that beyond the atoms everything else was void, and although Lucretius tried to relate the smoothness or roughness of their exterior to their taste (sweetness or sourness), and although Plato with his love of symmetry spoke of their geometrical shapes (the cube, the tetrahedron, etc), it was not for many centuries that any serious progress was made Herapath (1790–1868) and J J Waterston (1811–83) had thought of collisions between the atoms, and in fact it was Waterston, himself a keen billiards player, who introduced the idea of a hard, or 'billiards ball' atom This particular idea was to play a large part in the development of the theory of gases, and it illustrates the general theorem, that the initial development of scientific ideas (though not necessarily their final form) owes more than is sometimes supposed to non-scientific factors It was suggested on one occasion, I think by Lord Rayleigh, that if Maxwell and Clausius and others had also been billiards fans, we might have seen more rapid progress in the early stages of the theory

So it came about that in 1859 when the British Association met in his own city of Aberdeen Maxwell read a paper in which he began by saying that a gas consists of many little aggregates, not yet to be closely defined, moving in all directions Up to this date it had apparently been assumed, by Clausius and others, that as these minute particles flew around in all directions, they maintained always the same uniform speed But Maxwell thought otherwise There must be some sort of distribution function At present it was unknown, but shortly it was to be discovered To quote from that first paper

> "In order to lay the foundation of such investigations on strict mechanical principles, I shall demonstrate the laws of motion of an indefinite number of small, hard and perfectly elastic spheres acting on one another only during impact
>
> "If the properties of such a system of bodies are found to correspond to those of gases, an important physical analogy will be established, which may lead to more accurate knowledge of the properties of matter"

The time was now ripe for a more careful discussion of this velocity distribution But before it could be worked out, it was evident that the hard billiards ball analogy would have to be abandoned For if two equal and perfectly elastic spheres collide not only is the total energy conserved (as it obviously must be), but the two spheres merely exchange velocities along their line of centres Furthermore, the number of collisions which result in a given deflexion will be directly proportional to the velocity This leads to an unsatisfactory variation of the coefficient of viscosity with temperature It had therefore to be modified So in 1866 we have Maxwell's most classical memoir devoted to this topic Let us see how he begins

> "In the present paper I propose to consider the molecules of a gas not as elastic spheres of definite radius, but as small bodies or groups of smaller molecules repelling each other with a force whose direction always passes very nearly through the centres of gravity of the molecules, and whose magnitude is represented very nearly by some function of the distance of the centres of gravity I have made this modification of the theory in consequence of my experiments on the viscosity of air at different temperatures, and I have deduced from these experiments that the repulsion is inversely as the fifth power of the distance

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* The substance of one of the lectures delivered at King's College, London, to mark the centenary of the publication of Clerk Maxwell's papers on electromagnetic radiation These lectures will be published in book form

> "If we suppose the molecules hard elastic bodies, the numbers of collisions of a given kind will be proportional to the velocity, but if we suppose them centres of force, the angle of deflection will be smaller when the velocity is greater, and if the force is inversely as the fifth power of the distance, the number of deflections of a given kind will be independent of the velocity Hence I have adopted this law in making my calculations"

It sounds very good, and it is certainly attractive But alas, even though it be almost an act of impiety, truth requires us to add that not only was there a slip in the calculations, his experimental values for the variation of viscosity with temperature were also wrong So we can see that, whatever Maxwell may have thought, it was purely a fortuitous situation that led him to propose an inverse fifth power law, and then to proceed with a series of further calculations based on that assumption

The possibility of some sort of power law for the mutual attraction or repulsion of two atoms was not unthought of before this time Maxwell himself, in his *Encyclopædia Britannica* article, refers to the ideas of Boscovich, who supposed the atoms to be of zero size—mere centres of some sort of force-field—such that there was an r^{-2} attractive force down to some minimum distance $r_{\min}$, and then at closer distances an infinite repulsion But this was rejected on the grounds that atoms were not of zero size, and an r^{-2} attraction did not fit observations of the viscosity–temperature relation

This same memoir (the '*Phil Trans* Memoir', as it has sometimes been called) goes on to consider the results that would follow from the assumption of a distribution law of velocities It has about it an almost Euclidean character, as theorem follows theorem with impeccable correctness But by far the most important of these is the first derivation of the distribution law Assuming the independence of the three components ξ, η, ζ of the molecular velocity in three mutually perpendicular directions, it follows quite simply that if the distributions of ξ, η, ζ are each of the form $f(\xi^2)d\xi$, then

$$f(\xi^2) = Ae^{-m\xi^2/2kT}$$

where T is the absolute temperature A knowledge of f, now quite properly called the Maxwell–Boltzmann distribution function, enabled Maxwell to calculate all the necessary mean and relative velocities which were involved in his earlier general formulæ The essentials of the kinetic theory of gases had now been established

(3) Problems raised by Maxwell's Work

Having briefly outlined Maxwell's successes, it may be appropriate to turn to the difficulties which he exposed, and the nature of the problems which he left unsolved

First there is the word 'molecule' itself As one of the earlier quotations shows he could not decide what sort of aggregate a molecule could represent In a lecture to the British Association at Bradford he realizes some of these problems

> "But we must now go on to molecules Molecule is a modern word It does not occur in Johnson's Dictionary The ideas it embodies are those belonging to modern chemistry"

A little earlier in the same lecture

> "An atom is a body which cannot be cut in two A molecule is the smallest possible portion of a particular substance No one has ever seen or handled a single molecule Molecular science, therefore, is one of those branches of science which deal with things invisible and imperceptible by our senses, and which cannot be subjected to direct experiment"

One might almost say that he had become involved in the positivist, or operational, controversy that was to become effective some decades later In another British Association address he remarks that mathematics "is an operation of the mind", and physics is "a dance of molecules", with "laws of their own' But the connexion between the two takes us to a region where it is dangerous to tread

> "But who will lead me into that still more hidden and dimmer region where Thought weds Fact, where the mental operation of the mathematician and the physical action of the molecules are seen in their true relation? Does not the way to it pass through the very den of the metaphysician, strewed with the remains of former explorers, and abhorred by every man of science?"

This is a strangely modern cry to come from an early Victorian Yet it is a cry that was often to be heard in later years, and still is heard, chiefly from the mouths of those who do not understand the nature of scientific discovery For the "den of the metaphysician" is every scientist's study

The second problem arising from Maxwell's work, and to the solution of which he could contribute nothing, was the origin of the forces which one atom exerted on another How could one atom exert forces on another at some distance from it? Surely one of the arguments in favour of these atoms was that they explained the way in which forces, such as viscosity and pressure, could operate without the need to invoke action-at-a-distance? "It is in questionable scientific taste", he once said, "after using atoms so freely to get rid of forces acting at sensible distances, to make the whole function of the atoms an action at insensible distances"

Looking at this matter from the added experience of a later century, it is easy now to see that, at that particular stage, there was no conceivable chance that Maxwell should have solved this problem We have merely to recall that the very word 'electron' was only coined—by G Johnstone Stoney—in 1891, twelve years after Maxwell's death, and then it was nothing more than "a natural unit of electricity", with electrolysis as its basis A fairly well-developed theory of the isolated atom is an inescapable pre-requisite to any adequate account of the mutual interaction of two such atoms

But this leads to a third problem, in which Maxwell took considerable interest, and to which he frequently referred in his public lectures This is the apparently uniform and identical nature of every atom or molecule of a given kind Let us put this problem in his own words, taken from an address to the Mathematical and Physical Sections of the British Association

> "In fact one of the first conditions which a molecule must fulfil is, apparently, inconsistent with its being a single hard body We know from those spectroscopic researches which have thrown so much light on different branches of science, that a molecule can be set into a state of internal vibration, in which it gives off to the surrounding medium light of definite refrangibility—light, that is, of definite wavelength and definite period of vibration The fact that all the molecules (say, of hydrogen) which we can procure for our experiments, when agitated by heat or by the passage of an electric spark, vibrate precisely in the same periodic time, or, to speak more accurately, that their vibrations are composed of a system of simple vibrations having always the same periods, is a very remarkable fact"

In all this Maxwell was reacting to one of the new thought-forms of his age Darwin's *Origin of Species* was published in 1859, the year before Maxwell went to King's College, London In 1861 there had been the famous debate between Huxley and Bishop Wilberforce at the Oxford meeting of the British Association Scientific thinking had been deeply coloured by this new way of regarding Nature So Maxwell ponders on why the characteristic frequencies of emission and absorption of light do not themselves differ from one atom or molecule to another He returns to it again and again

> "Each molecule, therefore, throughout the universe, bears impressed on it the stamp of a metric system as distinctly as does the metre of the Archives, or the double royal cubit of the Temple of Karnac
>
> "No theory of evolution can be formed to account for the similarity of molecules, for evolution necessarily implies continuous change, and the molecule is incapable of growth or decay, of generation or destruction"

Again we can see that, with knowledge as it was at the time, this was bound to be an insoluble problem With our greater insight now, we can understand that without some form of quantum theory there could be no answers to questions about the identical nature of all atoms and molecules of the same substance Maxwell had got himself to the position where he was wanting to ask one of the most important questions physics ever has had to ask This is Why do we get quantization in certain phenomena, why are there quantum effects, showing discrete and not continuous variations ? This question lies at the very heart of modern physics, it dominates the whole of chemistry, and shows every sign of being no less important in biology But although Maxwell had indeed brought himself to the position where he would have liked to ask these questions, he could not do so Ideas of this kind were not 'in the air' at this time It was necessary to wait another forty years until Planck and Einstein had prepared the ground, only then could these questions be posed

We come to the fourth—and last—in this series of unanswered problems of Maxwell's day This concerns the formation of a molecule Atoms may —almost by definition—be unbreakable, but how did they ever get formed into molecules ? Surely no discussion that we can hope to bring forward will ever be able to pierce this mystery So he writes

> "The form and dimensions of the orbits of the planets, for instance, are not determined by any law of nature, but depend upon a particular collocation of matter The same is the case with respect to the size of the earth, from which the standard of what is called the metrical system has been derived But these astronomical and terrestrial magnitudes are far inferior in scientific importance to that most fundamental of all standards which forms the base of the molecular system Natural causes, as we know, are at work, which tend to modify, if they do not destroy, all the arrangements and dimensions of the earth and the whole solar system But though in the course of ages catastrophes have occurred and may yet occur in the heavens, though ancient systems may be dissolved and new systems are built, the foundation stones of the material universe remain unbroken and unworn They continue this day as they were created—perfect in number and measure and weight "

Those last two sentences are a wonderful illustration of pure Victorian prose Yet there is—for me—a certain irony in the fact that I read them the day before I saw the colour film of the first Eniwetok explosion of an atomic bomb For what I was witnessing in the disappearance of an island a mile or so in length was the simultaneous shattering of a very large number of these "unbreakable foundation stones"

Maxwell could not be blamed for not foreseeing this His distinguished contemporary, Prof Tait of Edinburgh, had written that "the great test of the reality of matter" was its "indestructibility" Molecules, and atoms, were to be regarded as part of the given world At that stage of inquiry it was pointless to be overmuch disturbed about their origin

(4) Recent Developments : Origin and Nature of Interatomic Forces

The time has come to look at the changes that have taken place since Maxwell's day It will be necessary to skate rather lightly over the arguments and the justifications for our present beliefs But perhaps this will not matter, since it is with the nature of these beliefs rather than the evidence for them that we are concerned

The four problems which were listed in section 3 can be summarized (1) What is a molecule, and its relation to its constituent atoms ? (2) What is the origin of intermolecular or interatomic forces ? And what is their law of dependence on distance and orientation ? (3) Why are molecules so invariable in character with no evolutionary or continuously varying properties ? (4) How does a molecule form ?

There is only one of these four problems in which Maxwell could have been expected to have made further progress than he did This is the form of the interatomic or intermolecular force and the progress would have been achieved by making more use of Clausius's virial theorem Clausius (1822–88) was nearly ten years older than Maxwell, and outlived him by nearly another ten years (Maxwell's dates were 1831–79) He is best known by his clear statement of the second law of thermodynamics, and his development of the concept of entropy But he had introduced the virial theorem, and so made possible an experimental estimation of the forces which led to the laws of imperfect gases In particular, if we suppose that the atoms of a gas, when at distance r apart, exert mutual forces on each other derivable from a potential energy $U(r)$, then the virial law leads to the virial expansion

$$pV = A + B/V + C/V^2 + \qquad (1)$$

where the successive coefficients A, B, C, are such that

$$A = nkT$$

(T = absolute temperature, n = number of atoms)

$$B = 2\pi nkT \int_0^\infty r^2(1 - e^{-U/kT})\,dr$$

Kammerlingh Onnes called these the first, second, virial coefficients B is due to collisions between pairs of particles, C is due to ternary collisions, etc Corrections are needed in the virial expansion (i) if the interacting species do not exert central forces on each other, and (ii) if the temperature is so low that quantum corrections are involved For the interaction of rare gas atoms at room temperatures neither of these is serious It follows that by a careful experimental determination of the constants in equation (1) it is possible to get information about $U(r)$. Since B is a function of U and of the temperature T, a complete knowledge of B as a function of T, more particularly if supplemented by some knowledge of C, should be sufficient to infer the form of $U(r)$. If Maxwell had been able to follow up this line of attack, the inadequacy of the hypothetical inverse-fifth power law would soon have been apparent.

As we said earlier, Maxwell could in principle have made progress along these lines. But he did not, and it was left until later before much real progress was achieved. This progress consisted in recognizing that the interaction $U(r)$ is best thought of as the combination of an attractive potential and a repulsive one. Van der Waals approximation to equation (1)

$$(p + a/V^2)(V - b) = nkT \qquad (2)$$

suggested that there were attractive forces (and the existence of surface tension confirmed this), and also that something similar to an atomic radius existed to show that one atom could not approach closer to another atom than a certain distance without overcoming considerable repulsive forces.

One part of the origin of these repulsive forces is easy to recognize. For if two atoms approach so close that the nucleus of one gets within the greater part of the charge-cloud of the other, the mutual interaction will be dominated by the ordinary classical Coulomb repulsion between the two positive nuclear charges. There is, however, another important contribution to this repulsive force, which will be described later. But it follows that at small distances the repulsive force is larger than the attractive one. This suggests an inverse power law of the form

$$U(r) = C_1/r^n - C_2/r^m \qquad (3)$$

where C_1, C_2, n and m are constants. The first term in equation (3) is the repulsive one, the second term is the attractive one, so that the repulsion at small distances requires that $n > m$. The most commonly adopted form of equation (3) is the particular case when $n = 12$, $m = 6$. This is the Lennard-Jones potential.

$$U(r) = C_1/r^{12} - C_2/r^6 \qquad (4)$$

A potential of this kind also fitted crystal dynamics, though the index n was not critical, and values in the range 9–15 could be chosen almost arbitrarily.

With such a potential it was possible to choose C_1 and C_2 so that the second virial coefficient was well represented. It could then be argued that, in this empirical fashion, the effective law of force had been determined. All this could in principle have been accessible to Maxwell.

However, an empirical knowledge of $U(r)$ is not sufficient by itself. We want to know more about the origin of these attractive and repulsive forces, and we want to be able to calculate them—in any event sufficiently accurately as to feel confident that we are giving the correct explanation of their origin. There is also a further point closely related to the first and last of the four problems listed earlier. Why is it that some pairs of atoms interact with a potential energy of the general type of equation (4), and other pairs of atoms actually join together to form stable molecules? The subject of interatomic forces is thus linked with the subject of valence. In this field Maxwell was under the disadvantage that official chemists were only just beginning to establish any serious theory of valence. The old electrostatic picture of Berzelius, in which the molecule HF would be treated as if it were H^+F^-, was manifestly unable to account for covalent bonding, as in H–H or F–F. In fact, it was only in 1861 that Kekulé was talking of fixed atomic valences, and the traditional symbol for a chemical bond, the familiar horizontal line as in H–H, was being introduced by Odling, Crum Brown, Frankland, Couper and others. It soon became quite clear that there must be some entirely additional factor to be considered, not included in equation (4): this factor would enable us to decide whether two or more atoms would or would not form a molecule, and whether two molecules would join to form a larger molecule, or (more commonly) interact according to some such law as equation (4).

There are thus three issues to settle. (1) What is the origin of the attractive term in equation (4) and why is it an inverse sixth-power potential? (2) What is the origin of the repulsive term in equation (4)? (3) Why is there sometimes an attractive force instead of the repulsive term, and this attractive term is sufficient to lead to the formation of a stable molecule? We must consider these in turn.

(5) Long-range Dispersion Forces

If two atoms or molecules carry net charges there will be quite straightforward forces of attraction or repulsion due to conventional Coulomb (that is, inverse-square law) forces between these charges. We neglect this possibility, since it is unusual and trivial. We have to show, instead, why two neutral atoms or molecules exert long-range attractive forces on each other.

Consider first two hydrogen atoms, as in Fig. 1,

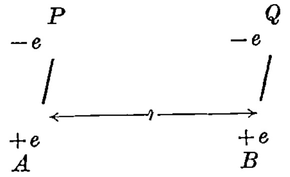

Fig. 1

the nuclei of which A, B are a distance r apart. Each atom has one negative electron (at P and Q respectively). The combination of $+e$ at A and $-e$ at P is nearly equivalent to an electric dipole of magnitude $e \times AP$. Similarly, the combination of $+e$ at B and $-e$ at Q is nearly equivalent to an electric dipole of magnitude $e \times BQ$. These two dipoles will exert forces on each other. For the mutual potential energy of two dipoles $\mathbf{m}$ and $\mathbf{m}'$, separated by a distance $\mathbf{r}$, is

$$\frac{\mathbf{m} \cdot \mathbf{m}'}{r^3} - 3\,\frac{(\mathbf{m} \cdot \mathbf{r})(\mathbf{m}' \cdot \mathbf{r})}{r^5} \qquad (5)$$

It is obvious that this varies with distance according to the law r^{-3}. Now the electrons are moving, so that we must take some sort of average of this potential. In wave-mechanical language, we treat the expression in equation (5) as a perturbation acting on the two atoms The first-order energy change is zero, and so we need to go to second order As a result the change in energy is of dimension $\left(\frac{1}{r^3}\right)^2$, that is, r^{-6} Following London, who first set out this analysis, we call this the dipole–dipole interaction There are, naturally enough, dipole–quadrupole, quadrupole–quadrupole interactions, for which the energy varies, with a higher power of $1/r$ When the atoms or molecules have more than one electron each, the instantaneous dipole moments in equation (5) are obtained by summation of the separate one-electron terms

In this way we can see the origin of the attractive term C_2/r^6 in equation (4) But in addition the analysis shows that the coefficient C_2 is proportional to the product of the polarizabilities of the two interacting systems In favourable cases the precise value of C_2 can be determined by purely theoretical means We describe these forces as dispersion forces because by far the greater part of the energy comes from the outer and most loosely bound electrons these are the same as the electrons responsible for the dispersion of light

There are two other contributions to these long-range attractive forces both are dependent on the existence of a permanent dipole moment in the interacting systems If one molecule has a permanent dipole moment, this will interact with the permanent moment of the other It will also polarize the other Both these phenomena lead to a net force of attraction In both cases the associated energy varies as r^{-6} Thus the constant C_2 in equation (4) represents the sum of three separate contributions All three are often of the same order of magnitude, with the induction force tending to be the smallest

More recently, it has been shown (first by Casimir and Polder) that on account of the finite speed with which the electromagnetic field is transmitted from one molecule to another, the dispersion energy varies as r^{-7} rather than r^{-6}, when r is large Fortunately, this result, however amusing, is scarcely of any experimental significance

(6) Overlap Repulsive Forces

To understand the repulsive forces in equations (4) and to be able to decide in what cases this repulsive force should be replaced by a very powerful attractive force, leading to the formation of a stable molecule, we need the wave mechanics of Schrodinger, and the exclusion principle of Pauli A simplified account of these, as they affect an isolated atom, can be given as follows

Each electron in an atom must no longer be described in terms of some sort of planetary orbit around the nucleus, but in terms of a charge-cloud These charge-clouds, or atomic orbitals as they are usually called, have size, shape and energy If we build up an atom by first removing all the electrons and then feeding them back to the nucleus, one at a time, we shall presumably begin by first filling the lowest energy levels The Pauli principle tells us that each allowed orbital will only accommodate two electrons, and then they must have opposed spins This means that the second pair of electrons will have to go into a higher energy orbital, and this process continues until all our electrons have been accounted for One important result of this is that the last few electrons to be added to the atom have much higher energies than the first few, also, they are forced away from the first ones, and take up places farther out from the nucleus It is thus that the periodic shell structure of a heavy atom is explained We could almost summarize by saying that Nature is so designed that we cannot have too many electrons in the same region of space without having to pay for it in terms of higher energies (This is the wave-mechanical analogue of the semi-classical theorem that in any volume h^n of phase space, where n is the number of spacial co-ordinates, there is just one allowed energy level)

Now let us see what happens if we bring two helium atoms together from a large distance Each separate helium atom has two electrons, which complete its inner shell (the K-shell) and so lead to the familiar inert gas structure In Fig 2a are shown the two atoms a long way apart, and the spheres within which each separate charge-cloud is effectively confined We have to say 'effectively confined' because strictly the density of the charge-cloud extends to infinity However, on account of its exponential decay with distance we can draw boundary surfaces such as those of Fig 2a, such that a large fraction, for example 95 per cent, of the charge lies within those surfaces It is in this way that we define the size (or Van der Waals radius) of an atom, a matter about which, of course, Maxwell was powerless to say anything really fundamental These two pairs of electrons so successfully 'occupy' the volumes shown in Fig 2a that no other electrons can get within this volume without raising the energy

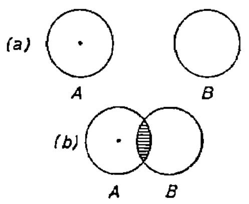

Fig 2 (a) Two atoms A and B well separated from each other (b) The same two atoms brought so close that their charge-clouds overlap The overlap region is shown shaded

Next consider what happens when the two atoms are brought closer together After a while their boundary surfaces overlap, as indicated in Fig 2b But this causes trouble, since in the shaded volume, which is common to both atoms, there are now too many electrons In a rough sort of way we can say that some of them must go into (locally) higher energy states This means that when the atoms get within each other's boundary, the energy rises The prime cause of this rise is the Pauli principle But, of course, a rise in energy of this kind is entirely equivalent to a repulsive force Thus, the origin of the repulsive force (equation 4) is essentially the influence of the exclusion principle, operating as soon as the separate atomic charge-clouds overlap We may therefore call these repulsive overlap forces

A similar argument will apply whenever we bring together atoms or molecules with completed electronic shells, since, when the two boundary surfaces overlap, the Pauli principle will begin to operate, just as in the case of two helium atoms just described Furthermore, it appears that the energy rises very rapidly as the overlap increases The Lennard–Jones formula (equation 4) represents this by the term C_1/r^{12}, which increases rapidly as r decreases Accurate calculations by Bleick and Mayer for helium and neon, using wave-mechanical methods, suggest that a better form may be an exponential one, so that instead of equation (4) we should write

$$U(r) = C_1e^{-ar} - C_2r^{-6} \qquad (6)$$

Fortunately, however, there is little real difference in predicted behaviour when we use the 6–12 formula (equation 4), or the exp-6 formula (equation 6)

(7) Valence Forces

The third problem enunciated at the end of section 4 was concerned with the fact that sometimes, instead of a repulsive force as described in section 6, we find a strong attractive one This is, of course, nothing other than what the chemist calls a valence force

To understand these valence forces let us suppose that in Fig 2a we were bringing together two hydrogen atoms instead of two helium atoms The essential difference is now that each atom has only one valence electron rather than two Each boundary sphere can therefore hold one further electron without the operation of the Pauli principle necessarily involving large repulsive energies The two atoms may therefore approach closer together, as in Fig. 2b, so that their boundary surfaces overlap But this is only possible on one condition—that the spins of the two electrons are opposed (that is, antiparallel) If the spins are parallel, there is anti-bonding with a large repulsion, if the spins are opposed, there is bonding Thus the role of the spin is now quite crucial

We still need to show that, when the two spins are opposed, there is bonding What we have so far shown only amounts to a proof that there is no overlap repulsion. The simplest way to see this is by means of the diagram in Fig 3. Let us consider just one electron in the presence of two separate attracting nuclei A and B If we are going to apply this argument to the hydrogen molecule the nuclear charges will be $+e$, and the electron at P will experience a potential energy $-e^2\left(\frac{1}{r_a} + \frac{1}{r_b}\right)$. Let us now compare the values of this potential energy when P moves on the sphere around A, represented by the dotted circle The potential energy is lowest (largest negative value) when P is at Q, on the axis of the molecule, between A and B, and it is highest when P is at R This means that the electron will tend to concentrate itself around Q rather than around R Alternatively, we may say that the charge-cloud will build up a greater density around Q, drawing charge from the outer regions of the molecule towards the centre of the molecule, as represented schematically in Fig 4 Now the negative charge at Q will serve to pull both nuclei A and B towards it In this way there is a force of attraction If it is sufficiently strong, it will lead to the formation of a stable molecule.

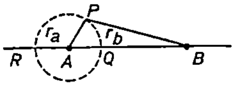

Fig 3

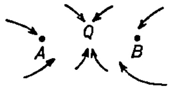

Fig 4

It may now be objected that in H_2 there are two electrons This modifies the argument, but without altering the result For both electrons will tend to concentrate their charge-clouds around Q However, the mutual Coulomb repulsion between these electrons will cause them to 'elbow' each other away from Q But some excess charge will still remain around Q, leading to the same result as before In a strict calculation we should have to calculate this distortion of the original charge-clouds, and we should also need to take account of the kinetic energy of the electrons Here, too great a concentration operates to produce an effective repulsion For by concentrating an electron in a small region (for example, a rectangular or spherical box), we reduce the wavelength λ of the associated de Broglie waves Now the momentum p is related to λ by the de Broglie formula $p = h/\lambda$ So a decrease in λ implies an increase in p, and therefore in the kinetic energy $p^2/2m_1(p^2/2m = \frac{1}{2}mv^2)$. Fortunately, however, this increase in kinetic energy is less than the decrease in potential energy The argument for this assertion follows from the virial theorem previously discussed, where it can be proved that in the equilibrium configuration the kinetic energy gain relative to the two isolated atoms is only one-half of the corresponding decrease in potential energy So, in an almost totally unexpected way, the same theorem which Maxwell could have used to obtain empirical information about interatomic forces in a gas can now be used to deal with situations such as are involved in chemical bonding, of a type that Maxwell could not conceivably have discussed

(8) Conclusion

We can now see how impossible it was that Maxwell should have been able to describe either the dispersion attractive forces, or the spin repulsion and attractions For, implicit in the account of sections 5–7 there lies the discovery of the electron by J J Thomson, the nuclear atom of Rutherford and Bohr, the idea of electron shells associated with Moseley, the concept of stationary states with no radiation (how appalled Maxwell would have been at the almost cavalier way in which his superb development of the equations of the electromagnetic field had to be abandoned at the microscopic level !), the wave equation with its quantum conditions, and the Pauli exclusion principle These concepts, which are the peculiar glory of modern physics, have changed our way of thinking about interatomic forces so utterly that, although the problems are the same, the accounts given in the first and second parts of this account appear to have almost nothing in common Indeed, there are those who, when hearing of wave equations, wave functions, charge-clouds and their overlapping, or spins and their pairing, and realizing that not one of this whole catalogue has the objective character which will permit us to hold it in our hand and look at it, will want to recall some of the words of Maxwell which I quoted earlier If Maxwell himself had been here to-day, he might have said "Did you read that sentence of mine about passing through the metaphysician's den ?" We should have had to reply "This way may, in truth, be strewed with the remains of former explorers, but it is only because they have passed along it that we can complete the story which you began to tell".

THE EARLY DAYS OF CHEMICAL FERTILIZERS

By G E. FUSSELL

BEFORE the third decade of the nineteenth century farmers used nothing that could properly be called a chemical fertilizer The just reason for this was that none was available Most of the fertilizing agents applied to the soil were organic—farmyard manure, the sheep fold, waste products of the house, animal and plant refuse, wool and hair waste, shreds of leather, tanner's bark, and organic wastes from soap and sugar boiling In addition to these the farmers used the so-called fossil manures, lime, slaked and unslaked, chalk, marl, sea shells, and mixed their soils as they deemed it necessary and rewarding They sanded the clay and clayed the sand and the fen All this was traditional, and most of it had been used for centuries, if not millenia

These materials were used empirically according to the lessons of experience derived from practice No farmer would, I suppose, have claimed to understand the chemical processes which occurred as a result of his action indeed, the chemists themselves had been contented with speculation until the seventeenth and eighteenth centuries when the new experimental work so widely extended our knowledge of the world around us, and provided a groundwork of the science of plant nutrition

The early philosophers who devoted themselves to plant chemistry were handicapped by their acceptance of the traditional four elements of classical times, and the three principles of Arabic thinking but several thought that nitre or saltpetre was the prime promoter of vegetation Francis Bacon, writing in 1627 [1], postulated "it is true, as I conceive, that such Earth as hath Salt-Peter bred in it, if you can procure it without too much charge, doth excel" Evelyn repeated this more emphatically in 1675. If saltpetre was plentiful and cheap, "we should need but few other composts to meliorate our ground", and Sir Kenelm Digby actually made an experiment by watering barley plants with a weak solution of nitre in 1675 "to their great benefit"[2] Other distinguished chemists of a later date expressed something of the same opinion; but their ideas could not be put to the rough test of practical trial because supplies of the material were unobtainable The quantities to be obtained were wanted by the 'armament industry' for making gunpowder

In the seventeenth century, J R Glauber, whose name is immortalized in Glauber's salts, obtained saltpetre from the floors of cattle sheds, then one of the main sources of supply He argued that it derived from the droppings and urine—the floors were only earth in those days Consequently, it must have been a component of the feed, which was, of course, plants He made some trials of saltpetre as a fertilizer, and obtained higher than average yields He concluded that saltpetre was "the essential principle of vegetation", and argued that the effect of all manures commonly used was the result of the saltpetre they contained[3]

If any farmers ever heard of Kenelm Digby's or Glauber's experiments, which seems to be extremely unlikely, they could not have profited by them, because they could not have purchased the material Only about one hundred and fifty years later did sufficient supplies come on the market—by way of importation from the East Indies A firm, Forman and Hadow, of 51, Lime Street, London, sold the material Hadow had a house at Colney, Herts, where he used it as a fertilizer between 1824 and 1828 He may have done so before because some farmers in the county had already been using it for some years What, apart from the fact that they could get it, caused these men to begin using it is now impossible to discover Probably some of the older among them had attended Humphrey Davy's lectures on agricultural chemistry given to the Board of Agriculture between 1802 and 1812 He had said that saltpetre or nitre might possibly furnish azote (nitrogen) to form albumen in plants, and that it should therefore be useful for wheat The fact, however, as was said at the time, seems at variance with the theory Davy thought that the nitrous salts were too valuable for other purposes to be used as manure[4]

Some farmers in the neighbourhood of Hitchin evidently did not agree with him because several of them were using it in 1828, and one or two had been doing so for a fairly long time For example, T Wilshire had used it for about twelve years on his heavy clay soil He applied one hundredweight per acre, and made comparisons with dressings of salt and soot His observations on the results are lost to us, but he found the saltpetre effective on cereals and grass, not on roots

John Lee of Ippolits had used this fertilizer even longer He spread 1–2½ hundredweights per acre He found the result was to stimulate a large bulk of straw, but he did not think white wheat yielded so well The saltpetre caused mildew, or so he imagined He preferred soot as a top dressing for wheat, and he certainly had a fair experience to guide him. He had found that if barley was dressed with saltpetre the following crop of clover and grass did well

J Curling of Offley Hales was more in favour of saltpetre He thought it good on all soils, and for both cereals and grass It increased the straw, which was anything but a nuisance then, and the yield was far superior to that produced by any other manure Unfortunately, neither of these men gives any actual figures, and there is no measure to judge the exactitude of their impressions Curling sowed the saltpetre well after the crop was up, but before doing so he mixed it "carefully and intimately" with ash in the proportion of 1¼ hundredweight to a small cartload (rather a vague measure) of ash One wagon load of 3 tons of saltpetre dressed 50 acres whereas an equal quantity of soot would only do 4 or 5 acres There was a saving of at least 15 shillings an acre

Other farmers who used saltpetre were Mr Sharpe bailiff to E H Delme Radcliffe, Esq , H Crabbe of Temple Dinsley, Mr Crawley (no address given), Mr Oakley of Preston and John Cook of Hillend The last-named was a cautious man, and went to the

East India Dock direct for his supplies, which were tested at Apothecaries Hall The inferior sort proved to be mixed with common salt Mr Roberts, steward to W Hale, Esq , and Mr Lucas of Hitchin also used it Lord Dacre had done so, and found it encouraging The last would, William Hawkins thought, "be peculiarly important in the eyes of those who might have hesitated making experiments on the credit of gentlemen whose names were unknown to them"[5] It is not impossible that a conversation between Lord Dacre and Lawes led the latter to produce chemically his superphosphate of lime

The news of this practice and these experiments must have been pretty well spread abroad The notion reached, what was then far-away, Scotland Thomas Bruce of Grangemuir, who was acquainted with Forman and Hadow, learned all about it, and was so much impressed that he imported some saltpetre from London He used it on wheat sown after potatoes, and the results were so good that he tried to persuade the Highland and Agricultural Society to institute some definitive experiments[6]

Those farmers who tried saltpetre are to be commended for their initiative, but their efforts were brought to nought by the importation of the first cargoes of Chilean nitrate of soda late in the 1830's Lord Dacre, who had tried saltpetre on the grass in his park at The Hoo, near Welwyn, for two or three years about 1820, found that it produced a heavy crop at the expense of the finer herbage For a while he gave it up, but then tried it again and changed his opinion of its worth In 1839 he tried some of the newly imported nitrate of soda, applying an equal money cost of each to an equal area—quite an unusual way of conducting an experiment The nitrate was about 30 per cent cheaper than saltpetre The first was sold by William Mitchell, Commercial Sale Rooms, Mincing Lane, at 19*s* 6*d* per hundredweight, whereas saltpetre (nitrate of potash) cost 25*s* duty paid Another advantage of the nitrate of soda was that it rarely contained more than 5 per cent of 'alloy' while saltpetre was impure to the extent of 30–50 per cent Mitchell had sold nearly 2,000 tons of nitrate of soda in the previous year, and had a stock of less than 1,000 tons at the docks Of saltpetre he was carrying between 4,000 and 5,000 tons, but considered that a demand for 1,500–2,000 tons for agricultural use would raise the price too high for it to be economic for that purpose[7]

Other farmers became interested James Everitt of North Creake, near Fakenham, Norfolk, was one He used saltpetre in 1838 as a top dressing on growing crops, which was usual, at 1 hundredweight per acre This was on an experimental plot of two 'rigs' each 1¼ acres The increase over the untreated land was at the rate of 6½ bushels an acre plus one-sixth more straw In 1839 he estimated the increased yield on treated plots at 4 bushels wheat and 15 bushels oats plus 11 hundredweight straw above average Nevertheless, he was so impressed by the opinion of an eminent professor of agriculture that American nitrate of soda was "better calculated for agricultural purposes" that he bought 5 tons for use in 1840 [8]

Saltpetre remained the interest of a select few farmers only, and one of them, George Kimberley of Trotsworth, Surrey, thought the history of the material was that of all artificial manures, especially the saline, which, successively and hastily adopted after a few fortunate experiments "have started it into a fashion" It had been used since the Hertfordshire experiments, but only by the enthusiastic minority, not as a standard auxiliary manure Kimberley had himself become sufficiently interested to try the material for a couple of years in 1826 and 1827, using a plot isolated in the middle of a seeds crop, which had received the usual dressing of ten good cartloads of dung per acre The untreated piece was weak and poor in the spring compared with a thick, rank growth on the dunged area, but fourteen days after the trial plot had been treated with saltpetre it looked very much better than the other There was no difference in the succeeding wheat crop, but the cost was £5 12*s* an acre for the horse manure including spreading, while that of saltpetre was only £2 15*s* 6*d*

Kimberley had formed no theory about it, practical facts were, in his opinion, of more service and better understood On the basis of these and other experiments he recommended the substance, finely pulverized, as a top dressing on all crops, at the rate of 1–1¼ hundredweight per acre on the first spring growth He remarked that Cuthbert Johnson, a respected farming writer of the time, thought its agricultural use had not been examined so carefully as it ought to have been It is probable that the scanty supplies to be had were a sufficient reason for this neglect In 1834 the anonymous author of *British Husbandry* declared that it was rarely used as a manure for that reason He only mentioned it as a matter of secondary interest to a few speculative farmers For the rest he quoted Davy and the Hertfordshire experiments[9] Subsequent writers of text-books displayed an equal lack of enthusiasm All that John Donaldson could find to say was that "of late years nitre has attracted some notice as a manure" As a top dressing on wheat it had exceeded the value of rock salt, but not invariably On hay grounds it had promoted one-third more produce its effect on chalk grounds was generally good J W F Johnston could only say that besides being made into gunpowder it had "also been recommended and frequently and successfully tried by the practical husbandman, as an influential agent in promoting vegetation"[10]

Only a small minority of farmers tried saltpetre Even if those recorded is multiplied by five or ten to include those whose efforts did not see print the number would still be an insignificant proportion of the total Many more tried nitrate of soda Not only was it cheaper but also more of it reached the market, although some of the quantity imported was used for chemical manufacture The speculative or advanced farmers who bought it were more widely scattered over the country, too

In May 1839, the Earl of Zetland had no less than 1 ton sent to Upleatham, Yorkshire, and used it at 1¼ hundredweight per acre on wheat, turnips and meadow land Nothing came of it on the turnips, it was applied too late on the wheat The results on grass were astonishing A treated plot of 90 square yards produced 30 stone (14 lb) of hay the same area untreated only 14 stone There was a great increase of aftergrass, too, which the cattle and sheep ate greedily Of course, this was only one year's results what later consequences would be it was impossible to say[11] Sir George Legard, another Yorkshire landowner, found the results of his trials were inconclusive He made comparisons between nitrate of soda on one acre of 40 acres of wheat after clover ley on wold land, eaten off by sheep Rape dust at 8 bushels an acre was sown the nitrate at 1 hundredweight The acre treated gave a larger bulk of straw than the rest, but the grain was lighter In a comparison with soot, the sooted wheat was better Against bones on turnips nothing the results were quite inconclusive Angus of Neswick, near Driffield, was more ambitious, and made comparative trials with various manures, but did not include nitrate of soda[12]

David Barclay of Eastwick Park, near Leatherhead, Surrey, was a prophet, or, rather, a far-seeing man Though he made trials of nitrate of soda and recorded others, he felt that the investigations of the effects of its use were likely to advance science rather than practical farming, a division of function that the Royal Agricultural Society's motto had joined together Manure was, he said, loosely classed as real or nutritious manure, that is, dung, and stimulating manure, that is, lime or other mineral substances exciting other substances to make plant food The German writers now say, he went on, that dung acts by reason of its chemical elements, some of the minerals that constitute the food of plants This was, of course, the theory advanced more particularly by Liebig, but suggested before him by Sprengel and Schubler Barclay went on to the argument from the results of applying urine, soot and gas liquor, all of which contain ammonia Nitrate of soda, he said, made it possible to go a step further Ammonia is a combination of nitrogen with hydrogen Either of these might be the source of its power over vegetation "but we have seen that saltpetre or nitrate of potash and cubic saltpetre or nitrate of soda, act on crops in the same peculiar manner as fermented urine, soot or gas liquor, which do not contain hydrogen but do contain nitrogen in the form of nitric acid Nitrogen therefore appears to be the active principle in all five substances Liebig in proving the efficacy of ammonia has shown the necessity of nitrogen in forming gluten" This he condemned as only guesswork, and demanded scientific research into the action of manure and the food of plants "We should all", he said, "try nitrate of soda"

Many farmers were already doing so, not to speak of Barclay himself His own experiments led to very different results on different crops The standard of judgment was severely practical Not only were the comparative yields studied—though I gather the comparison was on the basis of accepted averages rather than control plots—but the financial result, that most important factor, was taken into account Barclay, too, collected all the accounts he could of other people's trials Several of these were Surrey farmers Walter Calvert of Ockley Court, George Dewdney of Castle Mill, Drewitt and Sons of Piccard Farm, Guildford, these last well known in Government circles having given evidence to at least two Select Committees Besides these there were John Burgess, Elmhurst, Great Missenden, Bucks, John Raymond Barker, Fairford Park, Glos , John Grey of Dilston, Northumberland, W H Wilson, Didlington Hall, Brandon, Suffolk, William Greaves, Bakewell, Derbyshire, John Muskett, Fornham, Suffolk, W H Hyett, Painswick, Glos , Joshua Rodwell, Alderton, Woodbridge, Charles Newman, Court Farm, Hayes, Middlesex

It would be tedious here to recite all the results these people noted Suffice it to say that Barclay found it impossible to reconcile the different results given by his various correspondents, an experience which, had he known it, is not unusual in the initial stages of a co-operative trial of new material Disappointedly he felt that these conflicting records were calculated to discourage the use of it as manure "The experiments which have been made with this new manure", he said, "require to be repeated and their accuracy established before any practical conclusions can with certainty be built upon them", a statement the justice of which few would deny He thought that judiciously applied it would prove a most valuable manure, and had decided to carry out further trials on a larger scale[13]

Other people continued to carry out comparative trials as between saltpetre and nitrate of soda, but the price and adulteration of the first made "it a poor competitor", and by the end of the 1840's few persons would have thought of using it Some of the men who made trials included the so-called Peruvian guano, salt, nitrate of potash, various types of manufactured guano put out by fertilizer firms, and the usual organic manures Lime was also used, and such things as rape dust Among them were such eminent men as John Grey of Dilston and John Hannam, who won a prize of £50 from the Highland and Agricultural Society[14] As might have been expected nothing conclusive could be determined from this hotch-potch of uncorrelated field trials, not carried out to a standard pattern under one supervision Some optimism was rightly expressed, and the general opinion formed that nitrate of soda was a useful top dressing for a variety of crops in a variety of conditions, but some of the most careful observers among those who used the materials remarked sadly that the results were not always the same This did not prevent the use of nitrate of soda, for example, in increasing quantities

But what aroused most interest was the treatment of bones with sulphuric acid to make superphosphate of lime The use of bone waste had been written about from the 1770's because some observers had not then been acquainted with its use in the neighbourhood of Sheffield, where it was the refuse from the manufacture of knife handles and such things

Fifty years later the use of bones had become popular in several parts of the country For example, they were used on the dairy pastures of Cheshire to replace the phosphates lost through the dairy industry The demand was so large that "the import value rose from £14,395 in 1823 to £254,600 in 1837"[15] They came in mainly through the port of Hull, where they were ground down into the sizes preferred by the farmer During the twenty years prior to 1834 the farmers on the calcareous soils of Lincoln and Yorkshire had generally adopted bones as a dressing for turnips The best results were said to be obtained when this material was mixed with vegetable or coal ash The growth of the young plant stimulated and thus escaped the ravages of the 'fly' Long before this time, but probably no more than half a century or so, some people had used them after burning the bones into ash, when they had the same effect as sulphate of lime or gypsum, but the effect was not then considered so good as uncalcined bones If placed at the bottom of the farmyard, that is, where the cattle fed, the urine might be expected to decompose the bones, or they could be partially broken with a hammer Some farmers had installed their own grinding machines Similarly it was said that bones, after being broken and boiled for grease, were sold to the farmers near London It was agreed that the smaller they were ground the better The application of 120 bushels per acre of small bones was estimated to be equal to 40–50 cartloads of 'fold' manure, that is, that produced by wintering cattle in yards or covered sheds

In Warwickshire the farmers had formerly bought about 5,000 bushels of horn shavings, waste from the comb and horn lanthorn industry at Kenilworth, but had largely forsaken this by 1830 in favour of bones[16] The farmers, who used bones by themselves or in conjunction with other material as compost or decomposed as suggested here, recognized that their value lay in their content of phosphate of lime This took some time to become available as plant food in the soil, the second crop often getting more benefit than the first

Among chemists it had long been generally known that soluble phosphate of lime was produced by adding sulphuric acid to bone ash Berzelius, the great German chemist, made a careful study of this and other phosphates[17], but it was Justus von Liebig, in a paper read to the British Association for the Advancement of Science in 1840, who pointed out the value of this preparation, made by adding sulphuric acid plus three or four parts of water at the rate of half the weight of finely crushed bones After allowing the mixture to digest for some time 100 parts of water was added, and the whole spread as a liquid manure, or, at least, that was the treatment that Liebig's experiments had indicated The question of priority of discovery, if such it could be called, has long been argued Lawes had made some pot experiments in 1839, used the material on half an acre of turnips in 1840, and on a much larger area in the following year[18] He took out a patent for the manufacture of superphosphate of lime in 1842

The taste for comparative experiment must have been stimulated by the novel accessibility of the new manures, Chilean nitrate, Peruvian guano, ground bones, as well as by the efforts of the new Royal Agricultural Society, which were supplementary to, and possibly productive of more enthusiasm than, those of the societies, great and small, formed in the eighteenth century Something, too, must be attributed to the expansion of chemical knowledge that promised the possibility of gaining more definite information about the value of the different substances It is perhaps significant that farmers in the most improved parts of the country were those who carried out the experiments

John Marshall of Riseholme, near Lincoln, made a trial of six different systems of manuring in 1841, whale oil, bones, night soil and crushed oyster shells with and without burnt ash, and one plot with burnt ash only Marshall planned this experiment well, but, of course, could not base final conclusions on one year's work He did, however, decide to reduce his consumption of bones from 2,000 bushels to 200, and by substituting other manure hoped to save £175 His personal preference was for night soil compost dried sufficiently so that it could be drilled[19]

But after Liebig's paper and Lawes's patent dissolved bones impinged most strongly on the agricultural imagination Lawes himself abandoned the use of bones as a base, and substituted other forms of phosphate in his factory Members of the Morayshire Farmers Club carried out trials of this material, prepared, I gather, on their farms, and the Club issued a report Comparison was made between bones and guano, bone dust and bone watered with sulphuric acid Bones alone, dung and bone dust were applied as a contrast The Duke of Richmond ordered an experiment to be made, and found the treated bones gave the best results Philip Pusey, in his comments on these trials, referred to Sprengel and Hannam, and declared the results "wonderfully according to the predictions of chemical science, but not yet (in 1843) completely established"[20] That this was so is demonstrated by the fact that farmers went on making comparative trials, and publishing their results[21]

John Hannam, who became something of an authority on fertilizers at this time, not only made trials but also discussed theory He was somewhat doubtful of the conclusions drawn from Sprengel's experiments that only the inorganic part of the bones was the fertilizing agent The opposite opinion was held by Prof Johnston Hannam made the best of both worlds by asserting that both might be considered fertilizers In his opinion burnt bones acted more rapidly than fresh, and fresh when reduced to fine powder "Burnt bones absorb a large quantity of water by means of which the organic acids in the soil more easily act upon the phosphates", and rendered them soluble Oddly enough, Hannam knew of Lawes's experiments, but had not made himself acquainted with them[22]

When Lawes decided to abandon bones and to seek other sources of phosphate, he imported some Spanish phosphorite from Estremadura, and made trials with it Prof Charles Daubeny, who had visited Spain in this connexion, tried it at the Botanic Garden, Oxford, and concluded that since Spanish phosphorite was wholly destitute of organic matter, the benefit derived from its use came from the phosphate of lime Harry Verney made trials in Buckinghamshire, as did a couple of farmers in Hereford[23]

By this time, brief though it was, Philip Pusey was able to say that Dr Liebig's great discovery of dissolved bones in sulphuric acid for manure had been clearly established by the experiments of the Duke of Richmond and others Nothing was wanting in 1846 but a plan for bringing it into the ordinary routine of farming, a necessity which was met by the establishment of Lawes's factory for the manufacture of superphosphate of lime Indeed, Lawes had a measure of priority over Liebig in the discovery of the process, as well as successful sales of his product, whereas Liebig's patent manure failed to meet expectations for reasons, that have only been understood more recently

The early days of chemical fertilizers may be considered to have ended by 1850 Supplies of Chilean nitrate and Lawes's superphosphate were by then to be had in generous quantities, and sulphate of ammonia was derived from what was known as gas liquor, a waste product of the expanding gas-light industry

By that date (1850) the effect of the use of these fertilizers was already noticeable in the increased yields per acre on British farms, and perhaps in other European countries, although farmers on the Continent began to use them at a slighter later date than those in Great Britain For example, the yield of wheat in England had risen from about 20–22 bushels an acre in the late eighteenth century to about 26 bushels in 1850 [25] Comparable increases had probably occurred in the yield of other cereal crops, but the yields in the eighteenth century have not so far been fully established With the continued and expanding production and use of chemical fertilizers during the second half of the nineteenth century the yield rose to about 32 bushels an acre and has become rather more at the present day Similar increments in the acreage yield have been obtained both in wheat and other crops in Britain, the Continent, and the great grain-producing countries of America and Australasia.

It cannot, of course, be claimed that all this increase has been due to the effect of the use of chemical manures only Science has performed other functions in breeding new and more productive varieties, and has made great advances in the control of plant pests and diseases, all of which have helped the farmers of the world to produce more and more adequate supplies of food for society, though the whole world is not yet free from hunger Some of the credit for this great advance, too, must be given to the engineers who have provided the modern power units and the machinery used with them as well as means of moisture conservation and disposal So many factors are involved that it is difficult to make a real assessment of the effect of each, but the chemical fertilizers the use of which was directed by science, which is also guiding their production, certainly played a major part in the early days of the nineteenth century, and have continued to be most important, indeed increasingly important, to the present day

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[21] *J Roy Agric Soc Eng* , 443 (1845) Richmond, Duke of, Wagstaffe, E , Westerholm Fen, Purchas, R W , St Briavels, Morayshire Club, *ibid* , 601 (1843) John Barton did not think these chemical manures repaid cost in additional food

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OBITUARY

Dr. H. Hamshaw Thomas, M.B.E , F.R S

HUGH HAMSHAW THOMAS, who died on June 30 at the age of seventy-seven, was the senior British palæobotanist He spent his whole working life in Cambridge apart from the two World Wars In the First he served with the Royal Air Force in the Near East, and in the Second was in charge of a team of Air Force Intelligence men near Henley-on-Thames

His first important palæobotanical papers published in 1909 and 1911 were careful studies of petrified Calamite leaves and in the well-established tradition of Scott, and gave no sign of what was to come However, Seward suggested that he should re-investigate the British Jurassic plants—and it must then have seemed a simple and short task At that time an ordinary fossil plant, 'impression with carbon', as it was called, was just a black mark on a rock and observation with a hand lens was the limit However, Nathorst in Stockholm was developing the intelligent study of fossil cuticles, and Thomas went there to fit himself for advanced work He came away with a robust view at the opposite extreme such a fossil was no mere black mark but a whole organ, albeit compressed, and failure to see every cell of its structure is failure of technique or observation.

Thomas was also inspired by Robert Kidston with whom he collected in the Scottish coalfields From him he learnt that the tradition that the proper subject for a palæobotanist's studies is the specimens provided by a geologist or by an early amateur collector is no good He must collect for himself, study his material and then go back and get more; he ought not to publish until he has a great collection confirming every point repeatedly

These perfectionist ideas dominated Thomas's work, but they had their perils, for like Kidston, Thomas only described a fraction of the good things he discovered However, they combined happily in his famous paper published in 1925 on the Caytoniales, those tantalizingly Angiosperm-like plants This family is Thomas's creation, for although small pieces were known as isolated and uncommon fossils they were hopelessly misunderstood Thomas gathered together hundreds of specimens over the years and patiently attempted to section them His success was only moderate, for he could see the more robust cells in about a tenth of the specimens he treated, but it was much the best work of its kind that had been done A good many shook their heads over Thomas's synthesis of *Caytonia* but later work has confirmed it

The first important fruit of his work with Nathorst was a paper (in 1913 with Dr N Bancroft) on the cuticles of "Fossil Cycad" leaves, that welter of confused genera and species They showed that the cuticles divided them sharply into two groups, the Bennettitales and the Nilssoniales or true cycads Others working over the same material have merely sharpened the distinction which remains the beginning of orderly treatment

Thomas's last major paper in palæobotany was on the Corystospermaceae (1933), another entirely new family of Mesozoic Gymnosperms, this time from South Africa, where he spent a good deal of time with his wife These are perhaps more important than the Caytoniales, for they are by no means rare, but apparently dominant over the southern hemisphere throughout the earlier Mesozoic His election to the Royal Society happened in the next year Apart from these contributions, which opened entirely new fields, he wrote a good many other papers which made valuable contributions to knowledge In his later years, indeed since *Caytonia*, he became increasingly pre-occupied with general problems, the origin of the Angiosperms, the nature of morphology, and indeed the philosophy of natural science, for his outlook was anything but restricted

Although an obituary in *Nature* of a distinguished scientist should deal mainly with scientific work we must also recall his deep appreciation of beauty, his gentle manner and his kind thoughtfulness.

Tom M. Harris

NEWS and VIEWS

The Russian Satellites *Vostoks 3* and *4*

The third Russian manned Earth satellite, *Vostok 3*, carrying Major Andrian Nikolayev, was launched at 08 30 h U T on August 11 The satellite, designated $1962\alpha\mu1$, entered an orbit inclined at 64° 59′ to the equator at 16 00 h U T on August 11 its orbital period was 88 32 min, the perigee and apogee heights being 180 and 234 km respectively At 08 02 h U T on August 12, *Vostok 4* ($1962\alpha\nu1$), carrying Lieut.-Colonel Pavel Popovich, was launched into an almost identical orbit. Initially, the two satellites were very close together, and Major Nikolayev saw *Vostok 4* through his porthole, but since the orbital period of *Vostok 3* was slightly lower, it slowly drew ahead of *Vostok 4*, at a rate of about 2 min a day At 18 00 h U T on August 12 the periods of *Vostoks 3* and *4* were 88 2 and 88 3 min respectively, their perigee heights 177 and 178 km, their apogee heights 228 and 235 km, and their orbital inclinations 64° 59′ and 64° 57′ On August 15 the two astronauts successfully returned to Earth, close to the intended point, south of Karaganda in Kazakhstan, within a few minutes of each other, at 06 55 and 07 01 h U T respectively

The flights of *Vostoks 3* and *4* mark several impressive new achievements in the development of space exploration Both Major Nikolayev and Lieut.-Colonel Popovich lived, slept and worked in conditions of weightlessness, apparently with efficiency and without ill-effect, for 4 and 3 days respectively, much longer than the 25 h of their predecessor Major Titov For the first time live television pictures of a man in orbit were publicly shown on Earth, and there was the first radio communication between men in space, as well as the first double descent from orbit The placing of *Vostok 4* into such close proximity with *Vostok 3* shows that a satellite can be launched into a specified orbit at a specified time, and the experiment goes a long way towards demonstrating the feasibility of a rendezvous between two spacecraft

Eightieth Birthday of Prof. James Franck

Prof James Franck, who celebrates his eightieth birthday on August 26, had his first great achievement fifty years ago, when he showed (with G Hertz, with whom he shared the Nobel Prize in 1926) that the energy loss of electrons in mercury vapour changed sharply at particular electron energies Here was direct evidence for the existence of quantum states in atoms, preceding Bohr's model of the hydrogen atom Atomic and molecular energy-levels remained Franck's field of study for another twenty fruitful years at the University of Göttingen, where he was professor and head of a flourishing school. many of his students from abroad became distinguished physicists (such as Blackett, Condon Oppenheimer, to mention a few) After 1933, though being a Jew, he could have stayed on because he had done distinguished service as an officer during the First World War But Franck preferred exile, first for a short time in Copenhagen, then in the United States There he devoted much of his effort—and still does—to the study of what is perhaps the most complicated and most important molecular process the photosynthesis of living material in plants For this work he was quite recently awarded a prize from the Academy of Gottingen

What distinguishes his work is his amazing physical intuition—rivalled only by people like Faraday and Rutherford—which has repeatedly enabled him to achieve penetrating insights with scarcely any use of mathematics or complex theories The 'Franck–Condon' principle', which explained many fine details in molecular spectra and which appeals directly to the imagination of the physicist, is a good example During the Second World War, Franck helped with the development of the atom bomb But when it was achieved he did his best—as chairman of a committee of scientists brought together for that purpose—to prevent its use and to forestall the arms race which he could foresee This is but one example of the deep humanity and unfailing kindness that have made him loved by everybody who knows him

Aviation at the Imperial College of Science and Technology : Prof. P. R. Owen

Prof P R Owen, who has recently been appointed to the Zaharoff chair of aviation in the Department of Aeronautics of the Imperial College of Science and Technology, is an eminent successor to the previous holders of this chair L Bairstow, A A Hall and H B Squire (see *Nature* obituary, 193, 421, 1962), who graced this important appointment Prof Owen graduated with an honours B Sc degree in aeronautics at the Queen Mary College in 1940 and worked afterwards in industry and the Royal Aircraft Establishment for a number of years While in the Royal Aircraft Establishment he became, in 1948, a principal scientific officer and head of the Fluid Motion Section, at which position he remained until 1953 After this period he joined the University of Manchester as a reader and director of the Fluid Motion Laboratory and was ultimately appointed professor of mechanics of fluid motion Prof Owen is well known for a number of significant contributions to the science and art of aeronautics His researches extend over a wide range of theoretical and experimental investigations, both so far as fundamental and practical problems are concerned Thus, he covered supersonic aerodynamics, where he proved during the War one of the pioneers in Britain, the mechanism of boundary layer transition, the laminar separation bubble on aerofoils and other topical aeronautical applications From 1953 onwards his interests became even more catholic and he investigated a number of fluid motion problems as far apart as hypersonic flow, turbulence, heat transfer, meteorology and such unusual sidelines as dust-transport in gas streams He is a member of a number of Government committees and well known as an inspiring teacher and leader of men He will, no doubt, broaden still further his research activities in the largest Department of Aeronautics existing in Britain at a university

Physical Chemistry at Southampton :

Prof Graham J Hills

Dr Graham J Hills, at present lecturer in the Imperial College of Science and Technology, has been appointed to the newly instituted chair of physical chemistry in the University of Southampton Dr Hills, who is thirty-six years of age, graduated from the old Birkbeck College in the City of London Having initially trained as an analyst, he undertook electrochemical research, as a London University Postgraduate Scholar, at that College and this work led to the now classical papers of Hills and Ives on the calomel electrode The association of these authors has continued over the past decade and is to be seen in the recent book *Reference Electrodes* Dr Hills left Birkbeck in 1949 to join the staff of Imperial College, first as assistant lecturer and then as lecturer in analytical chemistry On the departure of Dr Bockris, he transferred to physical chemistry and continued the long tradition of electrochemistry at Imperial College He extended the high-temperature investigations into molten salt systems and made a particular study of ionic mobilities by tracer-diffusion, conductance and polarographic techniques

During the period 1955–59, another comprehensive study was made of polyelectrolytes, ion-exchange resins and membranes, and in 1957 there began the present school of research into electrochemistry at high pressures This has involved the measurements at high pressures of electrolytic conductance, transport number, double-layer capacity and rate constant and is aimed at a better understanding of kinetic processes in condensed phases With considerable support from the Department of Scientific and Industrial Research and the Central Electricity Generating Board, these investigations are being extended to high temperatures, and this major undertaking is being launched at Southampton In 1959, Dr Hills was visiting lecturer in the University of Belgrade, and in 1961 visiting research associate in the University of Illinois For his published research in electrochemistry, he was awarded this year the degree of D Sc

The British Coal Utilization Research Association

Dr D T A Townend, C B E

Dr D T A Townend, director-general of the British Coal Utilization Research Association (B C U R A) for the past seventeen years, retires on September 30 During the First World War he served in the Royal Naval Air Service, afterwards he returned to the Imperial College of Science and Technology, where he was for many years associated with Bone and Newitt in research work on combustion problems, particularly at high pressures Towards the end of this period he elucidated the two-stage process of combustion of higher hydrocarbons and its relation to the problem of 'knock' in internal combustion engines In 1938 he was appointed to the Livesey chair of coal-gas and fuel industries in the University of Leeds and became associated with research work undertaken collaboratively between the University and the Institution of Gas Engineers (later the Gas Research Board), his research work also threw considerable light on the phenomena concerned with the stability of burner flames During the Second World War he was engaged on research problems for a number of Ministries, and as a scientific adviser to the Ministry of Home Security he made notable achievements in the field of incendiary warfare In 1945 he was invited to his present post During the War the B C U R A staff had been widely dispersed in temporary accommodation, and he was largely responsible for the present organization at Leatherhead Following nationalization, in 1947 the National Coal Board took the place of the Mining Association of Great Britain as the principal member of the B C U R A , membership by the other nationalized industries soon followed, and to-day much of the B C U R A 's work is supported by them, as well as by Trade Associations and other industrial concerns Dr Townend is a past chairman of the Committee of Directors of Research Associations and at present vice-chairman of the Parliamentary and Scientific Committee

Mr. D. Hicks

Dr Townend is to be succeeded by Mr D Hicks, now carbonization and scientific director of the East Midlands Division of the National Coal Board and director of the Operational Research Unit and Pneumokoniosis Research Unit of the Board He joined the staff of the Fuel Research Station in 1925 and was appointed officer-in-charge of the Cardiff Coal Survey Laboratory in 1929 In 1946 he was appointed national superintendent of the Coal Survey Organization with responsibility for co-ordinating the work of the eight Coal Survey Laboratories in the Coalfields In 1947 he was appointed director of scientific control of the National Coal Board The responsibilities of this post included the direction of the Coal Survey Laboratories and the creation of a Scientific Service in the coalfields He was also responsible for forming the Board's Operational Research Group and for the scientific programme of the Pneumokoniosis Field Research Unit Mr Hicks is also chairman of the Research Committee of the British Coke Research Association, of the International Standards Organization (Solid Mineral Fuel Committee) and of the B C U R A Domestic Appliances Committee and a member of Council and of the Research Committee of the Coal Tar Research Association He is a Fellow of the Institute of Fuel and of the Royal Institute of Chemistry

Mr R L Brown

Mr R L Brown, director of the B C U R A 's Basic Research Laboratories since 1950, has been appointed deputy director-general Mr Brown won an open scholarship in mathematics to Trinity College, Cambridge, in 1934 and was a wrangler, taking a first in Part 2 and passing with distinction Part 3 of the Mathematical Tripos He joined the B C U R A in 1938 and was appointed head of the Mathematics and Mechanics Department in 1946 and superintendent of the Physics Department in 1949 He is a Fellow of the Institute of Fuel and of the Institute of Physics and was awarded the Students Medal of the Institute of Fuel in 1939 He is a member of the Publication Committee of these Institutes and was chairman of the Organizing Committee of the Institute of Fuel Conference on "Science in the Use of Coal", and from 1956 until 1960 was honorary secretary of the Committee of Directors of Research Associations He, and Mr Hicks, will take up their new posts on October 1

Great Britain and Space Research

IN a written answer in the House of Commons on July 30 the Minister of Aviation, Mr J Amery, stated that the United Kingdom, together with Australia, Belgium, France, the Federal Republic of Germany, Italy and the Netherlands, was participating in the development of a three-stage satellite launcher by the European Launcher Development Organization A preparatory group had been set up to co-ordinate the work proceeding in the member countries, pending the ratification of the Convention to establish the Organization A technical mission was at present in Australia examining the problems connected with firing the *Blue Streak* first stage and the three-stage launcher from the Woomera Range The Postmaster-General, Mr J R Bevins, stated on July 31 that the Post Office expected to spend about £700,000 in 1962–63 on the development of communications by means of artificial satellites, and tentatively provision of £1 million was provided in each of the next four years

On August 2 it was stated by Lord Hailsham in the House of Lords and by the Parliamentary Secretary for Science in the House of Commons that a Space Research Management Group was set up under the Office of the Minister for Science, with effect from May 1, 1962, which had assumed the responsibilities for the management of the programme of experiments in rockets and satellites, previously discharged under *ad hoc* arrangements by two scientists and one engineer on loan to the Royal Society from the public services The new Group would comprise these three officers, five additional officers and supporting staff This is an *ad hoc* arrangement pending the outcome of the present inquiry into Government and scientific organization and had been introduced in agreement with the Royal Society The British National Committee on Space Research, set up by the Royal Society, would continue to advise on the scientific content of the programme and the Steering Group on Space Research would continue to advise the Minister on policy matters in its field In the House of Commons, Mr Freeth added that the massive flow of coded data from the satellite *Ariel* would take time to process and analyse Those concerned with the experiments were not yet ready to express considered views although they had indicated that much interesting and valuable information about the structure of the ionosphere and the constitution of the high atmosphere was now available Recently, transmission of data had been interrupted irregularly and the reason for this was being investigated

Nuclear Power Reactors in Britain

IN written replies to questions in the House of Commons on July 31, the Parliamentary Secretary for Science, Mr D Freeth, stated that the Atomic Energy Authority had spent about £10 million on the development of beryllium as a canning material for advanced gas-cooled reactor fuel Including supporting services, 630 people were employed on the Dounreay fast reactor Comprehensive inspection during the five and a half years since the first nuclear power station at Calder Hall came into operation had given no evidence that the life of magnox stations would be limited to 20 years, there was as yet no final proof of the life of a reactor, but an assumption of 30 years could be as realistic as 20, particularly for the later stations in the programme

Professional Qualifications and the European Community

IN a debate in the House of Lords on Britain and the Common Market on August 1, Lord Cohen of Birkenhead directed attention to the implications of the Treaty of Rome for all those professions which require a qualification registrable by law Section 1 of Article 57 empowers the Council to issue directions regarding mutual recognition of certificates, diplomas and other qualifications Lord Cohen was concerned that in consequence a medical or other professional qualification obtained in any of the member States of the European Economic Community would be acceptable as a criterion of a registered medical practitioner Although vice versa British qualifications would be recognized by the 'Six', Lord Cohen said that on account of the wide disparity in standards of medical qualification and practice among members of the European Economic Community this Article had given rise to grave disquiet in the medical, dental and para-medical professions in Britain Lord Cohen entered no claim for any sectional interest but was concerned at the effect on the public interest of any debasing of professional standards In replying on the debate, the Lord Chancellor pointed out that the Council of Ministers could only issue directives for the mutual recognition of qualifications on proposals made to them by the Commission and after consulting the European Assembly Such directives had no effect for the medical, para-medical and pharmaceutical professions, until conditions for the exercise of these conditions had been co-ordinated Further voting on these matters must be unanimous and he thought that these were substantial safeguards The Government understood the importance of this subject, and if Britain joined the Community the Government would keep in close touch with the General Medical Council and all other interested bodies, while playing her part in the Community's proceedings Regard would be had, as the General Medical Council had urged, to the standards achieved in Britain

The Commonwealth Development Corporation

THE Minister of State for Colonial Affairs, Lord Lansdowne, announced in the House of Lords on July 31 that it had been decided to extend the activities of the Colonial Development Corporation which have hitherto been confined to dependent territories It is proposed henceforth to authorize the Corporation to undertake new development schemes in countries in which it was previously empowered to operate even after they have become independent members of the Commonwealth The

Corporation will be renamed the Commonwealth Development Corporation The necessary legislation will be introduced as soon as possible A similar statement was made simultaneously in the House of Commons.

M R C. Committee to study the Methodology of Toxicity Testing

In reply to a question in the House of Lords on August 2, the Minister for Science, Lord Hailsham, said that the Minister of Health sought advice on testing new drugs generally through his Standing Medical Advisory Committee, which was the appropriate body Since the recent events relating to thalidomide, Lord Hailsham said he had himself inquired of the Medical Research Council concerning the part it could expect to play in advancing methods of testing The Council had that week announced its intention to appoint a committee, under the chairmanship of Sir Charles Harington, to study the methodology of toxicity testing Lord Hailsham attached great importance to the work of this Committee, although it was not the Medical Research Council's responsibility to carry out routine testing programmes on drugs or other chemicals from the point of view of either efficiency or toxicity It did, however, sponsor many clinical trials of new preparations of particular promise for specific purposes and had for many years undertaken fundamental toxicological research, especially with regard to the difficult methodological problems which arise in devising tests to demonstrate possible long-term effects which might be produced, for example, by food additives The Council had a special responsibility for advising the Minister of Health on the scheduling of drugs under the Therapeutic Substances Act. So far as thalidomide was concerned, he was advised that there was general agreement that in the existing state of knowledge it was extremely unlikely the most rigorous programme of screening tests would have suggested an effect was to be expected on the developing human embryo

U S. National Committee for the International Council for Building Research, Studies and Documentation

The National Academy of Sciences–National Research Council has announced the organization of a U S National Committee for the International Council for Building Research, Studies and Documentation The Academy–Research Council was requested to become the official adhering body for the United States instead of the Housing and Home Finance Agency in order to broaden and strengthen participation by the United States in the activities of the International Council As a non-governmental organization, the International Council for Building Research, Studies and Documentation promotes international co-operation in its areas of interest and is concerned with not only the technical but also the social and economic aspects of building Membership in the U S National Committee includes representatives of nine national organizations and five federal agencies, provisions have also been made for additional representatives and three members-at-large No chairman has yet been named The Committee will be administered within the Division of Engineering and Industrial Research of the Academy–Research Council Staff support is being provided through a Secretariat consisting of an executive secretary, Mr Robert M Dillon, and a technical secretary, Mr Robert W Spangler Further information can be obtained from the executive secretary, U S National Committee for CIB, National Academy of Sciences–National Research Council, 2101 Constitution Avenue, N W, Washington 25, D C

Leicester Museums and Art Gallery

It is seldom that a provincial museum can finance the publication of a list of figured and described material in its geological collections This has recently been accomplished by the Leicester Museums and Art Gallery, which has published *A Catalogue of the Figures and Cited Specimens in the Department of Geology, 1962* (Pp 46 Leicester The Museums and Art Gallery, 1962). The list has been compiled by the curator of geology, C A Sizer, who, in an introduction, rightly stresses that the formative period of the collections covers a long time In spite of different curatorial standards through the decades, it has been possible to list a large number of specimens A few are holotypes, a few more are figured, but the great majority are cited, though mentioned is the term more generally used These lists will be of service to palæontologists, especially those working in the Leicester area, and, although the information is of a negative character, it is of value to know that unfortunately certain specimens have been lost The publication also includes a few petrological specimens and the relevant references

Refraction of the Eye and its Components in Twins

Two earlier monographs by Prof Arnold Sorsby *et al* have established that relatively few cases of long sight and short sight are the result of anomalies in the dimensions of the optical components, for the most part, these are within the normal range but are inadequately correlated Three questions thus arise First, what determines the anatomical anomaly seen in the 3–5 per cent of the population who have 'component' ametropia? Secondly, what causes 'correlation' ametropias which are seen in some 55 per cent of the population and which represent varying degrees of failure, during the growth of the components, of a physiological co-ordinating mechanism ? Finally, how do the 40 per cent or so who are emmetropic come to have perfect correlation ? A new investigation carried out by Prof Sorsby, M Sheridan and G A Leary on twins is an attempt to assess the significance of heredity in the determination of these three types of refraction (*Medical Research Council Special Report Series*, No 303 *Refraction and its Components in Twins* Pp viii + 43 London H M S O , 1962 7*s* net) It is based on an analysis of data not only concerned with refraction as a whole but also on the individual optical components For both refraction and its components, uniovular twins in all the refraction groups were found to show a significantly higher incidence of concordant values than biovular twins and unrelated pairs Such discordance as was found in uniovular twins was of the same order as that known to occur with many morphological features, and which is generally regarded as due to variation in the expression of the genes rather than to environmental influences. It may therefore be taken as established that the dimensions of the optical components, the efficiency of the mechanism co-ordinating the growth of the components, and thus the refraction of the eye, are all genetically determined The modes of inheritance and the possibility that environmental factors have a minor modifying influence are the principal problems now awaiting clarification

The Institution of Electrical Engineers

THE following officers have been elected to the Boards of the Institution of Electrical Engineers' new Divisions, which will replace the former Specialized Sections on October 1 *Electronics Division Board*, Mr. J A Ratcliffe, director of the Department of Scientific and Industrial Research, Radio Research Station at Slough (chairman), Mr G G Gouriet (deputy chairman), Dr R C G. Williams and Mr. A J Young (vice-chairmen) *Power Division Board*, Mr D B Irving, chairman of the London Electricity Board (chairman), Mr C. D Wilkinson (deputy chairman), Mr C H Flurscheim and Mr D T. Hollingsworth (vice-chairmen) *Science and General Division Board*, Dr D Taylor, co-ordinator of research and development for the Plessey Group of Companies (chairman), Dr J R Mortlock (deputy chairman), Mr. C G Garton and Prof J M Meek (vice-chairmen)

Sixth-Form Biology in the Future

A CONFERENCE on sixth-form biology is to be held by the Joint Biology Committee, in the Senate House, University of London, on November 10 Interest will be directed less to details of syllabuses than to considerations which are in keeping with contemporary progress in biological science and the flow of educational thought Those taking part include Dr. L M J Kramer, senior biology master, Eton College, Miss M Going, headmistress, Church of England College, Birmingham, Miss B G Ashton, head of the Science Department, Edgbaston High School, Birmingham, Mr P Kelly, head of the Biology Department, Bexley Grammar School, Dr J T Aitkin, reader in the Department of Anatomy and Embryology, University College, London, and Dr C D Pigott, lecturer in the Department of Botany, Cambridge Further information can be obtained from the Association of Agriculture, 36 Victoria Street, London, S W 1

Nuclear Studies at the Liverpool College of Technology

THE prospectus of post-graduate courses and research in nuclear studies to be held at the Liverpool College of Technology during the 1962–63 session is now available The programme again includes the well-established series on "Radiation Safety and Health Physics" These courses, which have received the full support of the United Kingdom Atomic Energy Authority Health and Safety Branch, are of seven months and two weeks duration The long course leads to a College Diploma in Health Physics and has been recognized by the Department of Scientific and Industrial Research as suitable for the tenure of its advanced course studentships Two-week courses in radiobiology and one-week courses in radiation chemistry and radiometric analysis are also being offered The prospectus is available from the principal lecturer in nuclear studies, J W. Lucas, City of Liverpool College of Technology, New Building, Byrom Street, Liverpool 3

Burroughs Wellcome Research Fellowships

THE Australian Academy of Science is inviting applications for Burroughs Wellcome fellowships Awards will be made in such a way that there are not more than three fellowships occupied at any time, each tenable for two years The fellowships may be awarded in any branch of medical sciences. They must be occupied in one of the following professorial departments of an Australian university medicine. psychological medicine, preventive medicine, experimental medicine, pediatric medicine (child health) pharmacology, or pharmaceutical science Applicants should, in general, hold the Ph D degree or have equivalent status and experience Preference will be given to applicants who are under the age of 30 years on July 1 in the year in which the application is received The fellowships are valued at £2,000 per annum There is no printed form of application Each applicant will be required to state the general nature and scope of the research he wishes to undertake, and the university department which is prepared to accept him He should submit copies of scientific papers that he has already published and should furnish the names of three referees who can speak from personal knowledge of his capacity for research Applications, which will close on September 30, should be sent to the Assistant Secretary, the Australian Academy of Science, Gordon Street, Canberra City, A C T

Announcements

THE death is announced from Stuttgart of Dr Otto Fauser, a Regierungsbaudirector of the pre-war German Reich, a well-known member of the older group of soil scientists, and a regular and enthusiastic participant in their conferences and excursions

MR T L BIRRELL has been elected president of the Plastics Institute for 1962–63 At present, Mr Birrell is a part-time director of Yarsley Research Laboratories, Ltd He was one of the original signatories to the British Plastics Moulding Trade Association (the forerunner of the British Plastics Federation) and was chairman of the Plastics Institute in 1942–43, and president for one year in 1952–53

THE Bradford Institute of Technology is holding a short course for graduates on "Polymer Structure and Properties" during November 2–3 Lecturers include Dr C W Bunn, Dr L B Morgan, Dr A Keller, Dr I Goodman and Dr M Gordon Further information can be obtained from Dr W R Moore, Department of Chemical Technology, Bradford Institute of Technology, Bradford 7.

THE Congress on the History of Pharmacy at the University of Nottingham, a notice of which appeared on p 546 of the August 11 issue of *Nature*, has been postponed and will now be held during September 20–23, 1963 In its place, the third British Congress on the History of Medicine will be held in London during September 26–28, 1962, the theme of which is "The Evolution of Hospitals in Britain" Further information can be obtained from Dr. F N L Poynter, Wellcome Historical Medical Library, the Wellcome Building, Euston Road, London, N W 1

ERRATUM In the article entitled "Effect of Strain and Group Size on the Response of Mice to 'Seconal' Anæsthesia", by J H Mackintosh, which appeared on p 1304 of the June 30 issue of *Nature*, the numbers of animals used in the experiments have been omitted Each figure in Table 1 represents at least 27, but usually about 40 mice

DENTAL HYGIENE AND FLUORIDATION OF WATER

NEARLY fifty years ago two American dentists directed attention to a condition of the teeth, characterized by unsightly mottling of the enamel, which occurred in the inhabitants of certain well-defined areas The cause of this abnormality was later shown to be the presence of fluoride in the drinking water, the topographical distribution of cases being due to variations in naturally occurring fluorides in the water in different localities Lesions occurred only in persons who had been exposed as children to fluoride-containing water and did not occur in adults who had passed their childhood elsewhere The proportion of persons affected, and the extent of the lesions, were shown to vary directly with the concentration of fluoride in the water Later, it was found that though the enamel could be so severely affected that the teeth appeared to be quite badly damaged, they were at the same time much less susceptible to dental caries than were normal teeth These findings were confirmed in many subsequent investigations, and it has now been established that concentrations of fluorine salts of more than 1 0 part per million in drinking water may lead to the appearance of enamel lesions and also to increased resistance to caries At this level, however, the lesions are minimal and very few persons are affected With increasing concentrations the lesions become more pronounced and more members of the population show them Nevertheless, the resistance to caries which was found to be associated with the presence of very obvious lesions is still present when the level of fluorides in the drinking water is so small that only very occasional lesions of minimal proportions occur in a few people Investigations in the United States have shown that the degree of this resistance is such that children born and brought up in areas with 1 0 p p m of fluoride occurring naturally in the water have up to 60 per cent less dental caries than children brought up in areas where there is only a trace of fluoride in the water

Further investigations in the United States ,and Canada showed that the addition of fluoride to water sufficient to raise the fluoride content to 1 0 p p m had the same effect on the incidence of dental caries as water with naturally occurring fluoride As a result of these findings, a British mission to the United States and Canada in 1952 recommended that fluoride should be added to the water supplies of selected communities in the United Kingdom, as a pilot study The Ministry of Health report, *The Conduct of the Fluoridation Studies in the United Kingdom and the Results Achieved after Five Years**, now gives the results of these investigations carried out in Watford in part of Anglesey and in Kilmarnock Andover was originally designated as a fourth study area, but fluoridation was discontinued by the local authority after two years

The report gives details of the carefully controlled investigations which have now been in progress for five years Only the findings for the deciduous teeth of children aged 3–7 years are given, since the full effect of fluoride is only exerted when it is absorbed continuously during the whole period of tooth formation and calcification The data show that whereas prior to fluoridation in 1955–56 children aged three years had on average 3 80 carious teeth, the figure for this age in 1961 was 1 29 The corresponding figures for four-year-old children were 5 39 and 2 31, and for five-year-old children 5 81 and 2 91 These figures represent reductions of caries incidence of 66 per cent, 57 per cent and 50 per cent respectively There was also a reduction of incidence of caries in the control areas, but only in the region of between 2 and 7 per cent In children of six and seven years, some of whose teeth were already calcified before fluoridation commenced, the reduction of caries was of a lesser degree

In addition to these striking figures, the report deals with the question of the safety of fluoridation and also gives some details of the technical problems involved in the fluoridation of public water supplies With regard to possible toxicity, the fullest investigation of all available evidence has been made and it is concluded that no harm results from raising the fluoride content of drinking water to 1 0 p p m This agrees with the opinion of the World Health Organization's Expert Committee which, after examining the evidence, concluded that "All these findings fit together in a consonant whole that constitutes a great guarantee of safety—a body of evidence without precedent in public health procedures"

This century has witnessed many triumphs of preventive and curative medicine It is clear that this short report embodies yet another R B LUCAS

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* Ministry of Health Scottish Office, Ministry of Housing and Local Government Reports on Public Health and Medical Subjects No 105 *The Conduct of the Fluoridation Studies in the United Kingdom and the results achieved after five years* Pp iii + 50 (London H M Stationery Office, 1962) 4s net

ICARUS

A NEW journal, entitled *Icarus*, has recently been published* It is yet another of those intended to serve a part of that enormous new field of scientific activity popularly known as 'space research' According to the editors it is to deal with "the new interdisciplinary science of the solar system—which is emerging to claim its own identity at the cross-roads of the allied disciplines of astronomy, geology, geophysics, meteorology, geochemistry, plasma physics, and biology" Their aim is "to make a positive contribution to the cause of scientific communication in three distinct ways

"(*a*) by a selective concentration of subjects which would otherwise have appeared in a variety of widely scattered scientific periodicals to establish a large-signal, low-noise channel for information on most scientific aspects of the solar system,

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* *Icarus*, Vol 1, No 1 (May 1962) International Journal of the Solar System Edited by Z Kopal and A G Wilson Pp 93 Subscription rates (Vol 1, 6 issues) Institutional subscribers, 18 dollars, Private subscribers certifying that the journal is for personal use, 10 dollars (New York and London Academic Press, Inc , 1962)

"(*b*) by minimizing delay between the receipt of a communication and its publication to effect the promptness of communication essential in a so rapidly developing field of science,

"(*c*) by inclusion of a bibliographical section covering all fields of interest to investigators of the solar system to ease the task of monitoring the information"

There are 35 associate editors, most of whose names are already well known Their function is to collect papers and also to act as referees It remains to be seen whether this arrangement will prove more successful than that of anonymous referees whom we all find reasonably efficient in dealing with the papers of other authors, although so tiresome with our own

Fantastically great resources are at present being devoted to space research, but most of the effort is still being channelled into elaborate and lengthy preparations for difficult experiments, of which comparatively few have yet had time to produce results If all goes according to plan, ultimately, the amount of new material to be published and discussed may be very great But so far, relative to the scale of the effort, the volume of results to reach the stage of publication is small (although this is not to minimize their significance) Clearly, *Icarus* has been started in the expectation of taking part in the harvest, but in common with other similar journals it must, for the present, rely chiefly on papers that could equally well have appeared in one or other of half a dozen existing periodicals This at least seems true of *Icarus*, 1, No 1 It contains a long paper by A G W Cameron discussing the problem of the formation of the Sun and planets, and three much shorter papers on the interplanetary medium, on the behaviour of photoelectrons emitted by solid bodies such as the Moon, and about the blue haze on Mars There is also a letter discussing toroidal oscillations of the Moon

Science is to-day more than ever in danger of sharing the fate of the Tower of Babel However, the editors of *Icarus* believe that continued progress can be ensured by the formation of fresh, specialized sub-groups, each drawn from a fairly wide field of existing studies and provided with its own specialized journals This kind of approach is not exactly new. it has been fruitful in the past and perhaps it promises better than any other We are in a period of excessively rapid change in astronomy and in every other science connected with space research, affecting theories, instrumentation, administration, and ideas of all kinds, so that it is very difficult to say with confidence what policies are wisest But so far as publications are concerned, I question whether existing journals are so overloaded that they cannot absorb comfortably all the papers on astronomy likely to result from space research for at least two or three years yet By then we may be able to see the needs of the situation more clearly

The financial problems of existing journals are not negligible, and the extra support of space research papers, should it lead to a greater circulation, as it probably would, might in most cases be very welcome Further, I think it important that space research workers should know what is going on in the older astronomy, and that equally the older kind of astronomer should get to know about space research Ten or fifteen years ago, after some initial fumbling, radio and optical astronomers discovered that they were better off together than apart, and now to a large extent publish their papers together in the same journals I hope that this kind of development will come about in the case of astronomy conducted with rockets, artificial satellites, etc

As to rapidity of publication, the average interval between receipt of manuscript and publication is slightly greater in *Icarus*, 1, No 1, than in *Monthly Notices of the Royal Astronomical Society*, **124**, No 1, also dated May 1962, a journal now 135 years old. which still manages to cover most of astronomy at a satisfactory level of originality and technical quality

The bibliography mentioned by the editors include 349 papers in this issue, the great majority from astronomical journals They are classified, but no abstracts are given Policy for this section of *Icarus* is still fluid, and one must wait for further issues before being able to judge whether there will be a distinctive service not yet provided by existing abstracting journals

R O REDMAN

PLANNING NEW COMMUNITIES IN BRITAIN

THE report of the Carnegie United Kingdom Trust for 1960 gave some account of the study of the social problems of new communities in Britain which Dr J H Nicholson had made at the request of the Trust and of the National Council for Social Service, and stated also that the Trust had set aside £100,000 in the new quinquennium, which opened in 1961, in order to promote pioneer projects in the provision of social amenities in new communities The Trust has also appointed an Advisory Committee to consider applications, and where appropriate, to initiate action Up to the end of 1961 no project, however, had been brought to the point of qualifying for a grant from this fund, practically all failing to measure up to the required standard of innovation The fund is reserved for assisting work that is now on a national scale, and the Trust is prepared to wait for the right projects It is hoped that by the end of 1962 a pattern of expenditure will be planned which will provide assistance for promising projects in one or more of each type of new community in different geographical regions

Dr Nicholson's report was published in August 1961 *, and the importance attached by the Carnegie United Kingdom Trust to this work is well indicated by the fact that in its report for 1961 † the Trust includes Dr Nicholson's general recommendations *in extenso* as well as those on social development His report is concerned with the new communities on housing estates, in the fifteen new towns, in the planned extensions to certain small towns, and in the new housing estates built to replace slums now being demolished in the central areas of most large cities Dr Nicholson directs most attention to the new towns, considering their housing problems and those of employment and services in separate chapters, and to the new towns as communities in a third chapter Then he compares the four types of new communities and discusses the problems of community and leadership, assessing the factors which encourage the development of leadership and sources from which leaders are drawn

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* *New Communities in Britain Achievements and Problems* By J H Nicholson Pp 191 (London The National Council of Social Service, Inc , 1961) 10*s* 6*d*

† The Carnegie United Kingdom Trust 48th Annual Report, 1961 Pp viii+76 (Dunfermline, Fife The Carnegie United Kingdom Trust, 1962) (See also *Nature*, **195**, 338, 1962.)

These two chapters with their constructive suggestions as well as critical analysis are probably the most valuable part of the report, though Dr Nicholson's clear account of the actual achievements, as well as his lucid assessment of the situation, should contribute to the public understanding of the real situation and needs of the new communities Such an understanding, free from party political bias, is an almost indispensable pre-requisite to the formulation of the kind of proposals which the Carnegie Trust awaits

For the rest, the general content of the report is well indicated by Dr Nicholson's actual recommendations, which, as already noted, fall into two groups Taking the general recommendations first, the new towns are producing a new type of community, and this is likely to be true also of many expanded towns For social provision generally, these new communities will be unable easily to draw on the resources of personnel or material of the local authorities of the towns from which the population is being exported Dr Nicholson recommends accordingly that the needs of the newcomers rather than the existing fractions of the town or county should be the dominant consideration A balanced community, with the optimum degree of self-containment, should be envisaged from the start and be the guiding principle in policy and planning Future plans should provide for an adequate balance of age-groups, and if this cannot be achieved initially, the master-plan should provide for the building of the necessary accommodation to redress the balance later An adequate variety of types of accommodation to offer an effective choice between houses, maisonettes and flats is recommended, and to allow the possibility of purchase where desired The policy of providing employment with housing should be continued and extended, including further new towns and the possibility that one of the larger local authorities, or two of them jointly, might build a new town

Since the social structure of a community depends on the balance of occupations, a variety of industries and other kinds of employment should provide work for skilled and unskilled men, for women (full-time and part-time), and in particular, for juveniles Employment in commerce, offices, service, industry and shops, and in professional occupations is especially important It could be questioned whether the report takes account of long-term trends in employment, particularly the diminishing amount of unskilled labour that is likely to be required, and although this really lies outside the scope of Dr Nicholson's inquiry, the social effects of technological change certainly require careful study in the light of the needs of new communities Beyond this, Dr Nicholson recommends that the master-plan should provide for a town centre adequate to the needs of the community when fully developed, and the provision of services—including meeting-places and other amenities—should be related to the neighbourhoods and other planning units The master-plan should also provide sites for natural expansion, for churches, meeting-places, public houses and places of entertainment in each section These and adequate space earmarked for physical recreation, should be protected against 'infalling' with housing unless a comprehensive survey of social requirements shows they are no longer needed for social purposes

Nine recommendations on social development follow First, services and amenities should be provided concurrently with the houses, and great stress is laid on the need for the closest co-operation between the country and the developing authority as to provision and timing The need for community services, outside the scope of the statutory services, should be kept under constant review by statutory and voluntary bodies Provision should also be made ahead of needs for a meeting-place suitable for small groups, capable of extension if and when larger accommodation is needed and the necessary finance to build and maintain has been secured Community development should be fostered in various ways by canalizing action from representative bodies such as community associations, appointing a social development officer in every new town and large estate, and neighbourhood workers in smaller estates, by placing housing management in the hands of qualified officers able to make personal contact with tenants, establishing as soon as possible the normal range of social agencies, voluntary as well as statutory, providing in larger communities a joint centre for social workers with offices, meeting-room and shared services, and making every effort to elicit leadership from within the community itself

Further close consideration and experiment should be directed towards discovering the types of provision—typically in clubs—most likely to attract and hold adolescents, and use should be made of the experience of social workers trained in group work Community and neighbourhood associations are making a major contribution to many new communities, particularly by providing in their centres a wide variety of leisure activities, and in the experience they give in the techniques of democratic self-government and leadership The associations and their National Federation should continue to review the organization and policies of the movement in the present situation in new communities, in particular considering how the needs of the communities they serve can best be ascertained and met, continuing and extending the training of full-time community centre wardens and part-time voluntary officers The Churches should keep continually under review the balance between their own social activities and the work of their members in the wider community Close touch should be kept between the various authorities and agencies concerned in social development at officer-level and those appointed to special duties in this field should be consulted from the planning stage onwards The participation of the general community should be secured at local level through the community or neighbourhood association and at central level through a council of social service Further field surveys dealing with particular communities and particular problems are needed It is hoped that the bodies which have sponsored and financed studies of social development will continue to do so, and that other bodies will join them in making possible the further exploration of this wide and important field

EARTHQUAKE SWARM IN CHILE BEGINNING IN MAY 1960

BEGINNING in May 1960 and continuing with lesser intensity throughout the succeeding months a violent earthquake swarm ravaged large areas of Chile between latitudes 36° S and 48° S The readings obtained by Mr E F Baxter at the University of Durham Seismological Observatory were as shown in Table 1 The swarm was accompanied by a major eruption of the volcano Puyehue and by a tremendous sea wave (Tsunami) which extended from Chile to the most distant parts of the Pacific Ocean About 500 deaths from the effects of the earthquakes in Chile are known, and probably in the same country more than 1,000 died in consequence of the Tsunami Thousands of people were injured and recovery from property damage is even now not complete D Pierre Saint-Amand has recently published a vivid account of the happenings in Chile at the time[1]

Table 1

		G M T			
		h	m	s	
May 20, 1960	*e PPNZ*	11	32	25	
May 21, 1960	*e PZ*	10	17	25	
	i PPZ	10	22	10	
May 22, 1960	*i XZ*	10	49	48	
May 22, 1960	*e PZ*	19	10	33	
	i PPZ	19	15	09	
	MN	20	26		amplitude 0 8 mm

The immediate swarm consisted of more than 225 earthquakes and earth tremors, of which the chief are shown in Table 2

Table 2

	G M T			Lat	Long	
	h	m	s	°S	°W	Magnitude
May 21, 1960	10	02	50	37 5	73 5	7 5
May 22, 1960	10	32	43	37 5	73	7 5
May 22, 1960	18	55	57	38	73 5	7 8
May 22, 1960	19	10	47	38	73 5	7 5
May 22, 1960	19	11	20	38	73 5	8 4
June 20, 1960	02	01	08	38	73 5	7 0
Nov 1, 1960	08	46	1 9	38 4	74 4	7 3
Dec 2, 1960	09	10	41	24 5	69 9	7 2

Local investigations have been undertaken by the Chilean Geological Survey under the direction of Don Carlos Ruiz, by the Escuela de Geologia (University of Chile) under the direction of Don Humberto Fuenzalida, by the U S Geological Survey, by the University of California and by other organizations Instrumental data were obtained throughout the world

The first large shock of the swarm came about 6 a m local time on Saturday, May 21, 1960, with epicentre in the peninsula of Arauco, just south of Concepcion near the town of Curanilahue The damage was largely in the city of Concepcion and in the coal-mining towns of the Arauco Peninsula There was also some damage to towns in the central valley Shocks continued on Saturday and Sunday throughout southern Chile and people, being afraid, were fortunately out of doors when the 'Remezon' struck This was at about 3 15 p m local time Dr Saint-Amand reports that the motion of the ground then was slow and rolling like that of the sea during a heavy swell Parked cars in Concepcion rolled to and fro through a distance of ½ m and bobbed up and down The tops of the trees waved as in a tempest Buildings crashed The period of vibration of the ground is reported to have been of the order of 10 sec or more and the visible duration of the motion some 3½ min In the region of the lakes, at Llifen, a similar movement began smoothly and continued for about 2 min when, suddenly, a loud noise was heard underground This was followed by a sharp jarring motion and a more rapid, less regular, vibration of the earth Other places reported similar movements The seismograph at Concepcion was unhinged by the first shock, but the instruments at Santaigo continued recording It was clear that Chile was being subjected to a major earthquake swarm, and that the active area was about 160 km wide and 1,600 km long in southern Chile

About 20 min after the greatest earthquake on May 22, following an initial retreat of the sea from the land, the sea returned, advancing with tremendous force on the shore in a wave that was in some places more than 20 ft high The water rushed over the land doing very great damage The damage was especially great on the Island of Chiloe, and the maximum height of the principal wave in Chile appears to have occurred between Puerto Saavedra and Bahia Mansa Following this were several successive waves The waves, beginning near the coast of southern Chile, proceeded in all directions over the surface of the Pacific Ocean with a velocity of about 700 km/h, not only along the coast of Chile but to the shores of Hawaii, New Zealand, Australia, Japan and Kamchatka, causing damage in all these places The maximum height of the waves in Hawaii was at Hilo (35 ft), and in Japan north-east of Hondo (18 ft)

Between 1 30 p m and 2 45 p m local time on May 24, 1960 (some 47 h after the main earthquake), the volcano Puyehue erupted explosively, sending out a cloud of ash and steam to a height of about 20,000 ft The eruption, which was from a fissure about 300 m long on the north-west flank continued for several weeks The ash eruption was followed by a discharge of viscous lava, making several flows about 1 km long The lava was no longer in motion on July 22, 1960

Very little permanent surface faulting has been observed following the earthquakes, but it is probable that the first earthquake had its focus on the Arauco fault plane near Curanilahue at considerable depth Movement probably next took place at the Reloncavi fault, and later movement along a series of other faults in the central valley and along the coast ranges, giving rise to after-shocks The changes in elevation of the coastline and islands (maximum about 6 ft) seem to indicate movement along a fault between Guafo and Mocha Islands and the coast The whole fault movement began in the north and progressed southwards Recent work by Aki using Rayleigh waves indicates that the after-shocks of the earthquake took place on dextral strike-slip faults provided we assume the faults to run parallel with the coast The total length of faulting for the whole swarm probably extended to more than 1,200 km

The considerable flooding after the earthquakes was not only due to the tectonic movement altering watercourses, but also to the Tsunami, which blocked the mouths of rivers and streams with mud and sand

Landslides, occasioned by the earthquakes, also dammed many rivers, especially those swollen by heavy rain

The region affected by these earthquakes has been similarly affected in the past Dr F Greve of the seismological institute at Santiago has summarized his own work and that of Montessus de Balore Between the years 1520 and 1946 there were 47 notable earthquakes in southern Chile, together with millions of minor ones Seven of these shocks probably attained a magnitude (8 4) similar to that of May 22, 1960, and eight shocks probably had Tsunamis associated with them In 1575 an earthquake had a similar zone of influence, even to having produced a large landslide in Rio San Pedro

E TILLOTSON

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EFFECT OF OXYGEN ON THE ELECTRON SPIN RESONANCE LINE-WIDTHS OF AMORPHOUS CARBONS

By J. W. C SPACKMAN

Physics Branch, Royal Military College of Science, Shrivenham

THE electron spin resonance absorption of the carbon blacks has frequently been ascribed to free radicals Recent work indicates, however, that it is due to charge carriers Thus the line shapes corresponding to a fast diffusing paramagnetic species can be obtained from compressed samples, and such modified spectra cannot be explained in terms of free radicals Work on this topic is in progress The present article is concerned with discussing the variation of line-width with oxygen pressure in the light of this observation The line-width of carbon blacks and other carbonaceous materials has been shown to vary linearly with oxygen pressure over a fairly wide pressure range[1-3] The results obtained for a series of commercial carbon blacks is shown at Fig 1 The curves are linear down to pressures of < 1 mm and these results show no sign of departure from linearity due to absorbed oxygen as has been reported by Tikhomirova *et al* [1] working on sugar carbons The curves show an interesting 'fanning-out' effect, that is, those with the greatest line-width at zero oxygen pressure have also the greatest slope

These effects can be semiquantitatively explained on the theory of Dyson for the spin resonance absorption of conduction electrons[4] Dyson proposes that the total relaxation time T_2 (where T_2 is the inverse line-width) in particles small compared with the skin depth is given by an expression of the form

$$\frac{1}{T_2} = \frac{1}{T_B} + \frac{1}{T_S} \tag{1}$$

where T_B is the bulk relaxation time and T_S is a surface relaxation time, which, in the absence of oxygen, is given by an equation

$$T_S = \frac{4}{3}\frac{r}{\varepsilon v} \tag{2}$$

where r is the particle radius, ε is the probability of spin disorientation during the surface collision and v is the velocity of electrons at the top of the Fermi surface For pure metals T_S is large compared with T_B and its effect may be ignored, but in collision with an impure irregular surface an electron is subjected to strong spin dependent forces due to surface irregularities or to paramagnetic surface impurities and the effect of T_S may be considerable It is in fact likely to be preponderant

Now in the presence of oxygen an additional term must be added to the expression for the surface relaxation time, that is

$$\frac{1}{T_S'} = \frac{1}{T_S} + \frac{1}{T_{O_2}} \tag{3}$$

where T_S' is the surface relaxation time in the presence of oxygen, T_S is the relaxation time in the absence of oxygen and T_{O_2} is a term introduced to account for the increased probability of spin disorientation due to oxygen By analogy with equation (2) T_{O_2} will be given by an equation

$$T_{O_2} = \frac{4}{3}\frac{r}{\varepsilon_{O_2} v} \tag{4}$$

where ε_{O_2} is the probability of spin disorientation at the surface due to the presence of oxygen Now

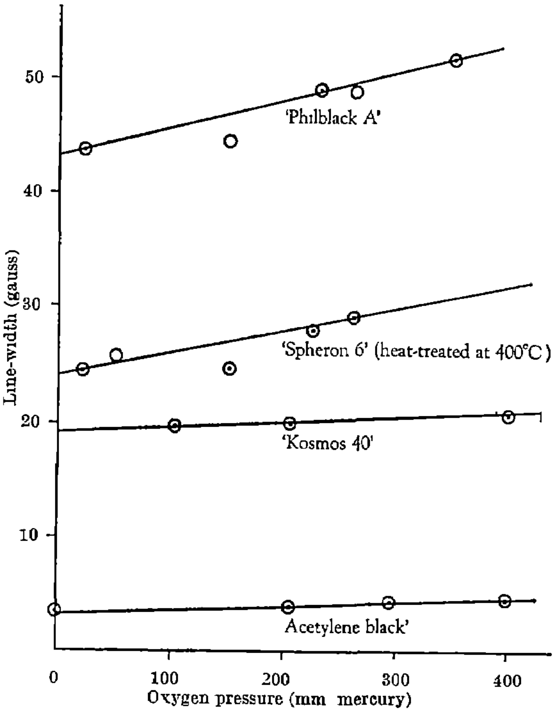

Fig 1 Variation of line-width with oxygen pressure

it is reasonable to suppose that ε_{O_2} is proportional to the concentration of paramagnetic gas molecules above the surface and to a surface factor representing the ease with which spin interaction can take place between the spin of an oxygen molecule and an unpaired spin in the material Thus if p is the pressure of oxygen above the material

$$T_{O_2} = \frac{r}{\eta p} \qquad (5)$$

where η is a constant for a particular surface and is a measure of the contribution of the nature of the surface to the probability of spin disorientation due to the presence of oxygen Thus re-writing the equations in terms of line widths

$$\Delta H = \Delta H_O + \eta p/r \qquad (6)$$

where ΔH_O is the line-width in the absence of oxygen. The term η may be further elucidated by the observation that η would appear to be proportional to ΔH_O for a given type of carbon, that is

$$\Delta H = \Delta H_O + \varphi \Delta H_O \frac{p}{r} \qquad (7)$$

Table 1 shows the values of ΔH_O, $\frac{d(\Delta H)}{dp}$ and φ for the range of carbon blacks excluding those with highly oxidized surfaces, that is, the channel blacks It can be seen that in view of the fact that these surfaces are far from identical the agreement for the value of φ is good

Table 1

Trade name	ΔH_O (gauss)	$\frac{d(\Delta H)}{dp}$ (gauss mm $^{-1}$)	$\frac{r}{(A)}$	φ
'Philblack A'	44	0 018	380	0 16
Kosmos 20'	15	0 004	650	0 17
'Kosmos 40'	15	0 004	525	0 14
'Spheron 6' (heat-treated to 400° C)	22	0 014	250	0 16
'Spheron 6' (heat-treated to 800° C)	52	0 032	250	0 15
'Vulcan 6'	40	0 033	230	0 19
'Vulcan 9'	46	0 045	200	0 19
'Shawinigan'	4 9	0 0016	400	0 13

The fact that $\frac{d(\Delta H)}{dp}$ is proportional to ΔH_O would seem to suggest that the spin interaction between oxygen and the spins of the system occurs via the surface imperfections that are responsible for the initial line-width

Table 2 shows the effect of a more highly oxidized surface on the values of $\frac{d(\Delta H)}{dp}$ and φ it can be seen that oxidation considerably increases the value of $\frac{d(\Delta H)}{dp}$ and at the same time produces a considerable variation in the value of φ

Table 2

Trade name	ΔH_O	$\frac{d(\Delta H)}{dp}$	r	φ
'Dixie 77'	4 7	0 028	300	1 7
'Spheron 6'	6 3	0 20	250	8
'Black Pearls 74'	5 0	1 4	170	48
'Black Pearls 2'	5 0	0 65	120	15
'Black Pearls 46'	5 0	0 28	280	16

It would appear that in this case the arguments no longer hold, probably because the free spins are much more likely to be localized in quinnoid structures and to behave more as a surface free radical Alternatively the presence of electron-attracting groups at the surface increases the length of time an unpaired spin is under the influence of the surface and thus effects due to variations between surfaces become magnified

At the same time, of course, the effectiveness of oxygen in broadening the line is increased It is very noticeable that heat treatment at 400° C , which removes much of the chemisorbed oxygen, brings 'Spheron 6' into the framework of the initial scheme (compare Table 1, items 4 and 5, and Table 2, item 2)

Equation (7) is likely to hold, therefore, in all cases of a reasonably pure non-oxidized carbon surface where the surface relaxation is small compared with the bulk relaxation time and where the particle radius is small compared with the skin depth

I thank Prof A Charlesby for his advice

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CRYSTALLINE FORMS OF CARBON: A POSSIBLE HEXAGONAL POLYMORPH OF DIAMOND

By SABRI ERGUN and LEROY E. ALEXANDER*

Pittsburgh Coal Research Center, U S Department of the Interior, Pittsburgh

THE number of valence electrons of elements belonging to Group IV in the Periodic Table, that is, carbon, silicon, germanium, tin, etc , or of two elements symmetrically arranged relative to Group IV, for example, AlN, BeO, ZnO, ZnS, etc , in principle permits the formation of a tetrahedral covalent bond between each atom and its four neighbours[1] Regular three-dimensional linking of tetrahedrons yields hexagonal-close-packed layers One of the two simplest forms of hexagonal-close-packed layer structures has a cubic symmetry and yields a diamond-type lattice, and the other yields a hexagonal wurtzite-type lattice

Close-packed layers can be characterized by a particular sequence of layers designated by *A*, *B* *C*, etc , the projections of the atoms in the layers designated by a common letter, for example *A* being identical Tetrahedral bonding yields layers that are alternately spaced by one bond-length and one-third of a bond-length The positions of the atoms in the adjacent layers separated by one bond-length are identical The layers form the (111) planes in the cubic lattice and the (00 2) planes in the hexagonal lattice These two structures differ in the arrangement of the adjacent layers which are separated by one-third bond-length In the cubic structure the sequence can be represented by three pairs, $/A\text{–}A/B\text{–}B/C\text{–}C\diagdown A\text{–}A/$, and in the hexagonal structure by two pairs, $/A\text{–}A/B\text{–}B\diagdown A\text{–}A/$ The positions of the atoms in these two sequences are illustrated in Fig 1.

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*Senior Fellow, Mellon Institute, Pittsburgh 13

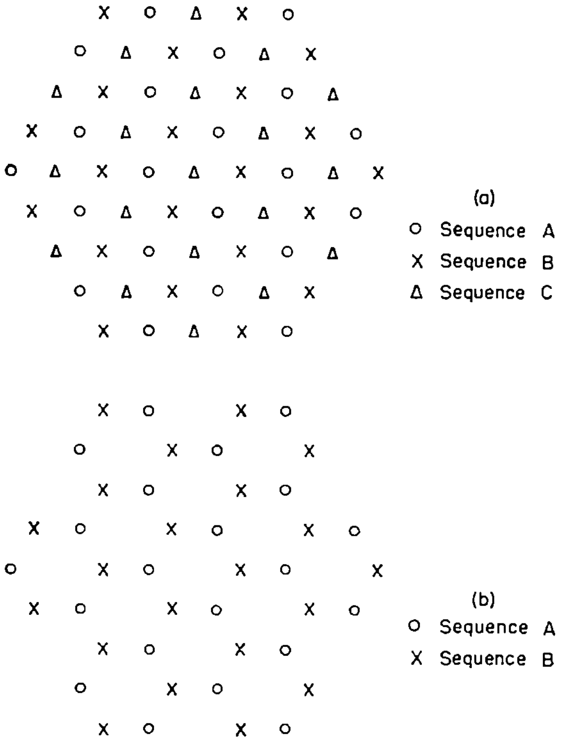

Fig. 1 Projections of atoms on (111) plane of face-centred cubic structure (*a*) and on (00 2) plane of hexagonal structure (*b*)

Reciprocal spacings ($1/d$), line indices (hkl), geometric structure factors (F_g) and relative scattering intensities (I_c) of the two crystalline forms of tetrahedral carbon are given in Table 1 The indices given cover those observable with copper $K\alpha$ radiation It is readily observed that the hexagonal structure, having less symmetry, gives rise to many additional reflexions

Many tetrahedral structures are known to exist both in cubic and hexagonal crystalline forms, and many form a variety of stacking sequences The classic example is ZnS The cubic crystal is known as sphalerite and the hexagonal as wurtzite Mixtures of SiC, ZnS and of many wurtzite-type compounds are known[1,2] The transformation from cubic lattice to hexagonal and vice versa, or the formation of mixed structures, can be accomplished by rotation or by translation of layers In addition to wurtzite-type compounds, other compounds, such as ice, are known to undergo such transformations Ice has the hexagonal crystalline form at ordinary temperatures, below −70° C it has the cubic form[3,4] It is rather remarkable that carbon, silicon and germanium have been reported to exist in face-centred-cubic form only It is of interest to know whether or not carbon can exist in the hexagonal arrangement under some conditions It should be pointed out that for a number of years graphite was believed to exist only in a hexagonal crystalline form having an *–A–B–A–B–* sequence Later work, however, indicated that the *–A–B–C–A–B–C–* sequence of stacking (rhombohedral graphite[5,6]) and/or other sequences[7] existed in graphites

It appears that the hexagonal form may exist in diamonds, causing what are termed layer faults and growth faults Recently it was shown that peak shifts and line broadenings observed in X-ray diffraction patterns of diamonds could be explained by altering the sequence of nearest layer pairs[8] That peak shifts and line broadenings would likewise be observed if diamonds contained some hexagonal layer sequences is also evident from an inspection of Table 1 The (10 0) and (10 1) reflexions of the hexagonal lattice would broaden and produce peak shifts of the (111) reflexions of the face-centred cubic lattice Similarly the (20 0) and (20 1) reflexions would influence the (113) reflexions, the (20 3) and (21 1) reflexions would influence the (133) reflexions, etc For every face-centred cubic reflexion shown in Table 1 there is a corresponding (that is, having the same d-spacing) reflexion of the hexagonal lattice (with the exception of the (400)), and each one is closely accompanied by additional reflexions

In both the cubic and hexagonal structures the closest layer pairs, that is, *A*/*B*, *B*/*C*, etc, form buckled hexagonal rings which are of chair type The buckled rings would project on the eight octahedral faces $\{111\}$ of a face-centred cubic crystal, every face having one parallel to it The normals to these face pairs are parallel to the four vectors connecting any one atom to its four neighbours In the hexagonal structure atoms form chair-type buckled rings on only the two faces that form the basal planes, namely, (00 2) and (00 $\bar{2}$) On the other three face pairs, denoted in the hexagonal model by (10 0) and ($\bar{1}$0 0), (1$\bar{1}$ 0) and ($\bar{1}$1 0), and (0$\bar{1}$ 0) and (01 0), boat-type rings are formed Since boat-type rings cannot be stacked on chair-type rings by normal tetrahedral bonding, mixed structures are possible only as a result of stacking variations along one axis, namely, the c-axis of the hexagonal lattice If hexagonal structures could be made to grow on the eight octahedral faces $\{111\}$ of a face-centred cubic crystal, a continuous and homogeneous crystal would not be formed Growth on two adjacent faces cannot be joined by tetrahedral bonding This is easily seen if one attempts to attach a few models of the hexagonal unit cell on a model of the face-centred cubic unit cell This limited miscibility of these two crystalline forms could result in growth faults in the formation of diamonds

Table 1 STRUCTURE FACTORS OF CUBIC AND HEXAGONAL CRYSTALLINE FORMS OF TETRAHEDRAL CARBON

Line No	$\frac{1}{d}=\frac{2\sin\theta^*}{\lambda}$	(hkl)	F_g	I_c†
		(*A*) Cubic diamond structure, $Z = 8$		
1	0 4856	111	$-\sqrt{32}$	256
2	0 7930	220	−8	768
3	0 9299	113	$-\sqrt{32}$	768
4	1 1215	400	−8	384
5	1 2113	133	$+\sqrt{32}$	768
6	1 3735	422	+8	1536
7	1 4569	511	$+\sqrt{32}$	768
8	1 4569	333	$+\sqrt{32}$	256
9	1 5860	440	+8	768
		(*B*) Hexagonal diamond structure, $Z = 4$		
1	0 4578	10 0	−2	24
2	0 4856	00 2	$+\sqrt{8}$	16
3	0 5182	10 1	+1 3257	21 088
4	0 6674	10 2	$-\sqrt{2}$	24
5	0 7930	11 0	+4	96
6	0 8604	10 3	+3 2005	122 912
7	0 9157	20 0	−2	24
8	0 9299	11 2	$+\sqrt{8}$	96
9	0 9473	20 1	−1 3257	21 088
10	1 0365	20 2	$-\sqrt{2}$	24
11	1 1701	20 3	−3 2005	122 912
12	1 2113	21 0	−2	48
13	1 2354	21 1	+1 3257	42 177
14	1 2975	10 5	+3 2005	122 912
15	1 3051	21 2	$-\sqrt{2}$	48
16	1 3735	30 0	+4	96
17	1 4135	21 3	+3 2005	245 823
18	1 4569	30 2	$+\sqrt{8}$	96
19	1 4569	00 6	$+\sqrt{8}$	16
20	1 5207	20 5	−3 2005	122 912
21	1 5271	10 6	$+\sqrt{2}$	24

*Bond-length is taken to be 1 5444 Å

†The $I_c = m\,|F_g|^2$

Of particular interest are the so-called amorphous carbons. Recent studies indicated that X-ray scattering intensities of non-graphitizable carbons could be explained by the presence of tetrahedral as well as trigonal and π structures[9]. Their non-graphitizability, hardness and sometimes density[10] favour the tetrahedral form. If under the conditions of carbonization, probabilities of formation of the cubic and hexagonal structures are not prohibitive in favour of one or the other, a skeletal network of interpenetrating crystallites would be formed; the closer the probabilities, the smaller the crystallites. Such a system could be termed polytype-crystalline in contra-distinction to the term polycrystalline, which is used to describe a solid composed of many crystals (or crystallites) of varying sizes but of the same type. Of course, the probabilities of formation of trigonal and π structures should not be overlooked. If tetrahedral and trigonal and π bonds are formed during the pyrolysis of carbonaceous materials, the resulting structures would be extremely complicated. Whether diamonds, graphites or polytype carbons are formed would depend upon the reaction time and the relative probabilities of formation of various crystalline forms, the probabilities being dependent upon the conditions under which the reaction takes place. It is interesting to note that under proper conditions of pyrolysis some carbon-containing materials can be carbonized to yield what are known as pyrolytic carbons in which the graphitic layers formed attain sizes of several thousand angstroms[11], where pyrolysis of polyvinylidene chloride[12] and of coals[13] yields crystallites of a few angstroms in size, that is 6–15 Å. Polytype crystallinity appears to offer a very reasonable explanation for the differences. Therefore, various crystalline forms of carbon should be considered in analysing the structure of the so-called amorphous carbons.

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MEASUREMENT OF CURRENTS BELOW THE SURFACE AT A LOCATION IN THE WESTERN EQUATORIAL ATLANTIC

By T. E. POCHAPSKY

Columbia University, Hudson Laboratories, Dobbs Ferry, New York

IN a communication to *Nature*, A. D. Voorhis[1] presented evidence for a strong eastward moving current just below the surface of the Atlantic in the eastern equatorial region from 10° W. to at least 20° W. We have recently completed some measurements at a fixed location on the western side of the Atlantic, and these revealed easterly currents which are weaker than those reported by Voorhis but which occur not only just under the surface but also at great depths. Voorhis found a long, narrow stream moving at a velocity near 100 cm sec^{-1} at a depth of 105 m. At our location, 2° 05.5′ N. and 35° 00.0′ W., we found an easterly current of 18 cm sec^{-1} at a depth of 91 m. The surface current was to the west and amounted to about 25 cm sec^{-1}. Rather than explore to find whether we were at the edge of a strong stream, we chose to examine how the current changed with depth. Measurements were made by following neutrally buoyant floats and were necessarily limited to a relatively small number of levels. At a depth of 130 m, the current was northerly at 3 cm sec^{-1} and shifted to southerly near 450 m. The current jumped to 24 cm sec^{-1} in an easterly direction at the 1,100-m level and continued in that direction at 12 cm sec^{-1} when the depth was 1,530 m. Below a depth of 2,000 m, the currents were weaker and to the north.

The entire series of measurements is summarized in Table 1.

Our knowledge of the flow below the surface in the equatorial Atlantic is theoretical and leans heavily on the work of Defant[2] and Wust[3]. Even so, their results are not completely applicable at the equator because the Coriolis force is small there and because their data emphasize meridional flows. Defant surmises the existence of a practically motionless layer of water below the surface. At our location, this would be 750 m down. An easterly countercurrent could exist in the top few hundred metres, and Neumann[4] has shown how the trade winds can produce a westerly surface current over such a countercurrent so as to produce a situation resembling that observed in our measurements.

Defant's analysis shows currents which are usually smaller than 3 cm sec^{-1} beneath the moving surface waters down to a depth of approximately 1,500 m. Continuing the study down to the bottom, Wust found two strong streams. One of these carries North Atlantic Deep Water southward at speeds approaching 15 cm sec^{-1} while hugging the continental slope off South America. The other stream is displaced 200–300 km to the east of this and moves Antarctic Bottom Water to the north at speeds up to 12 cm sec^{-1}. Our location should have been nearly over this latter stream. The measurements from 1,950 m down to 3,240 m revealed a northward current of approximately 5 cm sec^{-1}, which is consistent with Wust's results. On the other hand, the large zonal flows found at intermediate depths are unexpected.

By tracking neutrally buoyant, that is, Swallow-type[5], floats, it is possible to obtain velocities averaged over a long time, and tidal or other brief fluctuations in the measurements can be avoided. Our floats[6] telemetered their depths with an accuracy of a fraction of 1 per cent and have transponding features which allowed us to determine their positions relative to the ship with high accuracy. Determination of the position of the ship was made by dead reckoning

Table 1 DRIFT OF NEUTRALLY BUOYANT FLOATS AT 2° 05 5′ N, 35° 00 0′ W DURING FEBRUARY 1962

Time and date From —/5	To —/20	Depth (m) (0)*	Average float velocity cm sec^{-1} (15 – 50)	Direction of motion degrees† (220°–280°)
1513/13	0630/14	72	22 ± 3 5	047
1419/10	1240/11	84	18 0 ± 2 5	324
1420/10	1403/9	89	17 7 rel‡	320
1442/15	1417/16	91	17 ± 4 5	080
1630/15	0448/16	93	18 ± 4 5	088
1326/17	1030/18	130	3 ± 1 5	337
1325/17	1145/18	137	7 ± 1 5	307
1654/5	0530/6	440	16 2 rel	153
1655/5	1230/6	447	17 1 ± 2 5	152
1450/7	1450/9	1060	23 ± 2	082
1452/7	1042/9	1109	24 ± 2 5	108
1241/20	1100/22	1531	12 ± 2	076
1240/20	1000/22	1535	12 ± 2	076
1036/12	1120/14	1950	3 ± 1	035
1035/12	1650/14	2170	5 ± 0 5	033
1358/18	0830/19	2450	6 ± 2 5	010
1356/18	0830/19	2950	5 ± 2 5	005
1323/19	1645/21	3240	4 ± 2	320

* The numbers in parentheses are the sets of the ship They varied in the same fashion whether there was wind or not, excluding occasional small squalls

† This direction is accurate to ± 10°

‡ Two floats were dropped at the same time 'Rel' refers to measurements obtained by adding the relative vector distance between the floats to the position of the sister float

with respect to a buoyant float secured to the ocean floor by a mile length of cord This transponder float was 1,200 fathoms below the surface On occasion, the skies cleared so that we could locate the transponder by means of a star fix The origin of the track of a free float was known to an accuracy of ± ½ mile, and the final position was known to approximately ± 1 mile

The velocities reported in Table 1 were obtained by dividing the vector distance a free float moved by the total time at depth A float did not move with a constant velocity over its course, and the path itself tended to curve to the right Our precision of measurement did not allow investigating these features when the current was large The deeper floats remained near the anchored transponder, and it was apparent that they meandered in the quadrant northwest of the transponder One such float followed a clockwise path and passed near its initial position after 31 h Another one had a more nearly random counter-clockwise motion These trends in the movements were real, but details were obscured by errors in tracking Although measurements can be made to an accuracy of ± ½ mile relative to the transponder, it is possible that bottom currents could swing the transponder itself over this range We can, however, look on the results for the deep floats as reasonable approximations to the average velocities over periods of a day or so

This work was supported by the Office of Naval Research under contract *Nonr*–266(84)

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MEASUREMENT OF PARTICLES

By Dr C. N. DAVIES

(Medical Research Council), London School of Hygiene and Tropical Medicine, London, W C I

IRREGULAR particles, seen as silhouettes, can be measured in various ways What is meant by the terms radius, diameter, size, is frequently unspecified, and figures derived from such measurements are seldom related to the statistics of shape and then only by factors of doubtful meaning

Any of the common methods of expressing the sizes of the particles in a preparation can, however, be associated with the average of a series of analogous measurements of a single particle, oriented in different ways, this average has an exact geometrical significance

It is suggested, therefore, that if the repetition of the measurements be made not at random on a single particle, but once per particle over a number of particles lying randomly, then their average will have the same geometrical significance for the group as the average of the measurements taken on a single particle has for that individual

The validity of this proposition is obvious if the particles are all the same and favour all orientations equally The former restriction is rarely satisfied and the latter is notoriously untrue for orientation in three dimensions[1] Its validity can easily be tested, in any particular instance, by measuring a quantity S over a number of particles, 1, 2, 3, n and repeating the measurements in different directions, α, β, γ, . . . ν spread evenly over an azimuth of 180° The measurements comprise the array

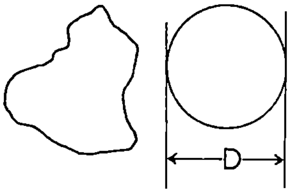
$$\begin{array}{cccc} S_{1\alpha} & S_{2\alpha} & S_{3\alpha} & & S_{n\alpha} \\ S_{1\beta} & S_{2\beta} & S_{3\beta} & & S_{n\beta} \\ S_{1\gamma} & S_{2\gamma} & S_{3\gamma} & & S_{n\gamma} \\ \\ S_{1\nu} & S_{2\nu} & S_{3\nu} & & S_{n\nu} \end{array}$$

If the averages of each row are identical, within an acceptable margin, they must match the mean of the averages of the columns, this proves the truth of the proposition for the material and technique of preparation in question This result will be referred to as group extension of the average

A popular measurement is that of the projected area diameter, D In this the problem of shape is evaded by requiring the observer to condense the outline of the particle into a circle of the same area (Fig 1) The measurement is usually made subjectively, it can be done with precision, either quickly, by light extinction, or slowly with a planimeter

Another measurement is the distance between parallel tangents to each particle (Fig 2) The average over a number of particles, all tangents being parallel, is known as Feret's statistical diameter, $\overline{F}$ (ref 2)

The distances, F, between pairs of parallel tangents to a single particle can be measured in different directions The distance between any pair is the sum of the projections of the elements, p_i, of either of the two parts of the perimeter between the points of contact of the tangents, provided that the perimeter is nowhere concave Concavities result in over-



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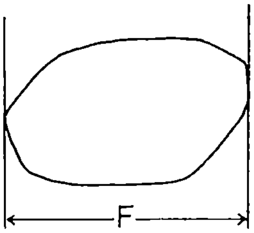

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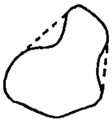

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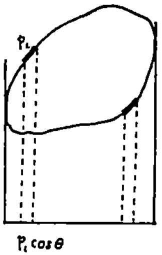

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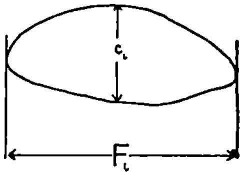

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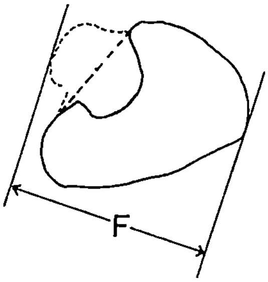

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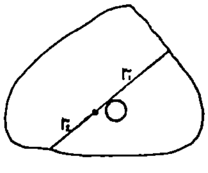

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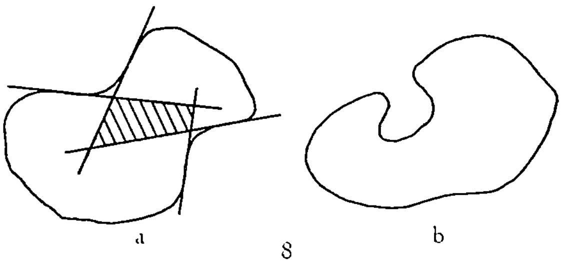

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Fig 1 Projected area diameter, D

Fig 2 Distance between parallel tangents F

Fig 3 Perimeter, P, of a particle which is not convex

Fig 4 Projection of elements of perimeter

Fig 5 Area is $F_i c_i$

Fig 6 Method of obtaining the true perimeter Note that the reflexion of the part of the outline which is inside the tangent bridging the convexity still leaves re-entrant regions Further reflexions are necessary until a convex outline results

Fig 7 Segments of a diameter through O

Fig 8 Re-entrant outlines *a*, Equation (4) is valid if O is in the hatched area, *b*, the entire area is swept by tangents to the outline and equation (4) does not hold

lapping of the projections of the elements, p_i, in some directions of projection The elements project additively only for convex outlines, an outline with concavities projects as a string drawn tightly round it (Fig 3)

When F is averaged over all possible directions of projection each element of projection is similarly averaged (Fig 4) and

$$2\bar{F} = \Sigma \frac{2}{\pi}\int_0^{\pi/2} p_i \cos\theta \, d\theta = \Sigma \frac{2}{\pi} p_i$$

$$\text{Hence} \quad \bar{F} = \frac{P}{\pi} \text{ for one particle} \qquad (1)$$

where P is the perimeter of the particle, if convex, or the length of a string drawn tightly round it if not convex[3]

Subject to group extension of the average being valid, the Feret statistical diameter of a number of particles is their average perimeter divided by π (ref 4)

It is obvious that the area, A, of a particle is given by the distance F_i between any pair of parallel tangents multiplied by the mean length c_i of chords parallel to the tangents (Fig 5)

Hence, for orientation i

$$A = F_i c_i \qquad (2)$$

Since c_i is weighted according to the length, F_i, the mean of c_i over all orientations is the mean length, $\bar{c}$, of a large number of chords drawn at random through the outline of the particle, hence

$$\bar{c} = \frac{\Sigma F_i c_i}{\Sigma F_i}$$

which, by (1) and (2), gives

$$\bar{c} = \pi A/P \qquad (3)$$

This result was obtained by Crofton[5], who also showed that

$$\overline{c^3} = 3A^2/P \qquad (4)$$

By group extension of the average the mean of single chords measured on a number of particles at random gives π times the mean of the ratio of their area to perimeter, P, defined here and there is an analogous extension of (4)

The effect of a particle having a re-entrant outline is to make the perimeter as estimated by equation (1) too short The correct perimeter is easily obtained by reflecting the concavity about the tangent to the outline which encloses it and measuring the chords for Feret's diameter to the reflected extension instead of to the tangent (Fig 6) The area of re-entrant particles can be correctly obtained from equations (2) and (3) by measuring the two or more parts of each chord which cuts a particle more than once and adding them together

If the lengths of the chords drawn at random are

$$c_1, c_2, \quad c_n$$

where

$$c_1 = \bar{c} + \delta_1, \text{ etc}, \text{ and } \Sigma\delta = 0$$

then

$$n\overline{c^2} = n\bar{c}^2 + \Sigma\delta^2$$

and

$$n\overline{c^3} = n\bar{c}^3 + 3\bar{c}(n\overline{c^2} - n\bar{c}^2) + \Sigma\delta^3$$

Hence, using (3) and (4)

$$A = \pi(\overline{c^2} - \frac{2}{3}\bar{c}^2) + \frac{\pi}{3n\bar{c}}\Sigma\delta^3 \qquad (5)$$

This enables the area of a shape to be computed from the lengths of random chords with group extension to the mean area of a number of particles

The last term in equation (5) is related to the third moment of the distribution of chord-lengths, that is to the skewness of their distribution; the term is usually small, being only a 2½ per cent correction for circles

Instead of drawing chords at random suppose they are all drawn through one point, O, inside the outline of a particle Let d be the length of a chord and suppose O divides it into segments r_1, r_2 (Fig 7) so that $d = r_1 + r_2$ If the chord rotates about O through an angle π it sweeps out the area and

$$\begin{aligned} A &= \int_0^\pi \tfrac{1}{2} r_1^2 d\theta + \int_0^\pi \tfrac{1}{2} r_2^2 d\theta \\ &= \frac{\pi}{2} (\overline{r_1^2} + \overline{r_2^2}) \\ &= \frac{\pi}{2} \overline{d^2} - \pi \overline{r_1 r_2} \\ &= \frac{\pi}{2} \overline{d^2} - \pi\rho \sqrt{\overline{r_1^2}\,\overline{r_2^2}} \end{aligned} \tag{6}$$

where ρ is the coefficient of correlation between r_1 and r_2

$$\rho = \overline{r_1 r_2} \Big/ \sqrt{\overline{r_1^2}\,\overline{r_2^2}} \tag{7}$$

This result is valid for all convex and some re-entrant outlines, subject to O not being on any tangent to the outline (Fig 8)

If O is a point on the periphery

$$A = \frac{\pi}{2} \overline{d_p^2} \tag{8}$$

where d_p is a diameter through a point on the periphery

This result is independent of the location of O on the periphery so long as it is not at a discontinuity

The correlation between r_1 and r_2 depends on the position of O within the outline, presumably the correlation will be greatest for a given outline when O is the centroid In this case

$$\overline{r_1^2} = \overline{r_2^2} = \overline{r^2} = A/\pi$$

Hence, from (5)

$$A = \frac{\pi}{2(1 + \rho)} \overline{d_c^2} \tag{9}$$

where d_c is a diameter through the centroid Notice that $\overline{d_c^2} = (1 + \rho)\overline{d_p^2}$

As before, this result extends to give the mean area of a number of particles from the mean square of the length of a chord through the centroid of each particle The mean of chords through the centroids of a group of particles is known as Martin's diameter[6]

The value of the correlation coefficient of the segments r_1, r_2 of a chord through the centroid of a figure depends on its symmetry It is unity for a figure having two perpendicular axes about which it is either symmetrical or skew symmetrical Thus $\rho = 1$ for circle, ellipse, rectangle and parallelogram The correlation coefficient has been calculated for three triangles with the following results

Triangle	ρ	$\delta A/A$ (per cent)
Isosceles, 20° at vertex	0.95	2½
Isosceles, 160° at vertex	0.97	1½
Equilateral	0.928	4

It is possible that the smallest value which ρ can take is that for an equilateral triangle, though this has not been proved The last column shows the error

$$\frac{\delta A}{A} = \frac{1 - \rho}{2}$$

which results from computing equation (9) with $\rho = 1$ instead of the true value, this gives too small an area

This method of finding the area of irregular shapes might be subject to less personal error than the usual one, based on projected area diameter, because it is probably easier to place a line through the centroid of an outline than to compress it to a circle having the same area, the two methods should be used to check one another, a further independent check is obtainable by measuring chords at random and applying equation (5)

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A SINGLY TERMINATED FREQUENCY FUNCTION WITH THREE PARAMETERS

By R ASH and E O POWELL

Microbiological Research Establishment, Porton Down, Salisbury

THE number of singly terminated continuous distributions which are applied to graduating biological data is rather few considering the great variety of such data Apart from the Pearson Type VI distribution with its limiting cases Types III and V and the negative exponential, only the 'lognormal' or Galton–McAlister[1] is in widespread use The lognormal distribution, because of its convenient sampling properties, is often applied without much regard for its suitability, and even when considered hypothesis would suggest a different form[2-4]

Apart from problems of statistical estimation, the lognormal distribution is not algebraically convenient (for example, its characteristic function is not expressible in a simple form) and the same is true of the Type VI in certain kinds of application The distribution to which we wish to direct attention is

$$dF = \frac{x^{\nu-1} \exp\left\{ -\frac{\alpha}{2}\left(\frac{x}{m} + \frac{m}{x}\right)\right\} dx}{2m^\nu K_\nu(\alpha)} \tag{1}$$

where $K_\nu(\alpha)$ is the modified Bessel function of the second kind It is singly terminated below at the origin, it is flexible because, like the Type VI, it has three disposable parameters, and it is fairly easy to manipulate It also lends itself to the elaboration of

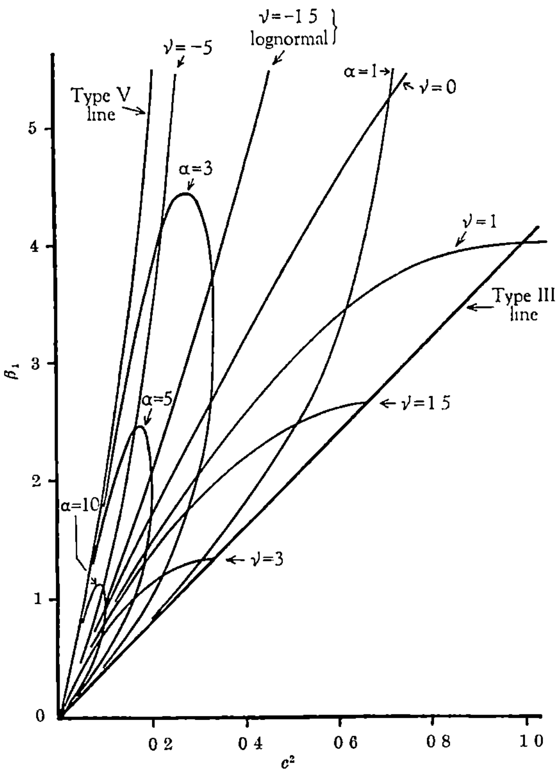

Fig 1 Frequency function of equation (1) Relation between squared coefficient of variation, c^2, and skewness, β_1, for various values of the parameters α, ν

compound distributions It arises naturally in discussing the distribution of the generation times of bacteria The generation time distribution can often be represented by a Pearson Type V, and when this is so, the 'carrier' distribution[5] takes the form (1) If the generation time distribution has the form (1), the carrier distribution is of the same form

The moments μ'_r of (1) about the origin are easily found by writing $x = me^{\theta}$ and using a standard integral for the Bessel function

$$K_\nu(\alpha) = \int_0^\infty e^{-\alpha\cosh\theta}\cosh\nu\theta \, d\theta \, ,$$

we find

$$\mu'_r = m^r \frac{K_{\nu+r}(\alpha)}{K_\nu(\alpha)} \tag{2}$$

The maximum likelihood equations for fitting (1) to a set of data can also be found by the usual procedure maximum likelihood estimates $\hat{m}$, $\hat{\alpha}$, $\hat{\nu}$, of the parameters are given by

$$A = \hat{m}\, K_{\hat{\nu}+1}(\hat{\alpha})/K_{\hat{\nu}}(\hat{\alpha}) \tag{3}$$

$$H = \hat{m}\, K_{\hat{\nu}}(\hat{\alpha})/K_{\hat{\nu}-1}(\hat{\alpha}) \tag{4}$$

$$G = \hat{m} \exp\{\mathscr{K}_{\hat{\nu}}(\hat{\alpha})\}$$

where A, H, G are respectively the arithmetic, harmonic and geometric sample means, and $\mathscr{K}_r(\alpha)$ has been written for

$$\frac{\partial}{\partial\nu}\left\{\log K_\nu(\alpha)\right\}$$

If in (1) α and m are allowed to tend to zero in such a way that α/m tends to a finite limit, say $2/\beta$, and if at the same time ν is positive, the limiting form reached is the Pearson Type III

$$\frac{x^{\nu-1}\exp(-x/\beta)}{\beta^\nu\,\Gamma(\nu)}$$

since near $\alpha = 0$, $K_\nu(\alpha)$ is about $\alpha^{-\nu}2^{\nu-1}\Gamma(\nu)$ Similarly the Pearson Type V is reached by making $\alpha \rightarrow 0$, $m \rightarrow \infty$ with αm finite and ν negative In fact the representative points of (1) in the Pearsonian β_1, β_2 diagram (see, for example, Pearson[6]) occupy the same region as those of the Type VI distribution The Type VI with lower terminus at the origin also has the same range of coefficient of variation and of skewness Fig 1 shows how the squared coefficient of variation, c^2, and skewness, β_1, are related to the parameters α and ν (m does not enter since it affects only the scale) Such a diagram can be used to obtain from the sample moments rough estimates of α and ν with which to begin an iteration to find $\hat{\alpha}$ and $\hat{\nu}$

(It is worth noting at this point that when the terminus of a distribution such as (1) is naturally fixed, the coefficient of variation acquires an intrinsic significance which it does not otherwise possess If the moments of a distribution are to be used to suggest a suitable functional form, it is better to infer the form from the coefficient of variation and skewness rather than the skewness and kurtosis—when this can be done—because of the relatively larger sampling variance of the fourth moment)

Since neither the moment nor the maximum likelihood equations can (in general) be solved explicitly for the parameters, the application of (1) demands the use of comprehensive and convenient tables of the Bessel function and its derivative with respect to the order Suitable tables do not yet exist, but Dr H J Gawlik, of the Royal Armament Research and Development Establishment, Fort Halstead, Kent, is undertaking their preparation

When ν can be assigned in advance and is a fixed integer n, or of the form $(2n + 1)/2$, and n is not too large, existing tables can be used There are several cases of fixed ν which give rise to distributions of special interest, thus $\nu = 1$ gives Keynes's[7] distribution Another particular case, that of $\nu = -3/2$, is immediately manageable and is remarkable as a very close approximation to the lognormal distribution

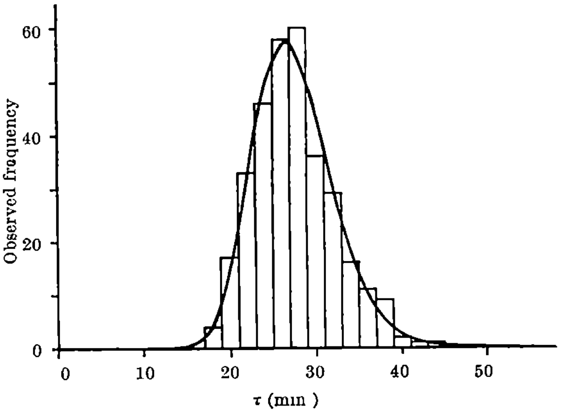

Fig 2 Generation time (τ) distribution of *Aerobacter aerogenes* with a fitted curve of the form of equation (1), $\nu = -3/2$

We have, since $K_{-\nu}(\alpha) \equiv K_{\nu}(\alpha)$,

$$K_{-1/2}(\alpha) = (\pi/2\alpha)^{1/2} e^{-\alpha}$$

$$K_{-3/2}(\alpha) = K_{-1/2}(\alpha)\,\{1 + 1/\alpha\}$$

$$K_{-5/2}(\alpha) = K_{-1/2}(\alpha)\,\{1 + 3/\alpha + 3/\alpha^2\}$$

and so from (3) and (4)

$$A/H = (\hat{\alpha}^2 + 3\hat{\alpha} + 3)/(\hat{\alpha} + 1)^2$$

$$A = \hat{m}\,\hat{\alpha}/(\hat{\alpha} + 1)$$

Also, from (2)

$$c^2 = 1/\alpha\,,\ \beta_1 = (1/\alpha + 3)^2/\alpha$$

Thus $\beta_1 = c^2\,(c^2 + 3)^2$ Now this relation between the skewness and coefficient of variation applies also to the lognormal distribution[8], if a distribution of the form (1) with $\nu = -3/2$ and a lognormal distribution have their first two moments equal, their third moments will also be equal On the c^2, β_1 diagram (Fig 1), the lognormal distribution is represented by the same line as (1) with $\nu = -3/2$ For the graduation of data, therefore, the two distributions will often be interchangeable

Example Observations of the generation time distribution of *Aerobacter aerogenes* growing on a synthetic medium at 35° (ref 9)

Distribution (1) with $\nu = -3/2$ and also a lognormal distribution were fitted by the method of maximum likelihood The sample statistics were

No of observations	323
Mean	27 511
Variance	21 965
3rd moment	54 912
4th moment	1544 418
Harmonic mean	26 742
Coefficient of variation	0 169
Skewness, β_1,	0 298
Natural logarithm of geometric mean, G	3 30035
Variance of logarithm of G	0 0285077
Estimates of parameters $\hat{\alpha}$ =	34 75
$\hat{m}$ =	28 30

(Unit of time 1 min)

The fitted curve of (1) is shown superposed on the histogram of the observations in Fig 2 (By the χ^2 test, the fit is good $\chi^2 = 3\ 6$, 8 degrees of freedom) The fitted lognormal curve is indistinguishable from it on the scale of the diagram

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ENZYMATIC REACTION SEQUENCES AND CYTOLOGICAL DIMENSIONS

By PAUL B WEISZ

Socony Mobil Oil Co Inc , Research Department, Paulsboro Laboratory, Paulsboro, New Jersey

OCCURRENCE of a number of sequential reaction steps catalysed by various enzyme systems is a characteristic of biochemical conversion processes Such polystep catalytic reaction sequences have been studied and experimentally demonstrated in hydrocarbon reaction systems It has been demonstrated[1-4] that, in a sequential reaction occurring on different catalyst entities, the physics of the transport process of the intermediates leads to a relationship between the attainable rate of the overall reaction, the thermodynamic properties of the intermediate, and the dimensions of the physical system

The trend of results of cytochemical investigation is the finding of considerable localization of certain enzymatic agents within specific parts of the cell[5]. It is therefore of interest that the aforementioned relationships should analogously apply to an enzyme system They should give us information concerning separation of participating components within such enzymatic apparatus, and therefore should also define a maximum feasible biological cell size

The Model and Critical Relationships

A simple first-order treatment of a sequential reaction step

$$A_{i-1} \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} A_i \xrightarrow{k_2} A_{i+1} \qquad (1)$$

leads to the reaction-rate

$$\mathrm{d}N/\mathrm{d}t = [A_{i-1}]\,k''\,,\ k'' = k_2\,[1/K + k_2/k_1]^{-1} \qquad (2)$$

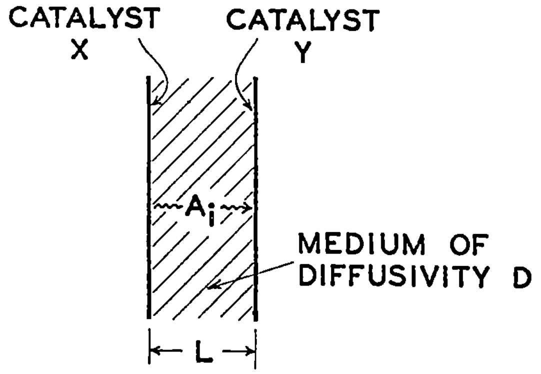

Fig 1 Reaction $A_{i-1} \overset{\text{at } X}{\rightleftarrows} A_i \overset{\text{at } Y}{\longrightarrow} A_{i+1}$

wherein $K = k_1/k_1' = [A_{i,\mathrm{eq}}]/[A_{i-1}]$ is the equilibrium constant characteristic of the formation of the intermediate A_i This rate is seen to be unlimited in magnitude provided the rate constants k_1 and k_2 are large enough, regardless of how unfavourable the $A_{i-1} \rightarrow A_i$ might be thermodynamically Now let us physically separate the sites on which $A_{i-1} \rightarrow A_i$ is catalysed (sites X) from those catalysing $A_i \rightarrow A_{i+1}$ (sites Y) (Fig 1) Here the concentration of the A_i species at X, $[A_i]_X$, will generally not be equal to that of $[A_i]_Y$ at the surface Y Instead, the two will be related to the overall reaction-rate through the diffusion equation

$$(\mathrm{d}N/\mathrm{d}t)_s = \frac{D}{L}\,([A_i]_X - [A_i]_Y) \qquad (3)$$

wherein $(\mathrm{d}N/\mathrm{d}t)_s$ is the overall rate of reaction per unit surface area, since in the steady state the rate of reaction for each sequential step has to equal the overall rate. When this is taken into account[5] in the kinetic derivation we obtain, for the rate of the system

$$(\mathrm{d}N/\mathrm{d}t)_s = [A_{i-1}]\,k''\,(1 + k''\,L/KD)^{-1} \qquad (4)$$

in place of equation (2). D is the diffusion constant for intermediate molecules A_i in the medium between X and Y, and L is the characteristic distance of separation between X and Y. With $k'' \rightarrow \infty$ the rate is seen to go to an absolute ceiling value of

$$(\mathrm{d}N/\mathrm{d}t)_{s,\ \max} = [A_{i,\mathrm{eq}}]D/L \qquad (5)$$

A volume element contains $1/L$ surface elements, so that the maximum possible rate per unit volume will be

$$(\mathrm{d}N/\mathrm{d}t) \leq [A_{i,\mathrm{eq}}]D/L^2 \qquad (6)$$

Thus we have a relationship between the velocity of the overall rate process, $\mathrm{d}N/\mathrm{d}t$, a thermodynamic characteristic $[A_{i,\mathrm{eq}}]$ of a reaction step and the physical properties of the multiple catalyst system, namely, the characteristic distance L between catalytic surfaces and D, the diffusion co-efficient in the intervening medium.

In previous work[4,5] it was shown that a diffusion criterion of the same form arises in a multiple catalyst system of quite another type, as regards the geometry of the model: catalytic sites are distributed throughout finite volumina of space, some of them contain only catalyst X, others only catalyst Y, as pictured by the model in Fig. 2. In this case again, equation (5) defines conditions for negligible diffusion inhibition, that is, for adequate utilization of the available catalysts.

It is well known[6] that the magnitude of calculated diffusion effects is not highly sensitive to the exact geometry of a system so that in any event L can be considered to be a typical length parameter. We may therefore examine the implications of equation (6) for systems where, for example, two different enzymatic materials are separated by a magnitude L across the cytoplasm of a cell, or by a membrane (for example, the mitochondrial membrane) of thickness L, or where one enzyme system may be distributed diffusely over a volume such as a mitochondrion, nucleus, etc., of characteristic size L, and another enzyme is available in the surrounding cytoplasm, and many similar situations.

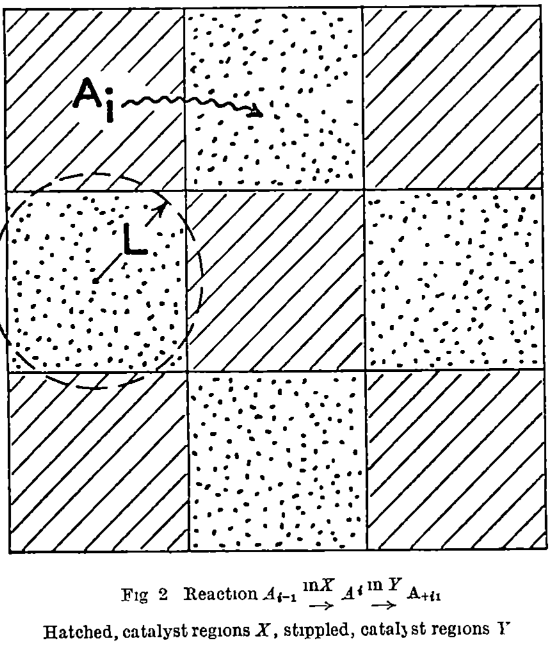

Fig. 2. Reaction $A_{i-1} \xrightarrow{\text{in } X} A_i \xrightarrow{\text{in } Y} A_{i+1}$

Hatched, catalyst regions X; stippled, catalyst regions Y

Cytological Implications

In a reaction sequence of several steps, the condition (6) must be satisfied by each intermediate $[A_i]$. If a number of steps is accomplished by intermediates 'shuttling' back and forth between the same pair of catalytic areas, that is, through the same distance dimension L, then, if for the intermediate existing at the lowest concentration the inequality (6) is satisfied, all other reaction steps will also proceed without difficulty. If various reaction steps are catalysed by various pairs of catalytic areas located at different characteristic distances, then conditions (6) must be satisfied for each pair individually. If we know the concentration of an intermediate involved in a reaction sequence proceeding at a given rate, then (6) will give us a maximum dimension of the apparatus which includes both enzymatic systems involved in its production and conversion.

Cell size. We might now estimate for the processes of cell metabolism the maximum dimension L, that is, a maximum allowable cell size, from the magnitudes of the reaction-rate $\mathrm{d}N/\mathrm{d}t$, and the concentration of the intermediate species $A_{i,\mathrm{eq}}$. We will assume that the actual concentration does approach the attainable equilibrium concentration, that is, that the enzyme catalyst has developed to fairly optimum activity. We may expect the diffusivity to be a magnitude typical for large molecular species in an aqueous medium, that is, $D \approx 10^{-6}$ cm ^{2}/sec.

The metabolic rate of oxygen uptake by various organs, cells, bacteria is of magnitude 10^{-2} c.c. oxygen/min./gm., or $\frac{\mathrm{d}N}{\mathrm{d}t} \sim 10^{-8}$ moles/sec. cm ^{3}. Let us take this as a typical rate magnitude.

We find the following dimensions L for various concentrations of reaction intermediates:

$[A_i]$ $\frac{\text{moles}}{\text{cm}^3}$	10^{-12}	10^{-10}	10^{-8}	10^{-6}	10^{-4}	10^{-2}
L cm	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1

Now, what are typical magnitudes for the concentration of intermediate reacting species in oxygen metabolism? Cell respiration is seriously inhibited by 0.5×10^{-8} moles/c.c. of a poisonous ion, such as cyanide, indicating that some important species exist at a concentration as low as this (see, for example, ref. 7). Furthermore, the concentration of cytochrome c in yeast cells is also found to be of magnitude 10^{-8} moles/c.c. With this magnitude, and assuming that the concentration of an enzyme specific for a given reaction will not greatly exceed in magnitude the concentration of the species to be reacted, we obtain for the maximum allowable dimension

$$L \sim 10^{-3} \text{ cm}$$

which is indeed of the order of actual cell dimensions, that is, from about 5×10^{-4} to 5×10^{-3} cm.

Equation (6) contains the quotient $\frac{dN}{dt}\frac{1}{[A_{i,eq}]}$ This allows us to use figures for reaction-rate of unit volume of matter (such as tissue) although the material may include not only the enzymatic apparatus but also other material which does not participate in the reaction, since the measured concentration is based on the same total volume It is interesting to note, therefore, that measured concentrations among samples may vary by orders of magnitude, but the same critical dimension for the specific enzymatic apparatus will be indicated by an approximately constant ratio of rate-to-concentration An example for this, applicable to oxygen metabolism and cytochrome *c*, is contained in the measurements of Pappenheimer and Hendee[8] for various rat tissues from whose data we have drawn Fig 3

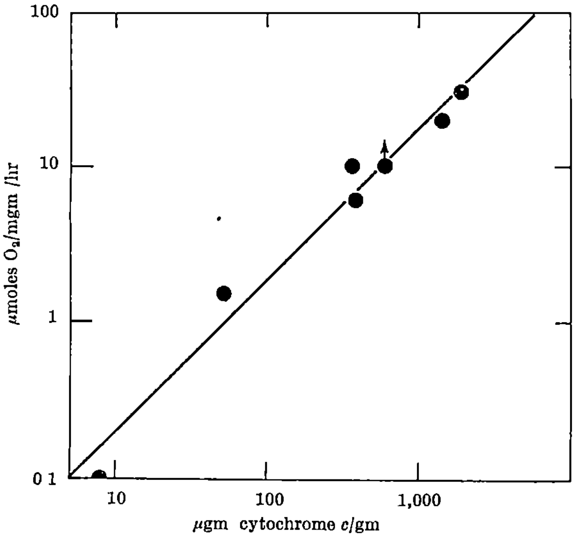

Fig 3

This finding, critically interpreted, establishes only a maximum possible dimension, not necessarily the actual dimension However, the consistent finding of the same order of magnitude rather than a range of magnitudes would strongly suggest that such dimension is in fact involved in the process

This example calculation here also demonstrates that reaction intermediates occurring at lower concentration than 10^{-8} moles/cm ^{3}, and participating in the same overall rate of metabolism, must be processed by catalytic agents physically removed from each other by distances smaller than the cell dimensions, namely, smaller by at least the square root of the concentration ratio

Turnover numbers and dimension of enzymatic apparatus It is difficult to find many specific instances with quantitative information concerning the concentration of intermediates However, the quotient $\frac{dN}{dt}\frac{1}{[A_{i,eq}]}$ can be transformed to the 'turnover number', T

$$T = \frac{60}{n}\frac{dN}{dt}\frac{1}{[A_{i,eq}]},$$

$$n = \frac{\text{molar enzyme concentration}}{\text{molar reactant concentration}}$$

From this and (6) it follows that

$$L \leqq \left(60 \frac{D}{nT}\right)^{1/2}$$

for the maximal distance magnitudes, or, with $D \sim 10^{-6}$ cm ^{2}/sec and n of order of magnitude unity, we have

$$L \leqq 8 \times 10^{-3}/\sqrt{T} \qquad (7)$$

For enzyme reactions characterized by turnover numbers of 10^2 to 10^6, we obtain

$$L \leqq 10^{-3} \qquad 10^{-5} \text{ cm}$$

The maximal value is again typical of actual cellular dimensions, while processes having very high turnover numbers require greater proximity Thus for processes having turnover numbers of the order of 10^6, the maximum characteristic dimension for the participating enzymatic apparatus approaches a value < 1000 Å , that is, a dimension smaller than the entire mitochondrion (See, for example, the active enzymatic regions identified, by electron microscopic methods[9], on the cristæ mitochondriales)

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PRESENCE OF SELENIUM-75 IN LIPOPROTEINS

By DR K P. McCONNELL and DR R S. LEVY
Radioisotope Service, Veterans Administration Hospital, and Department of Biochemistry,
University of Louisville School of Medicine, Louisville

IT has been shown[1] that selenium is as effective as vitamin E in preventing dietary liver necrosis in the rat, multiple necrotic degeneration in the mouse, and exudative diathesis in the chick Since 74 per cent of the serum vitamin E is carried by the serum lipoproteins in the human being[2], it would appear to be of interest to determine if selenium is carried by the serum lipoproteins In the first investigations[3] it was shown that in serum proteins from dogs injected 24 h earlier with selenium-75 and separated

Table 1 INCORPORATION OF SELENIUM-75 INTO SERUM LIPOPROTEINS*

Animal	Total ^{75}Se	Serum Protein-bound selenium		$\alpha\beta$-Lipoprotein fraction: Total activity fraction (A)	Total residual fraction (B)	^{75}Se in fraction	P-B Se† in fraction	β-Lipoprotein fraction: Total activity fraction (C)	Total residual fraction (D)	^{75}Se in fraction	P-B Se in fraction
	cpm × 10^5/ml	cpm × 10^5	per cent	cpm × 10^4	cpm × 10^5	$\frac{A}{A+B}$ (per cent)	per cent	cpm × 10^4	cpm × 10^5	$\frac{C}{C+D}$ (per cent)	per cent
Dog 14	4 21	4 09	97 2	3 30	22 12	1 47	88 4	1 04	21 60	0 48	44 4
	4 27	4 07	95 3	3 93	19 59	1 97	91 3	0 84	22 23	0 37	43 2
	4 01	3 76	93 8	4 90	22 16	2 03	89 6	0 82	21 85	0 37	42 3
	3 97	3 74	94 1	5 39	22 01	2 39	90 4	0 89	22 22	0 40	42 1
Average dog 14	4 13	3 97	95 1	4 33	21 47	1 97	89 9	0 90	21 98	0 48	43 0
Average dog 13	0 45	0 44	97 8					0 911	21 54	0 42	43 9
Average dog 12	0 56	0 55	98 2	1 09	3 00	3 40	91 3	0 047	3 54	0 13	—
Average dog 11	—	—	—	0 06	0 16	3 93	—	0 021	0 14	1 47	—
Average dog 10	0 42	0 38	90 4	1 30	2 45	5 00	—	0 117	2 71	0 41	—
Average rat 11	—	—	—	22 6	19 26	10 57	—	11 9	20 22	5 55	—
Average rat 12	2 88	2 71	94 1	14 1	45 75	2 99	90 3	—	—	—	—
Average rat 13	—	—	—	5 7	5 08	12 94	—	21 3	48 77	4 18	—

* In each case, except dog 14, average values are given In dog 14, a series of experiments has been listed to show the consistency of the method

† Protein-bound selenium by the trichloroacetic acid method

by paper electrophoresis, 60 per cent of the paper's radioactivity was equally distributed between the α-2 and β-1 globulins An additional 10 per cent was found in the albumin, β-2 and γ-globulin fractions In later time-distribution investigations[4] selenium was shown to be bound principally to albumin during the first hour, then later transferred and principally bound to specific globulin fractions, namely, α-2 and β-1 Since blood lipids are chiefly associated with the α- and β-globulin fractions, experiments were carried out on a series of dogs and rats in order to determine whether or not selenium was bound specifically to serum lipoproteins to the same degree as vitamin E by estimating the relative concentration of selenium in the various lipoproteins Apparently, both the α- and β-lipoproteins bind selenium-75 with the greater percentage in the α-lipoproteins The presence of selenium in lipoproteins, even though to a much lesser extent than vitamin E, raises the question as to the significance of this trace element in serum lipoproteins Normal dogs, about 20 kg, were injected subcutaneously with $H_2{}^{75}SeO_3$ in dilute hydrochloric acid which contained 1 mc selenium-75 (2 mg selenium) Male rats weighing 100–200 g were injected subcutaneously with a single dose of $H_2{}^{75}SeO_3$ in dilute hydrochloric acid containing approximately 0 2 mc of radioselenium and 40 μg selenium After 24 h whole blood was obtained, allowed to clot at room temperature, and the serum isolated by centrifugation

The lipoproteins were isolated by ultracentrifugation according to the method of DeLalla and Gofman[5] To recover the β-lipoprotein 6 66 ml of the serum were mixed at room temperature with 5 34 ml of a sodium chloride diluent having a density of 1 1364 g/ml The final mixture, with a density of 1 0689 g/ml at 26°, was centrifuged in a Spinco model *LH* preparative ultracentrifuge at 92,660*g* for 22 h Under these conditions, the lipoproteins having hydrated densities of 1 0689 g/ml or less float to the top of the centrifuge tube Paper electrophoresis shows that this fraction is free from albumin Using the Spinco tube slicer the top 2 ml, which contained all the β-lipoprotein, were removed For recovering both the α- and β-lipoproteins, 6 ml of the serum were mixed with a diluent having a density of 1 3884 g/ml (to give a final mixture with a density of 1 2046 g/ml at 26°) and centrifuged at 92,660*g* for 22 h Again, using the Spinco tube slicer the top 2 ml, which contains all the lipoproteins having a hydrated density of 1 2046 g/ml or less, were removed

Electrophoretic analyses were performed in a Spinco model *R* paper electrophoresis apparatus Separation of serum samples was carried out for 16 h at 2 5 m amp in veronal buffer, ionic strength 0 05, *p*H 8 6 in Whatman 3 *MM* paper strips The strips were then oven-dried at 120°–130° for 30 min and afterwards dyed with either bromphenol blue or oil-red-*O* The Spinco Analytrol recording densitometer and automatic integrator was used to evaluate the relative amounts of proteins in the stained material separated electrophoretically Protein-bound selenium was determined by the trichloroacetic acid method in which 20 per cent trichloroacetic acid was used as the protein precipitant, and the precipitate was washed twice with 10 per cent trichloroacetic acid The assay of selenium-75 was carried out in a well-type Berkeley scintillation detector[4]

In preliminary work the lipoproteins from serum were located on paper electrophoretic strips prior to counting by staining with oil-red-*O* Since there is considerable overlapping of serum lipoproteins with other serum proteins with this method, it was abandoned in favour of isolating the lipoprotein themselves by ultracentrifugation Under these conditions the radioactivity could be detected directly in a scintillation counter, with paper electrophoresis being used only as a method to determine the degree of homogeneity of the isolated lipoproteins

The results (Table 1) show that a small but significant percentage of the total serum protein-bound selenium is bound to lipoprotein Both the α- and β-lipoproteins of dog and rat serum bind selenium-75, with the greater percentage in the α-fraction In the dog, 3 58 per cent of the total serum selenium-75 is bound to the lipoproteins of which 2 23 per cent of the total serum selenium-75 is found in the α-lipoprotein and 0 58 per cent is found in the β- or α-2-lipoprotein In the rat 8 83 per cent of the total serum selenium-75 is bound to lipoproteins, of which 5 74 per cent of the total is found in the α-lipoprotein and 4 86 is found in the β-lipoprotein

Comparing the concentration of selenium and vitamin *E* in lipoproteins, we find striking differences in the concentration of these two substances For example, Lewis *et al* [2] showed that 74 per cent of the total serum tocopherol was bound to the lipoproteins in the human Of this, 54 per cent was bound to the α-lipoprotein and 20 per cent to the β-lipoprotein Investigations indicate that vitamin E and selenium have related antioxidant properties[6–9] In vitamin E deficiency peroxidation is widespread and is particularly damaging to the mitochondria and microsomes of the cells where lipid-free radical intermediates react at random with structural and functional proteins, lipids and other compounds The working hypothesis for vitamin E function is that it inhibits *in vivo* lipid peroxidation by reacting with free radical intermediates and with lipid peroxides Tappel *et al* [9] believe that the partial replacement of vitamin E by selenium compounds can occur in some animals because McConnell *et al* [10] have shown that selenium is incorporated into tissue proteins found in the mitochondria and microsomes where, because of their antioxygenic activity, they protect other compounds against *in vivo* peroxidation

Bieri[7] and Zalkin *et al* [9] have observed that dietary selenite, like vitamin E, inhibits *in vivo* lipid peroxidation in liver, heart and skeletal muscles of chickens on a vitamin E deficient ration Tissue antioxidant activity following administration of small amounts of selenium, cystine and vitamin E provides a possible explanation for the similar biological effects of these chemically diverse substances[11] Therefore, it is conceivable that in serum lipoproteins selenium compounds may act as antioxidants as they do in the tissues Lindgren *et al* [12], using gas chromatography, demonstrated a relatively high degree of unsaturation in the fatty acids of triglycerides, cholesterol esters and phosphotides

Whether the selenium molecule is bound in such a way as to be available as an antioxidant will be investigated at a later date Future work is being contemplated to determine whether the selenium was taken up by the protein or the lipid moiety or both

Investigations were carried out on dogs and rats to determine whether selenium was bound to serum lipoproteins A small fraction of the total protein-bound selenium was found in lipoproteins Both the α- and β-lipoproteins bind selenium-75 with the greater percentage in the α-lipoproteins A possible significance of selenium in serum lipoproteins is discussed

This work was supported in part by grants *A*-4445 from the National Institute of Arthritis and Metabolic Diseases (K P M) and *H*-3985 from the National Heart Institute (R S L), National Institutes of Health, U S Public Health Service

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IN VIVO INDUCED OXIDATION BY ADRENOCORTICOTROPHIC HORMONE OF REDUCED PYRIDINE NUCLEOTIDE IN THE ADRENAL CORTEX OF HYPOPHYSECTOMIZED RATS

By Dr. BRITTON, CHANCE, B. SCHOENER and Dr J J FERGUSON, jun

Johnson Research Foundation and Department of Biochemistry, University of Pennsylvania, School of Medicine, Philadelphia

METHODS have been described for measuring the fluorescence intensity of an 18-μ area of tissues in anæsthetized rats[1,2] More recently a method has been improved so as to permit the recording of fluorescence emission spectra from a 30-μ area[3]

In this article we describe a large decrease of fluorescence of the adrenal gland of hypophysectomized rats following the injection of a small amount of adrenocorticotrophic hormone (ACTH) into the vena cava of the anæsthetized animal The emission spectra corresponding to the change, the kinetics of the change, its sensitivity to the concentration of ACTH, and the concentration of ACTH giving half-maximal effects are described These results are correlated with independent chemical studies of corticosteroid secretion from the adrenal gland

The experimental methods are identical to those described elsewhere[1-4] In this series of experiments a 30-μ aperture was used In those experiments not requiring a recording of spectra, a broad secondary filter peaking at 450 mμ and giving half-maximal response of 400–500 mμ was used[4]

The rats were anæsthetized with 1 gm /kgm urethane, the adrenal cortex was cleared of fat The kidney and the adrenal were held very nearly motionless on a small platform The microscope was focused on the surface of the exposed adrenal gland, close to, but not coincident with, the region where the larger blood vessels are located The field appeared to be uniformly and brightly fluorescent in the hypophysectomized animals

As described previously, fluorescence intensity is recorded as a function of time or wave-length[3] The

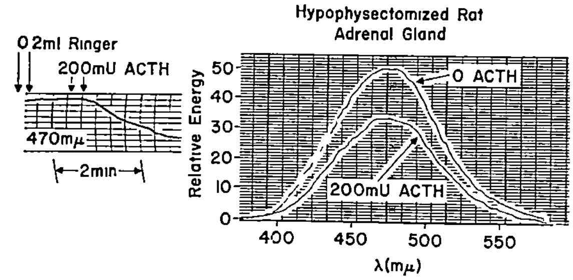

Fig 1 Emission spectra obtained with microspectrofluorometer focused on adrenal cortex of hypophysectomized rat (urethane anæsthesia) 366 mμ excitation, 30-μ observation aperture The upper spectrum represents the fluorescence emission with the rat breathing oxygen and prior to the injection of ACTH The inset on the left represents the time-course of changes following the injection of 0 2 ml Ringer solution and then 200 mU ACTH into the vena cava The decrease of fluorescence is indicated by a downward deflexion The spectrum labelled 200 mU ACTH is recorded after the effects of ACTH have reached a plateau The energy distribution is referred to a cæsium antimony photosurface and does not represent the absolute distribution of energy

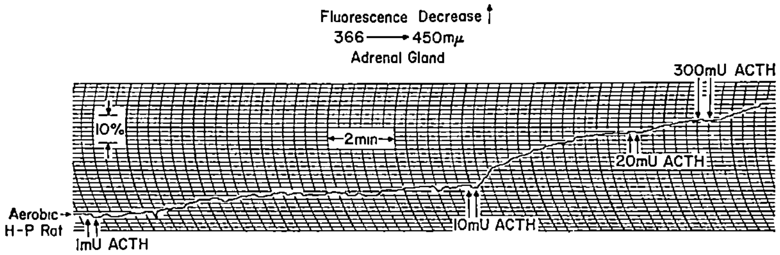

Fig. 2. Time-course of the effect of low concentrations of ACTH. Here the microfluorometer is used at a fixed wave-length with a broad-band secondary filter with a maximum at 450 mμ. The record shows the effects of 1, 10, 20 and 300 mU of ACTH on the fluorescence emission of the rat adrenal gland. Other experimental conditions similar to those of Fig. 1. A fluorescence decrease in this trace is indicated as an upward deflexion. Time proceeds from left to right

intensity at 470 mμ is recorded first as a function of time in order to ensure that experimental conditions are stable. Then, the wave-length drive is engaged and a spectrum is recorded in the region 400–600 mμ. The wave-length is reset to 470 mμ and ACTH is injected. After a plateau is reached, the spectrum is again recorded (Fig. 1).

Hypophysectomized male Sprague–Dawley rats weighing about 200 gm were obtained from Hormone Assay Laboratories, Inc., Chicago, Illinois, and were used 2–4 days after hypophysectomy. Hydrocortisone ('Cortef', Upjohn), 1 mgm, was injected intraperitoneally prior to adrenal exposure.

Commercial ACTH (Armour Laboratories 'Corticotropin') was used, and the manufacturer's batch assay data were utilized. The lot used was stated to contain 20 mU 'Pressor Material' per unit of ACTH. Independent controls showed 30 mU pitressin caused increased reduction of pyridine nucleotide. Thus, the observation of oxidation of pyridine nucleotide could not likely be due to the pressor content of ACTH.

Spectrum of the fluorescence change caused by ACTH. Fig. 1 represents an emission spectrum of the rat adrenal gland indicating the distribution of fluorescence energy prior to the injection of ACTH. The peak of the emission spectrum lies at approximately 475 mμ. Following the recording of the spectrum the monochromator was set back to 470 mμ and the time recording is shown on the left-hand side of the diagram. (Time proceeds from left to right.) Injection of 0·2 ml. Ringer's causes no measurable effect, but almost as soon as the injection of 200 mU of ACTH in 0·2 ml. Ringer's is complete, a downward deflexion of the trace occurs, which approaches completion in about 1·5 min. (A downward deflexion of the trace in this particular record corresponds to a diminution of fluorescence.) At this time, a second spectrum is run (200 mU ACTH), and it is seen that the fluorescence intensity has the same maximum but the intensity has diminished about 40 per cent.

Time-course of ACTH effect. In addition to the insert in Fig. 1, which indicates the rapid response to a high concentration of ACTH, we include in Fig. 2 the time-course of the response to small concentrations of ACTH. The experimental conditions are similar to those of Fig. 1, except that the monochromator for the fluorometer is not used and the secondary filter already described here is utilized. Time proceeds from left to right in the trace, and a fluorescence decrease corresponds to an upward deflexion. The experimental record begins with the hypophysectomized rat breathing oxygen. Double arrows indicate the initiation and completion of the injection of 1 mU ACTH into the vena cava. After approximately 1 min., the trace clearly shows an upward deflexion which reaches its half-maximal level in approximately 2 min. after the injection. After the plateau value has been established, 10 mU ACTH is injected and the trace deflects upwards at a much more rapid rate, the time for half-maximal effect being approximately 1 min., with the plateau value being established in approximately 5 min. Injections of 20 and 300 mU ACTH cause only small further changes.

ACTH concentration required for half-maximal effect. Although the concentration of ACTH giving half-maximal effect varies somewhat from preparation to preparation, more sensitive responses are obtained during the few days after hypophysectomy, and such a result is indicated in Fig. 3. Here, we have plotted the decrease in fluorescence as a percentage of the initial value against the logarithm of the ACTH concentration and find that half-maximal effect is obtained at 1·0 mU of ACTH. It is apparent from this curve that 0·1 mU could be detected, and this has been confirmed in other experiments. [*Note added in proof.* Drs. D. H. Nelson and G. F. Cahill have indicated in personal communications that a greater sensitivity is obtained a few hours after hypophysectomy. Recent experiments at 24 h. after hypophysectomy show half-maximal effect at 0·3 mU with 0·02 mU giving a detectable response.]

Correlation with corticosteroid production. Data from other laboratories on corticosteroid production in rat adrenals indicate that 0·02 mU can be detected and routine assays are carried out in the range 0·04–0·64 mU (ref. 5). The rate of corticosteroid production increases with the ACTH concentration at least up to 0·64 mU (ref. 5) or to 1 mU (ref. 6). Fig. 4 compares the time-course of the fluorescence decrease observed

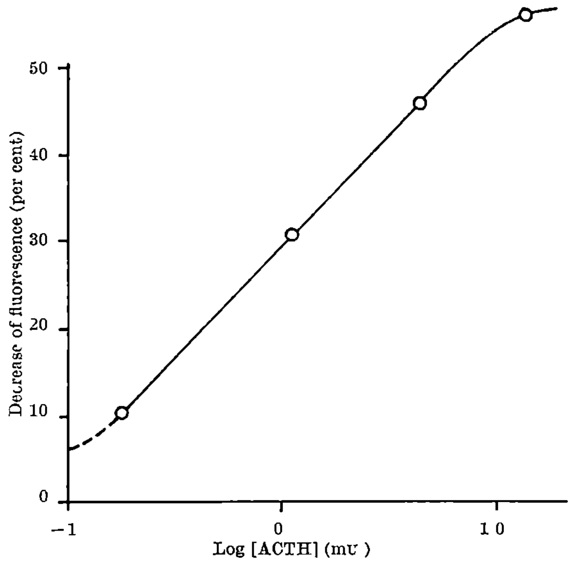

Fig. 3. Fluorescence response of the adrenal gland of the hypophysectomized rat to ACTH concentration. The values of the abscissa represent the logarithm of the total milliunits of ACTH injected and the ordinates represent the percentage decrease of the fluorescence prior to the injection of ACTH. Other experimental conditions similar to those of Fig. 2

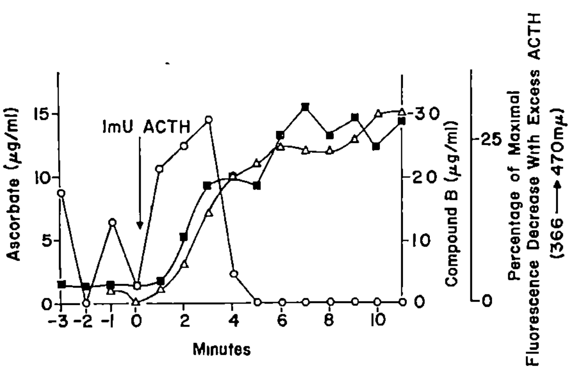

Fig 4 Correlation of adrenal fluorescence decrease and chemical assays of Lipscomb and Nelson (ref 6) for ascorbate and compound *B* released into adrenal vein caused by the injection of 1 mU of ACTH into the hypophysectomized rat
○, Ascorbate (Lipscomb and Nelson), ■, compound *B* (Lipscomb and Nelson), *Δ*, adrenal pyridine nucleotide

in these experiments with the time-course of compound *B* and ascorbate formation found by Lipscomb and Nelson[6] Assuming that the experiments on the two different animals are comparable, the correlation between the fluorescence decrease and the formation of compound *B* is as striking as is the lack of correlation with the formation of ascorbate However, simultaneous measurements on a number of animals are highly desirable in order to confirm this correlation

The change of the fluorescence emission spectrum caused by ACTH injection identifies the oxidation of reduced pyridine nucleotide as a primary biochemical response of the adrenal cortex *in vivo* A number of alternatives need to be considered in determining the biochemical origin of the changes First, this change resembles that observed in a resting-active transition of isolated mitochondria, which corresponds to an oxidation of reduced pyridine nucleotides of approximately the same fraction of the total (50 per cent)[7,8]

Note added in proof The histochemical studies of Greenberg and Glick[9] show a large increase of oxidized pyridine nucleotide (~ 40 per cent) in the glomerulosa, in agreement with our data However, they fail to find a statistically significant change of reduced pyridine nucleotide which is to be expected on the basis of our data Apparently more detailed histochemical assays for reduced pyridine nucleotide are desirable

However, the special role of triphosphopyridine nucleotide (TPN) must be considered Post-mortem chemical assays show a relatively small TPN concentration of this gland[10] Assuming that the post-mortem analytical results have validity *in vivo*, the low TPNH concentration in the normal animal (ACTH causes no further oxidation in our experiments with normal rats) indicates that TPNH was not the material oxidized in the experiments of Figs 1 and 2 The large concentration of diphosphopyridine nucleotide (DPN) in the normal animal indicates that oxidation of DPNH is consistent with the experimental observation *in vivo* (Assays based on quick freezing of rat liver[11] show the TPNH/TPN and DPN/DPNH ratios are higher *in vivo* than post-mortem) Present theories suggest that the prime effect of ACTH is on glycogen breakdown and the formation of TPNH by glucose-6-phosphate dehydrogenase, the initial effect of such a mechanism would be a reduction of TPN (ref 11) It is clear that if this effect occurs, it is too small to be observed in our studies In these experiments we observe the net oxidation of reduced pyridine nucleotide, which, of course, does not rule out simultaneous reduction of TPN at glucose-6-phosphate dehydrogenase and oxidation of TPNH and DPNH elsewhere in the cytoplasm and mitochondria Whether or not this net oxidation is due to increased intracellular ADP and phosphate-levels at the mitochondria reflecting increased energy requirements in the adrenal gland or to oxidation of TPNH in steroid hydroxylation in the cytoplasm cannot be resolved at the present time

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ULTRASONIC RELAXATIONS IN AQUEOUS SOLUTIONS OF ALIPHATIC AMINES

By J F McKELLAR* and J H ANDREAE†
Akers Research Laboratories, Imperial Chemical Industries, Ltd , Welwyn, Herts

A NUMBER of aqueous solutions of non-electrolytes exhibit a maximum in their 'excess' sound absorption at intermediate concentrations Several workers[1-3] have given explanations for this effect In particular, Schneider[4] has put forward an interesting explanation for the alcohol–water mixtures This involves perturbation by the sound field of the equilibrium

$$AA + BB \rightleftharpoons 2AB \qquad (1)$$

where $AA \equiv$ hydrogen bonded solute molecules, $BB \equiv$ hydrogen bonded water molecules and $AB \equiv$ hydrogen bonded complex between unlike molecules

Barfield and Schneider[5] have interpreted the 'excess' absorption in diethylamine–water in terms of this mechanism

We have found that, for a number of aqueous solutions of non-electrolytes, the 'excess' absorption gives rise to relaxations at frequencies above about 20 Mc/s An account of the relaxations obtained with the amines diethylamine, triethylamine, methyldiethylamine and *n*-amylamine will be the subject of this article

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* Now at Imperial Chemical Industries, Ltd , Dyestuffs Division, Manchester 9

† Now at Standard Telecommunications Laboratories, Ltd , London Road, Harlow, Essex

The ultrasonic techniques have been described[6] The frequencies used were 1 5, 4 5, 7 5, 13 5, 30, 70, 110 170 and 230 Mc/s Sound velocity measurements were made with an acoustic interferometer operating at 2 Mc/s

For the primary and secondary amines, the measurements α/f^2 were described by the relation for a single relaxation

$$\frac{\alpha}{f^2} = \frac{A}{1 + (f/f_c)^2} + B \qquad (2)$$

where α is the absorption coefficient, f is the frequency of measurement, A and B are constants and f_c is the relaxation frequency Values of $\mu_{\max}$, the maximum absorption per wave-length, defined by

$$\mu_{\max} = \tfrac{1}{2}A\ f_c\ C \qquad (3)$$

were calculated C is the sound velocity

For diethylamine-water the results extend the measurements of Barfield and Schneider[5] into a region of relaxation at higher frequencies, and cover the concentration range over which the excess absorption is readily measured The variation of $\mu_{\max}$ with concentration is shown in Fig 1 The variation of $\mu_{\max}$ and f_c with temperature at a mole fraction of 0 1 diethylamine is shown in Fig 2*a* The temperature dependence of $\mu_{\max}$ and f_c for *n*-amylamine–water is similar to that found with diethylamine For methyldiethylamine-water the results are of particular interest and require further explanation

Previous workers[2,7] have shown that, within the frequency-range 0 6–52 3 Mc/s, α/f^2 at intermediate concentrations of triethylamine–water, decreases rapidly with increasing frequency Further, it was found that α/f^2, measured at a constant frequency, increases rapidly with temperature until the lower critical solution temperature (18° C) of the mixture is reached This dependence on temperature of α/f^2 is quite distinct from that found with diethylamine–water[5] (and from the corresponding behaviour of $\mu_{\max}$ shown in Fig 2*a*)

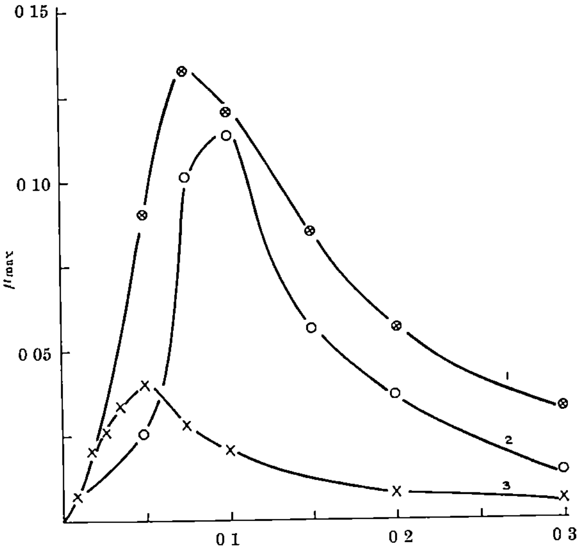


Fig 1 Variation of $\mu_{\max}$ with concentration (1) methyldiethylamine + water (0 0 °C), (2) diethylamine + water (0 0 °C), (3) *n*-amylamine + water (5 0 C)

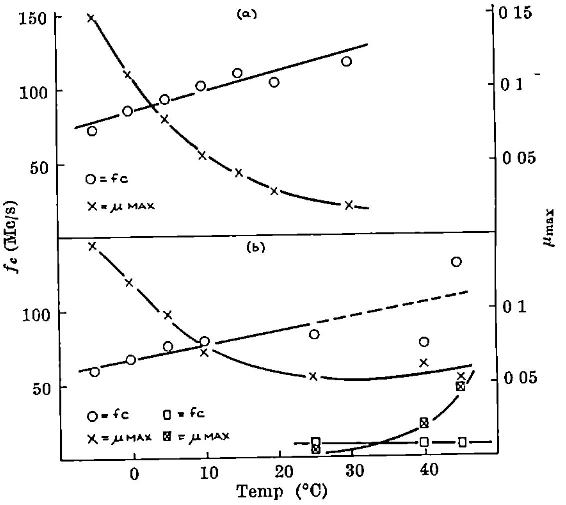


Fig 2 Variation of $\mu_{\max}$ and f_c with temperature (*a*) mole fraction of diethylamine = 0 1, (*b*) mole fraction of methyldiethylamine = 0 1

Measurements were taken in triethylamine–water at 0° C and 15° C at frequencies between 1 5 and 230 Mc/s in order to cover the frequency range in which the single relaxational behaviour is obtained with the other amines Analysis of the results showed that they could not be described satisfactorily by (2) Instead, they were described approximately by the corresponding relation for a double relaxation

$$\frac{\alpha}{f^2} = \frac{A_1}{1 + (f/f_{c2})^2} + \frac{A_2}{1 + (f/f_{c1})^2} + B \qquad (4)$$

The values for A_1, A_2, B, f_{c1} and f_{c2} are given in Table 1

Table 1 CONSTANTS FROM DOUBLE RELAXATION ANALYSIS

Mole fract amine	Temp (°C)	A_1	A_2	B	f_{c1} (Mc/s)	f_{c2} (Mc/s)
		(cm^{-1} sec^2 × 10^{-17})				
		(1)				
0 125	15	24,270	3,770	209	3 9	45
	0 0	4,936	1,993	284	9 7	62
		(2)				
0 10	45	10,760	486	110	6 3	138
	40	3,649	1,138	175	7 9	75
	25	873	884	184	7 2	81

(1) Triethylamine, (2) methyldiethylamine

It is seen that the higher frequency relaxation occurs at frequencies similar to those obtained with the other amines Also, the close proximity of the lower critical solution temperature gives rise to a second relaxation at lower frequencies This makes analysis of the relaxations difficult and prevents a satisfactory examination of their behaviour with temperature It became apparent that, to accomplish this, a tertiary amine was required the aqueous solution of which would have a fairly high lower critical solution temperature so that the higher frequency relaxation in particular could be studied over a fairly wide temperature and concentration-range The system methyldiethylamine–water was chosen (lower critical solution temperature = 49° C (ref 8))

At 0° C, methyldiethylamine–water shows only a single relaxation with relaxation frequency greater than 30 Mc/s The concentration dependence of $\mu_{\max}$ derived from this relaxation is shown in Fig 1 and is seen to be similar to that obtained with the other amines At temperatures above 10° C, however, it was found that the relaxational behaviour could not be described by equation (2) but could be described approximately by equation (4) This situation is therefore similar to that found with triethylamine–water and the constants for the relaxations are also given in Table 1. The variation of μ_{max} and f_c with temperature is shown in Fig 2b for the two relaxations The similarity between the higher frequency relaxation and the relaxation in diethylamine–water is apparent

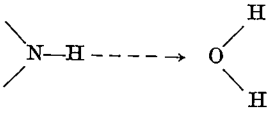
The foregoing results, for aqueous solutions of aliphatic amines, demonstrate the existence of two types of relaxation in the frequency range 1 5–230 Mc/s Possible mechanisms for these relaxations will be considered briefly

The first, which occurs at frequencies less than 10 Mc/s, is only observed when the temperature is close to the lower critical solution temperature of the mixture This suggests that some phase separation process is responsible Brown and Richardson[9], however, have shown that considerable sound scattering occurs in binary mixtures when these are at temperatures close to their consolute temperature In addition, our measurements, which terminate at 1 5 Mc/s, do not cover the whole region of the lower frequency relaxation In view of these factors, therefore, further analysis of the sound absorption data for this relaxation is likely to be difficult and we shall not consider this relaxation further

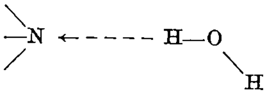
The second, which occurs at frequencies greater than 30 Mc/s, is observed with primary, secondary and tertiary amines This suggests that the mechanism responsible for the relaxation is one which is common to the three types of amine Following Schneider[4], we shall consider that the relaxation is due to perturbation of an equilibrium involving hydrogen bonding between unlike molecules However, the observations with the tertiary amines, for which the majority existence of the amine in the form of hydrogen-bonded dimers seems unlikely[10], causes us to reject the equilibrium (1) in favour of the simpler

$$A + B \rightleftharpoons AB \qquad (5)$$

In the case of the primary and secondary amines, hydrogen bonding in the complex AB can occur in two possible ways, shown diagrammatically by



(a)



(b)

The observations with the tertiary amines further suggests that the hydrogen bonding of the complex AB occurs by (b)

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EFFECT OF CERTAIN PHENOLIC ACIDS ON THE ELONGATION OF *AVENA* FIRST INTERNODES IN THE PRESENCE OF AUXINS AND TRYPTOPHAN

By J. H M HENDERSON* and J P NITSCH

Le Phytotron, Gif-sur-Yvette (Seine-et-Oise)

SEVERAL reports in the literature[1–5] have pointed out the synergistic role certain phenolic compounds can have in auxin growth responses It has been demonstrated that such compounds can interfere with the metabolism of 3-indolylacetic acid (IAA) (ref 6) Some of these reduce its destruction by IAA-oxidase polyphenols, especially ortho-diphenols such as caffeic and chlorogenic acids[7–9]. Others enhance IAA destruction and act as co-factors of IAA oxidase monophenols such as *p*-coumaric acid[10,11] In addition, certain phenols have been shown to increase the yield of IAA produced from tryptophan[12]

In the first internode test, numerous compounds act synergistically (that is, they promote more than an additive response) with IAA (ref 13) This effect could be due to the sparing of IAA or to the formation of a complex biologically more active than IAA. In order to elucidate this point, the following experiments have been made in regard to the first of these possibilities

(I) Auxin Experiments

(1) *Differential response to IAA, NAA and* 2,4-*D* Experiments were conducted in the same manner as described by Nitsch and Nitsch[13] for the *Avena* first internode test Concentrations of IAA ranging from 10^{-8} to 10^{-5} M were used Chlorogenic acid was used as the synergist at the concentration (10^{-4} M) shown to be most effective in a previous investigation[3] These results are shown in Fig 1 The lower growth at 10^{-5} M IAA was probably due to inhibiting concentration of IAA Similar results were obtained with coleoptiles The results confirm the previous reports that chlorogenic acid acts synergistically with IAA in promoting the elongation of oat first internode sections

Similar tests were made with two other auxins, 1-naphthylacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), at the same molar concentrations as IAA The results are summarized in Table 1 These experiments have been repeated several times In no case was the elongation produced by 2,4-D affected by chlorogenic acid, whereas on two occasions

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* Present address Carver Foundation and Department of Biology, Tuskegee Institute, Ala , U S A

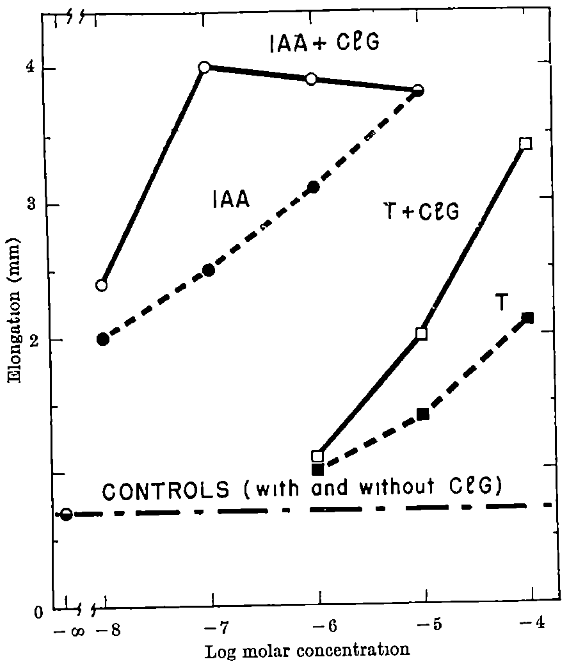

Fig 1 Elongation of *Avena* first internodes (var Brighton) in the presence of various molar concentrations of 3-indolylacetic acid (IAA) and L-tryptophan (T) with (solid lines) and without (dotted lines) chlorogenic acid (ClG) at the concentration of 10^{-4} M Initial length of sections 4 mm growth measured after above 20 h of incubation Each point is the average of two sets of 10 replicates each

the NAA results seemed to show a slight synergism. However, in comparison with the synergism shown by chlorogenic acid with IAA, the results with the other two auxins are negative There is the report in the literature by Reinders-Gouwentak and Smeets[2] that NAA acts as a synergist with ferulic and caffeic acid However, it would be unlikely that NAA could be involved in a system which is concerned with IAA economy through IAA-oxidase or a similar mechanism of destruction

In any event, the general lack of synergism observed with NAA and 2,4-D confirms the earlier results reported for NAA (ref 13), and suggests that the effect of chlorogenic acid is linked to a mechanism which is specific for IAA

(2) *Influence of chlorogenic acid on IAA destruction* In order to answer the question whether chlorogenic acid enhances growth through a sparing mechanism, the IAA remaining after the growth of internode sections was measured Since the IAA concentrations used in the internode test were too low for spectrophotometric detection, the *Avena* curvature test was used For spectrophotometric determinations, higher levels of IAA were used (5–10 μg/c c) The Gordon–Weber reagent[14] was used for the detection of residual IAA The results are given in Table 2

Table 1 EFFECT OF CHLOROGENIC ACID (10^{-4} M) ON THE ELONGATION OF *Avena* FIRST INTERNODE SECTIONS IN THE PRESENCE OF AUXINS

Auxin		Without chlorogenic acid	With chlorogenic acid
IAA	10^{-5} M	30	25*
,,	10^{-6} M	22	43
,,	10^{-7} M	15	31
NAA	10^{-5} M	38	31*
,,	10^{-6} M	22	20
,,	10^{-7} M	9	11
2,4-D	10^{-5} M	36*	29*
,,	10^{-6} M	36	32
,,	10^{-7} M	14	13
Controls		6	7

Initial length of sections, 4 mm, incubation medium, phosphate–citrate buffer, *p*H 5 0 containing sucrose (2 per cent) and 'Tween 80' (0 1 per cent) Readings taken after 20 h of incubation at 25° C

Increased growth of sections in mm (× 10) in one typical experiment

* Growth possibly inhibited by too high a concentration

Table 2 INHIBITION OF IAA DISAPPEARANCE FROM INCUBATING SOLUTIONS BY CHLOROGENIC ACID (ClG)

A *Avena* curvature (Went)

Medium	Degrees curvature	Residual IAA conc μg/l	Per cent
IAA 10^{-5}	0°	0	0
IAA 10^{-6} M + ClG	15°	30	1 7
IAA 10^{-6} M	0°	0	0
IAA 10^{-6} M + ClG	6°	12	6 9
IAA control (1 43 × 10^{-7} M) 25 μg/l	13°	—	—

B Spectral data

No of sections	IAA		IAA + ClG	
	Absorbancy*	Controls (per cent)	Absorbancy	Controls (per cent)
0 (controls)	54	100	30†	100
5	36	67	30	100
10	32	59	23	78
20	15	28	13	45

Avena curvature tests and spectral analysis on IAA remaining in medium after incubation for 20 h at 25° C in the dark (some green light) Chlorogenic acid conc 10^{-4} M Total vol sol , 0 5 c c

* Absorbancy in arbitrary units from linear chart paper of Beckman *DK*-2 spectrophotometer

† This value is lowered 45 per cent by the presence of chlorogenic acid

From the *Avena* curvature results it would appear that chlorogenic acid preserves a small amount of IAA activity at the higher levels From the spectrophotometric results it is obvious that IAA disappears in the presence of internode sections However, when chlorogenic acid is present in the medium, the level of IAA remains higher (Table 2) Chlorogenic acid in the presence of IAA lowers the absorption (about 45 per cent), possibly in the manner suggested by Platt and Thimann[15]

One may conclude that chlorogenic acid does reduce the disappearance of IAA from the solutions in the presence of first-internode sections

(II) Experiments with Tryptophan

The foregoing results strongly suggest that the first internode sections destroy the IAA which has been added to the solution and that this destruction is reduced when chlorogenic acid is added These results parallel those obtained by Rabin and Klein[7] with a preparation of IAA-oxidase Vendrig and Buffel[16] have recently questioned the likelihood that such a mechanism operates *in vivo* In order to elucidate this point, experiments were performed with a precursor which can be transformed into IAA *in vivo*, namely, L-tryptophan

L-tryptophan, at a sufficiently high concentration (around 10^{-5} M), elicits excellent growth of the mesocotyl section[17] During the incubation period several products are formed in the medium (Henderson, unpublished data) One of these substances is presumed to be IAA, for it has the following characteristics (1) it promotes curvature in Went's *Avena* test, (2) it produces a red colour with the Gordon–Weber reagent with maximal absorbancy in the visible range at 525 mμ, (3) 4–5 times more of the substance is formed when L-tryptophan is incubated at *p*H 7 5 than when it is incubated at *p*H 5 0, (4) an ether extract of the medium shows a spot at R_F 0 30 giving the characteristic pink colour with the Gordon–Weber reagent when chromatographed in isopropanol (80) + ammonia (0 1) + water (19 9) Blanks with the sample of L-tryptophan used did not reveal any contamination with IAA before incubation with the sections

The results in Fig 1 show that chlorogenic acid at 10^{-4} M has a synergistic effect with L-tryptophan as it does with IAA

By using the Gordon–Weber reagent it has been shown positively in six out of nine instances that more IAA is present in the medium containing chlorogenic acid + L-tryptophan than in the medium with L-tryptophan alone, despite the inter-reaction between chlorogenic acid and the reagent as pointed out previously (Table 2) One must consider that in the experiments with L-tryptophan both the synthesis and destruction of IAA are occurring simultaneously and continuously The presence of chlorogenic acid should doubly augment the presence of IAA by enhancing its biosynthesis[12] and by inhibiting its destruction[7]

(III) Antagonism between Mono- and Di-Phenols

The results shown here indicate that chlorogenic acid produces synergistic effects with both IAA and tryptophan Similar results can be obtained with caffeic acid, a component of chlorogenic acid (Table 3) If the promotive effect of these *o*-diphenols is mediated via the IAA-oxidase system, then a monophenol such as *p*-coumaric acid should act as an antagonist Coumaric acid, at the concentration of 10^{-4} M, inhibits the promotion of mesocotyl elongation by IAA (ref 13) When added together with chlorogenic or caffeic acids, it nullified the synergistic effect of these compounds (Table 3) Similar results were obtained with L-tryptophan

Table 3 EFFECT OF CHLOROGENIC (ClG), CAFFEIC (CAF) AND *P*-COUMARIC ACIDS (CUM) AT 10^{-4} M ON THE ELONGATION OF *Avena* FIRST INTERNODE SECTIONS IN THE PRESENCE OF IAA OR L-TRYPTOPHAN

	Additives					
	None	ClG	CAF	CUM	ClG + CUM	CAF + CUM
IAA (10^{-7} M)	22	29	30	13	20	14
L-Tryptophan (10^{-4} M)	16	30	30	19	18	16
Controls	4	7	4	5	—	—

Same experimental procedure as in Table 1 Increased growth of sections in mm (× 10)

The results presented here confirm and extend the results showing that *o*-diphenols such as caffeic and chlorogenic acids enhance the growth produced by IAA, but not that produced by 2,4-D and, possibly, NAA They have a sparing action on the IAA present in solution These phenolics exert a similar synergistic effect on tryptophan, which acts as a precursor of IAA Finally, the work presented here indicates that monophenols (such as *p*-coumaric acid), which act as co-factors of IAA oxidase, can completely nullify the effect of phenolic synergists All these results point towards the inhibition of the IAA-oxidase system as the origin of the synergistic action of polyphenolic compounds

We thank Mrs C Nitsch and Mr M Sandmeier, who assisted in various phases of the work One of us (J H M H) also wishes to thank the National Science Foundation for the opportunity to work at the Phytotron through the award of a Science Faculty fellowship for the year 1961–62

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HISTOLOGICAL DISTRIBUTION OF BRAIN GANGLIOSIDES: LYSOSOMES AS GLYCOLIPOPROTEIN GRANULES

By Dr HAROLD KOENIG

Veterans Administration Research Hospital, and Department of Neurology and Psychiatry, Northwestern University Medical School, Chicago

GANGLIOSIDES are acidic, hydrophilic glycosphingolipids that contain *n*-acetylsialic acid Although gangliosides occur in highest concentration in grey matter of brain, they are also present in white matter and seem to exist in other organs Recent observations implicating gangliosides in neural excitability have led to the suggestion that gangliosides are located in the cell membrane of neurones and are involved in ion transport and other aspects of membrane function[1,2] Gangliosides are greatly increased in Tay-Sachs disease, occurring here in granules of distinctive ultrastructure within neuronal cytoplasm[3] Little, however, is known concerning the histological distribution of gangliosides in the normal nervous system

Spherical inclusions are present in neuronal cytoplasm which are periodic acid–Schiff positive[4–6] and stain metachromatically with basic dyes after sulphation[6] These reactions are unaffected by diastase In the course of another investigation[7], I observed that these granules, which also occur in the sparse cytoplasm of glia, and in Schwann, leptomeningeal, adventitial, ependymal and chorioidal epithelial cells, are extracted by brief immersion of fresh nervous tissue in chloroform/methanol (2 1, v/v) at room temperature Formalin-fixation renders these granules resistant to chloroform–methanol and pyridine, even at 60° C for 18 h The inclusions are < 0 2–0 8μ in diameter, occur in dendrites but not axons, and are present in all species thus far examined, namely, rat, cat, dog monkey and man (Fig 1) Neuronal membranes are not stained by the periodic acid–Schiff method No other lipophilic, periodic acid–Schiff-positive constituent is evident in nervous tissue, except myelin lipids and neurokeratin (proteolipid protein)[7]

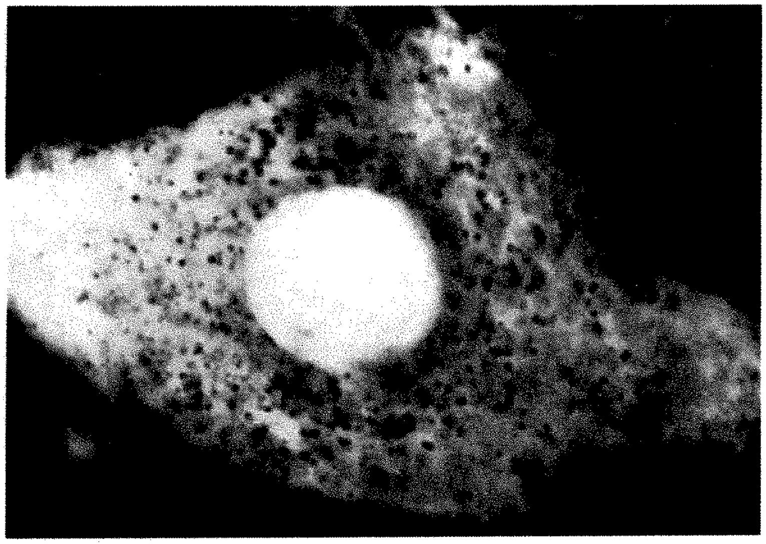

Fig. 1. Motoneurone of cat spinal cord stained by the periodic acid–Schiff method. Frozen section, calcium–formalin fixation. × 1,150

These histochemical properties are characteristic of lipophilic carbohydrate, that is, glycolipid. The lipid insolubility of the carbohydrate moiety after exposure of tissues to protein denaturants indicates that the glycolipid in these granules is complexed with protein. The protein matrix itself, like myelin protein[7], appears to be lipophilic in the undenatured state and thus would contribute to the proteolipid protein fraction[7,8]. Granules with these attributes are therefore designated GLP (glycolipoprotein) granules. Two classes of glycolipids are present in nervous tissue, cerebrosides and gangliosides. Cerebrosides are excluded on the ground that they are myelin sheath constituents, predominate in white matter, and do not survive paraffin-embedding. Gangliosides appear to qualify as the glycolipid moiety of GLP granules on several counts. The acidic character of gangliosides, which derives from *n*-acetylsialic acid, would incline them to form complexes with proteins. It furthermore could confer on GLP granules the negative charge which they seem to bear, judging from the facility with which they are stained intravitally by cationic dyes (*vide infra*). The topographical distribution of gangliosides in brain furthermore resembles that of GLP granules in that both are more plentiful in grey matter than in white matter, a correlation noted earlier[5].

These considerations led me to examine a known ganglioside–protein complex to determine whether it possessed the predicted histochemical properties. Gangliosides ('strandin'), isolated from cat brain by the partition-dialysis method of Folch *et al.*[8], were precipitated from aqueous solution with protamine, a basic protein, and spread on glass slides. The resulting dried films, stained intensely by the periodic acid–Schiff method, displayed strong sulphation-induced metachromasia and resisted extraction by chloroform–methanol. Ganglioside-protaminate thus exhibits the staining and solubility characteristics of GLP granules. Taken together, these observations lead to the conclusion that gangliosides are the glycolipids of GLP inclusions in brain.

Other staining methods appear to stain GLP inclusions. Early workers depicted fuchsiniphil, hæmatoxyliniphil, osmiphil and argentiphil granules in the cytoplasm of neurones ('neurosomes' of Held) and glia ('gliosomes' of Alzheimer) which resemble GLP granules in size, shape and distribution[9,10]. Similar neuronal inclusions are stained by the cationic (basic) dyes, neutral red and methylene blue, applied supravitally or injected *intra vitam*[11]. Baker *et al.*[12] correlated the neutral red inclusions, or 'lipochondria', of neurones with granules which, under appropriate conditions, are stained by heavy metals, acid fuchsin and sudan black B, give positive acid hæmatein and periodic acid–Schiff reactions, and are extracted by hot pyridine. They concluded that lipochondria contain phospholipid and 'cerebroside'. I have found that similar granules can be demonstrated, although inconsistently, by the Gram stain when applied to chromated tissues. There seems little reason to doubt that neuronal and glial granules which are stained by these, and other, methods, and the GLP inclusions discussed here, represent the same cytoplasmic constituents.

Granules with similar tinctorial and solubility characteristics also are present in cells of many other organs, for example, liver, spleen, kidney and intestine[13]. Because the periodic acid–Schiff-positive component of these granules in fixed tissues resists diastase and lipid solvents, it usually is identified as a mucopolysaccharide or mucoprotein[4]. Its extractability from fresh tissues by chloroform–methanol demonstrates clearly, however, that the carbohydrate

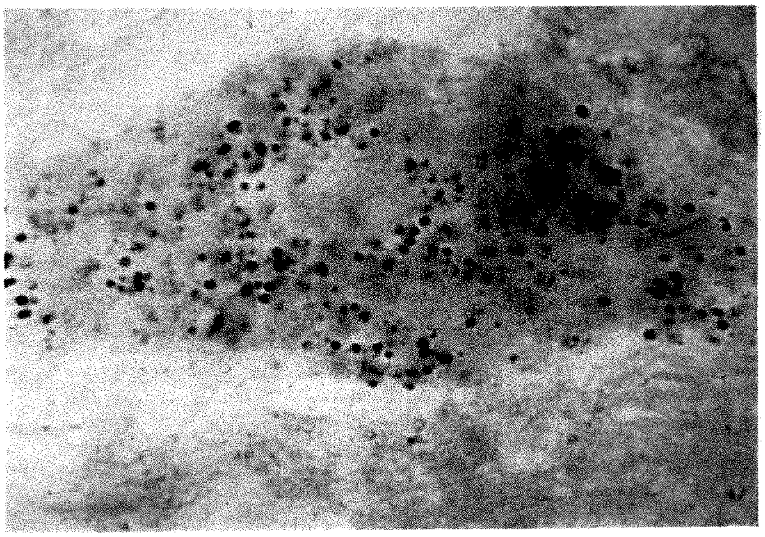

Fig. 2. Motoneurone of cat spinal cord stained for acid phosphatase by Gomori's method. Frozen section, calcium–formalin fixation, × 1,150

moiety is part of a protein-bound glycolipid. The glycolipid may be ganglioside, for gangliosides are present in spleen[14], liver[8] and kidney[15]. These probably differ from brain gangliosides in some respects; thus, they contain considerably less *n*-acyl sialic acid than their counterpart in brain[15]. Even in brain, gangliosides comprise a heterogeneous class of glycolipids[16].

The widespread occurrence of GLP granules suggests that they are a significant constituent of cells in general. It is proposed here that GLP granules are lysosomes, cytoplasmic organelles which contain acid phosphatase, acid deoxyribonuclease and certain other hydrolytic enzymes[17,18]. GLP granules bear a striking morphological resemblance to acid phosphatase granules[19] and acid deoxyribonuclease granules[20] in neurones (Fig. 2). Parallel increases in size and number of GLP granules and acid phosphatase granules have been observed in reactive macroglia and lipid phagocytes in several experimental and spontaneous[21] neural lesions. GLP granules are morphologically similar to acid phosphatase granules in liver, spleen, kidney and small intestine[21]. Acid phosphatase activity has been localized to neutral red inclusions in fibroblasts and astrocytes cultured *in vitro*[22] and to saliva-resistant periodic acid–Schiff-positive inclusions in liver[23]. Acid phosphatase, unlike glycolipid, is displaced or extracted from lysosomes in calcium–formalin fixed tissues by lipid-disrupting procedures[24]. This observation suggests that an appreciable fraction of lysosomal protein may be enzymatically inert. Biochemical studies, furthermore, have revealed a close correlation in the distribution of acid phosphatase and ganglioside-bound *n*-acetylsialic acid in sub-cellular fractions of brain; both constituents occurred in highest concentration in a fraction intermediate between the usual mitochondrial and microsomal fractions[25]. These findings support the inference that GLP granules are lysosomes. They also are consistent with the conclusion presented here that GLP granules in brain contain gangliosides.

The presence of glycolipids in lysosomes excites speculation concerning their role in these organelles. De Duve *et al.*[18] regard lysosomes as inert little bags delimited by a lipoprotein membrane. Their hypothesis ascribes to this membrane the function of restricting the physical freedom and substrate-accessibility of the enclosed enzymes. Release or activation of lysosomal enzymes, according to these workers, results from disruption of the lipoprotein envelope. Ultrastructural studies indicate, however, that although some may be vacuolated, lysosomes are essentially solid organelles[19,26]. Dense inclusion bodies occur in neuronal cytoplasm[27] which probably are lysosomes. Histochemical staining, moreover, indicates that protein-bound glycolipids are distributed throughout lysosomes.

The following hypothesis therefore is offered as an alternative to the membrane hypothesis of de Duve. It is proposed that lysosomes are composed of macromolecules of enzyme and non-enzyme protein conjugated to acidic glycolipids by ionic, covalent, and/or other bonds. The essential inertness of acid hydrolases *in situ* is viewed here as deriving from their sequestration within 'solid' glycolipoprotein complexes. Release or activation of these hydrolases is visualized as an attenuation or cleavage of glycolipoprotein-enzyme bonds, rather than a disruption of a membranous envelope.

It is relevant to inquire whether the action of known releasing agents[17,18] is consistent with this conceptual model of lysosomes. Brain gangliosides, and glycolipids from liver and kidney[15], are soluble in both aqueous and lipid solvents; acid hydrolases are water-soluble. Hypotonic aqueous media, lipid solvents and surface-active agents might reasonably be expected to dissolve lysosomal glycolipid or protein moieties, or both. Freeze-thawing, blenderizing and ultrasonic irradiation bring about a physical fragmentation of lysosomes. The consequent increase in surface area of comminuted particles would promote dissociation or solution of glycolipoprotein complexes. The releasing action of 'thermal activation' and proteases[28] suggests that non-enzyme protein is requisite to the physical integrity of lysosomes and their binding of hydrolases. It also would be anticipated that removal or blockage of the functionally important *n*-acyl sialic acid residue of acidic glycolipids might result in significant release of lysosomal hydrolases. Sialidase, basic proteins and metallic cations are suggested as potential release agents in this category. The results of preliminary experiments support this inference and suggest further that the intravital release of lysosomal enzymes may be responsible for structural and functional disturbances in some disease states[29].

These investigations were supported by grant *B*–1456 (*C*1) and (*C*2) from the National Institutes of Health of the U.S. Public Health Service, contract No. *AT* (11–1)–89 from the U.S. Atomic Energy Commission, and grant No. 304 from the National Multiple Sclerosis Society.

Note added in proof. Since submitting this article, it has been ascertained that the neuronal granules which possess acid phosphatase activity and GLP granules are identical. This was shown by photomicrographic recording of calcium–formalin-fixed sections stained successively by Gomori's glycerophosphate–lead nitrate method and by the periodic acid–Schiff method[13].

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A NOTATION FOR ALLOTYPY

By Dr S DRAY
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

Dr S DUBISKI
Instytut Hematologii, Zaklad Serologii, Warszawa

Dr A KELUS
Department of Experimental Pathology, University of Birmingham

Dr E S LENNOX
Department of Microbiology, New York University, College of Medicine, New York

AND

Dr J OUDIN
Institut Pasteur, Paris

We have agreed on a terminology and notation to be used by us in future publications on allotypy and have established correspondences among already identified allotypic specificities of rabbit γ-globulins[1-13]

Terminology

Allotypy is the property, possessed by certain proteins, of existing in several forms or *allotypes*[14] so far not distinguished by usual physicochemical and chemical criteria, and defined by two kinds of antigenic specificities isotypic and allotypic

The *isotypic*[15] *specificity* is that which is the same in all normal individuals of the same animal species, for example, there are antibodies against rabbit γ-globulin prepared in animals of species other than rabbit (for example, guinea pig), which do not distinguish one rabbit serum from another

The *allotypic specificity* is that which is not the same for all normal individuals of the species, for example, anti-rabbit γ-globulin prepared in some rabbits will precipitate sera of certain rabbits but not of others The fact that an antiserum reacts with some but not all individuals of a certain animal species is necessary but not sufficient to the demonstration of allotypy, for such a fact could be due simply to the presence or absence of the molecules carrying a given isotypic specificity. A demonstration of allotypy must include evidence that the observed immunochemical variation occurs in molecules carrying a common isotypic specificity

The *allotypic formula*, more or less complete according to the test antibodies at hand, of a biological fluid, of a preparation, etc , lists the allotypic specificities looked for and indicates their presence or absence

Immunochemical and Genetic Symbols

The choice of notation was guided by that suggested by Ford for the blood groups[16] Symbols in Roman type designate immunochemical specificities and those in italics refer to the genetic elements controlling these specificities (Table 1)

The *different allotypic specificities* are designated by Arabic numbers In order to avoid any confusion with numbers of quantitative meaning (frequency, etc), the numbers used as symbols are preceded by the capital letter A in Roman type, for example, A1, A2 In two different animal species, the use of the same number does not have any meaning of similarity or correspondence between the allotypic specificities designated by that number, or between the protein molecules which carry these numbered specificities It is assumed that the context will prevent any ambiguity, if not, the symbols of allotypic specificities can be completed by one or two letters designating the species (preferably from its Latin name) and added before the letter A

A *gene* controlling an allotypic specificity designated by a given number is designated by the same number in superscript to the block letter A, both letter and superscript in italics, for example, A^1, A^2

Distinct molecules carrying different allotypic specificities have systematically been found together in sera, as though controlled by the same allele or by closely linked genes In the case of such *families of allotypic specificities*, the family will be designated by a number and the different members of the family by the same number with the superscript ′ (prime) ″ (double prime), for example, A1′, A1″ Should further genetic experiments reveal that such allotypic specificities are controlled by different genes, these specificities would be designated by different numbers

The *locus* to which the gene controlling an allotypic specificity is assigned is designated by a small letter in italics, for example, A^1_a, A^9_b To include the symbol of the locus in that of the allotypic specificity, the symbol will be written, for example, Aa1, Ab9 in ordinary letters

Table 1 SYMBOLS

Meaning	Examples of symbols			Print
Allotypic specificity	A1		A2	Roman
Gene	A^1		A^2	Italics
Families of allotypic specificities	A1′	A1″	A1‴	Roman
Locus				
alone	a		b	Italics
in the symbol of the gene	A^1_a		A^9_b	Italics
in the symbol of the allotypic specificity	Aa1		Ab9	Roman
Genotype	$A^1_b A^1_b$	$A^4_b A^7_b$	$A^6_d A_d$	Italics
	A b($1^+2^-4^-7^-$)	A b($1^-2^-4^+7^+$)	A d(6^+10^-)	
Phenotype*		A b($1^+2^-4^-7^-$)d(6^+10^-)		Roman
		A($1^+2^-4^-6^+7^-10^-$)		
Allotypic formula†		A b($1^+2^-4^+7^+$) d(6^+10^-)		Roman
Allotype	A 3 A c3 A c3/Is X8		A 1,6 A a1d6 A a1d6/Is Y5	Roman

* The first examples of symbols of phenotype (or allotypic formula) correspond to the genotype on the line above The examples on the next lines are symbols of similar or more complete phenotypes expressed with or without the symbols of the locus

† Although the examples on the preceding lines are of either phenotype or allotypic formula, the example on this line is one of an allotypic formula and not a phenotype, since it includes three allotypic specificities controlled by three allelic genes

The *genotype* is written as follows $A_b^1 A_b^1$ or $A_b^4 A_b^7$ or $\mathrm{A}_b^9 A_d$ In the last example, the absence of symbol for the gene means an allele of the locus *d* the product of which is unknown Such a designation would be necessary if the individual is known to be heterozygous at the locus *d* despite the fact that only product Ad9 can be found

The *phenotype* is written with letters in Roman type The inclusion of a symbol of a given allotypic specificity indicates that the reaction suitable for determining the presence of this allotypic specificity has been carried out If this allotypic specificity is present, its symbol is accompanied by the sign + in superscript, or (in the contrary case) by the sign − in superscript, for example, $\mathrm{Ab}(1^{+}2^{-}4^{-}7^{-})$, $\mathrm{Ad}(6^{+}10^{-})$, or $\mathrm{A}(1^{+}2^{-}4^{-}6^{+}7^{-}10^{-})$

Allotypic formulæ In contrast to the phenotype which is characteristic for an individual, any mixtures (artificial, or natural such as those which occur in young animals) of proteins may have assigned to them allotypic formulæ In general, when a given individual is concerned, the allotypic formula is the same as the phenotype The same symbols are used as for designating phenotypes

Allotypes An allotype the molecules of which are known to carry one or more allotypic specificities will be designated, for example, as follows A 3 or A 1, 6, or to include the loci A c3 or A a1d6 The isotypic specificity (Is) of one allotype will be indicated as a denominator, the allotypic specificity (ies) being the numerator, for example, A c3/Is X8 or A a1d6/Is Y5 (the symbols X8 and Y5 are examples only and do not refer to assigned isotypic specificities) Other features characteristic of molecules of the concerned allotype, for example, the sedimentation constant, may also be indicated in the same manner, such as, A b1d10/Is Y8/7S

Present List of Allotypic Specificities in the Rabbit (Table 2)

In order to make correspondences among the allotypic specificities defined in the authors' several laboratories, antisera from these laboratories were tested with suitably chosen normal rabbit sera and their reactions compared in two ways

(1) By reacting them individually with single sera of non-immunized rabbits A criterion that the two antisera contain antibodies directed against different allotypic specificities is the finding of one or more normal sera reacting with one antiserum and not the other On the other hand, the larger the collection of normal sera giving similar reactions with the antisera in question, the stronger the presumption they do contain antibodies against the same specificity

Table 2 ALLOTYPIC SPECIFICITIES SO FAR FOUND IN RABBIT SERA DESIGNATED BY SYMBOLS OF THE PRESENT NOTATION, CORRESPONDENCE WITH THE SYMBOLS PREVIOUSLY USED IN DIFFERENT LABORATORIES

Present notation		Symbols of allotypic specificities previously used in different laboratories		
Allotypic specificity	Locus	Oudin (ref 2)	Dubiski and Kelus (refs 8–12)	Dray (refs 5, 6)
A1	*a*	b	E	L
A2		c	B	J
A3		d	D	K
A4	*b*	a	F	II
A5		g	A	I
A6		f	C	—

(2) By reacting them with one or several normal sera in neighbouring regions of the same gelled medium, the continuity or coalescence of zones of precipitation of these antisera with the same normal serum being another criterion that these two antisera contain antibodies against the same allotypic specificity

Similarity of reactions by the first test implies either the same specificity or the association of more than one specificity in the same sera Similarity of reactions by the second test implies either the same specificity or the association of more than one specificity on the same molecule

The specificities A1, A2, A3, on one hand, and A4, A5, A6 on the other hand, appear to be controlled by six genes belonging to at least two series of alleles The locus to which gene A^1 belongs will be called *a* and that to which gene A^4 belongs will be called *b* There is evidence that A^2 and A^3 belong to *a* and that A^5 and A^6 belong to *b* (refs 2, 5, 6, 8, 9, 10) Definite assignment to these respective loci will await further genetic experiments

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[14] From *ἀλλος* meaning other, and *τυπος*, meaning print or type (ref 2)

[15] From *ἰσος*, meaning equal or similar, and *τυπος*, meaning print or type, or more simply from *ἰσοτυπος*, meaning of the same form (ref 2)

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CHEMICAL STERILIZATION OF MOSQUITOES

By DR DONALD E WEIDHAAS

Entomology Research Division, U S Department of Agriculture, Orlando, Florida

LABRECQUE[1] has reported that three alkylating agents—aphoxide, hereafter designated as tepa (*tris*(1-aziridinyl)phosphine oxide), apholate (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine), and aphomide (*N,N′*-ethylene*bis*[*P,P*-*bis*(1-aziridinyl)-*N*-methylphosphinic amide])—caused sexual sterility in both sexes of house flies (*Musca domestica* L), and Weidhaas *et al* [2] showed that they also caused sterility in two species of mosquitoes, namely *Anopheles quadrimaculatus* Say and *Aedes aegypti* (L), when fed to adults in honey solutions These findings offer possibilities for the control or eradication of mosquitoes through the use of induced sterility[3,4] If the release of sterile males into natural populations can be shown to be as effective and practical as it has been with the screw-worm fly (*Cochliomyia hominivorax* (Coquerel)), sterilization by chemicals would be simpler and cheaper than sterilization by γ-radiation, and the facilities required would be more readily movable If sterilizing chemicals could be safely applied to natural populations, control or eradication could be realized by induced sterility without the necessity of rearing and releasing large numbers of insects

Administration of sterilizing chemicals in the adult food would not be a practical method for field use in the control of mosquitoes unless powerful attractants are developed, and other methods of administration might also be more effective for sterilizing laboratory-reared mosquitoes for release The experiments described here were therefore conducted to determine whether larval exposure and tarsal contact would also induce sterility

Experiments on the induction of sterility by exposure in the larval stage were made with *Aedes aegypti* Five hundred larvæ in the late third or early fourth instar were exposed in enamel pans containing 1 litre of tap-water treated with 10 p p m of tepa or apholate After 24 h, food, consisting of powdered dog checkers and yeast, was provided, and the larvæ were left in the treated water until they pupated The pupæ were segregated by sex, as determined by size, moved to untreated water, and allowed to emerge into small holding cages Pupæ from untreated larvæ were also segregated by sex to provide untreated adults for use in mating experiments

While the mosquitoes were still less than 24 h old, 40 treated or untreated males were combined with 40 treated or untreated females in screen cages 7 in wide, 10 in long, and 8 in high Water and honey solution were provided in cotton pads Three to four days were allowed for mating, then the females were allowed to feed on a guinea pig, and two days later egg collections were made The number of eggs laid and the percentage that hatched are given in Table 1

Table 1

Sex treated	Eggs laid	Per cent hatched
	Tepa	
Male	3,700	0
Female	153	16
Both	173	0
Neither	3,482	89
	Apholate	
Male	1,200	7
Female	1,000	53
Both	800	2
Neither	1,200	71

Tepa at 10 p p m completely sterilized males and almost completely sterilized females Normal females mated to treated males laid a normal number of eggs, none of which hatched Treated females mated to either untreated or treated males laid a reduced number of eggs, eggs from those mated to treated males did not hatch, but 16 per cent of the eggs from those mated to untreated males hatched Apholate at 10 p p m was not so effective

Anopheles quadrimaculatus adults were exposed to residual deposits of tepa on 'Masonite'* panels and glass surfaces On 'Masonite' the deposits were prepared by dissolving tepa in ethanol and applying a sufficient amount of solution with a small paint brush to give a deposit of 500 mg/sq ft For the applications on glass the tepa was dissolved in absolute methanol and 1 ml of solution was pipetted into a 3-in glass Petri dish The Petri dish was rotated until the methanol evaporated to give a deposit of 10 mg/sq ft Virgin males or females less than 24 h old were confined on these residues for 4 h Following this exposure various crosses of 40–50 treated and untreated males and females were made in cylindrical screen cages 12 in in diameter and 18 in long After 3–4 days a blood meal (from a guinea pig) was offered, and 2 days later 25 females were placed individually in glass vials containing water for egg deposition The results are given in Table 2

Table 2

Sex treated	Per cent of females laying eggs	Per cent of egg batches with hatch of 60–100 (per cent)	0
	'Masonite' surface		
Male	76	0	100
Female	4	0	100
Both	8	0	100
Neither	88	100	0
	Glass surface		
Male	92	0	100
Female	0	—	—
Both	0	—	—
Neither	84	100	0

Both males and females were completely sterilized by contact with residual deposits Unexposed females mated to males exposed to tepa on either glass or 'Masonite' surfaces laid a normal number of eggs, but none of the eggs hatched When treated females were mated to exposed or unexposed males, only a few females exposed on 'Masonite' and none of those exposed on glass laid eggs None of the eggs from females exposed on 'Masonite' hatched

Another test was run in which virgin, unexposed females less than 1 day old were mated to males which were exposed for 4 h to 10 mg/sq ft of tepa on glass when they were 4 days old These females laid a normal number of eggs, but none of them hatched, which showed that older males could also be sterilized by contact with residues

During June–August, a total of 88 wild females of *Anopheles quadrimaculatus* were collected from day-time resting stations in Lake County, Florida On return to the laboratory 57 of them were exposed for 4 h on 'Masonite' panels treated the preceding day with 500 mg of tepa per sq ft, and the other 31 were used for unexposed controls All females except three had blood or eggs in their abdomens The three that were unengorged were given a blood meal on a human arm 65 per cent of the unexposed females laid a normal number of eggs that hatched normally, 67 per cent of the exposed females laid a normal number of eggs, but not a single egg hatched Thirteen of the exposed females were dissected and the number of times they had laid eggs was determined by counting the ovarian relics Nine had laid their first batch of eggs, three, their second, and one, her fourth or fifth These results showed that older females that had previously mated, fed on blood, and developed partially or completely one to four or five batches of eggs, were completely sterilized by contact with a residual deposit of tepa

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* Mention of this proprietary product does not imply its endorsement by the U S Department of Agriculture

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ESTABLISHMENT OF FOUR STRAINS OF CELLS FROM INSECT TISSUES GROWN *IN VITRO*

By T D. C GRACE

Department of Microbiology, John Curtin School of Medical Research, Canberra,
and Division of Entomology, C S I R O , Canberra

MANY attempts to grow insect tissues *in vitro* have been made[1], and in the past few years there has been significant progress in the maintenance of cells for long periods[2] In 1960 a strain of cells from ovarian tissue of *Antheraea eucalypti* Scott (Saturniidae) was established, but due to an accident the strain was lost after it had been growing for six months, during which time it had passed through 22–25 generations and had been sub-cultured 18 times

This article reports the establishment of a further four strains of cells from ovarian tissue of *Antheraea eucalypti* As the development of the strains followed similar courses, only one strain will be described

The cultures from which three strains were developed consisted of cells from ovaries of diapausing pupæ of *A eucalypti* which had been dissociated in 0 25 per cent trypsin (Difco trypsin 1 250) in Puck's saline *A* After dissociation, the cells from four ovaries were placed in a Porter flask with 0 9 ml of insect tissue culture medium The other strain developed from cultures of ovaries which were placed in a Porter flask (4 ovaries/flask) with 0 9 ml of medium All the cultures were incubated at 27°–29° C

The medium was changed every 7–14 days for the first 7 months, but after the cells began to multiply rapidly it became necessary to change the medium every 4–5 days and sub-cultures were made at 8–10 day intervals The cells were removed from the bottle for sub-culturing in the following way the medium was removed from the bottles and the cells were washed once in Puck's saline *A* Then 2 0 ml of 0 1 per cent trypsin in Puck's saline *A* was added and the cells incubated at 25° C As soon as a few cells had come free from the glass, 1 0 ml of insect tissue culture medium was added and gently pipetted to free the cells from the glass After centrifugation at 200*g* for 2 min the supernatant was removed and the cells were re-suspended in fresh medium Approximately 50,000–100,000 cells/ml were placed in a 2-oz half-flat medicine bottle with 3 0 ml of medium

The medium used was a modification of that described by Wyatt[3] Its composition is shown in Table 1 The medium was prepared as follows

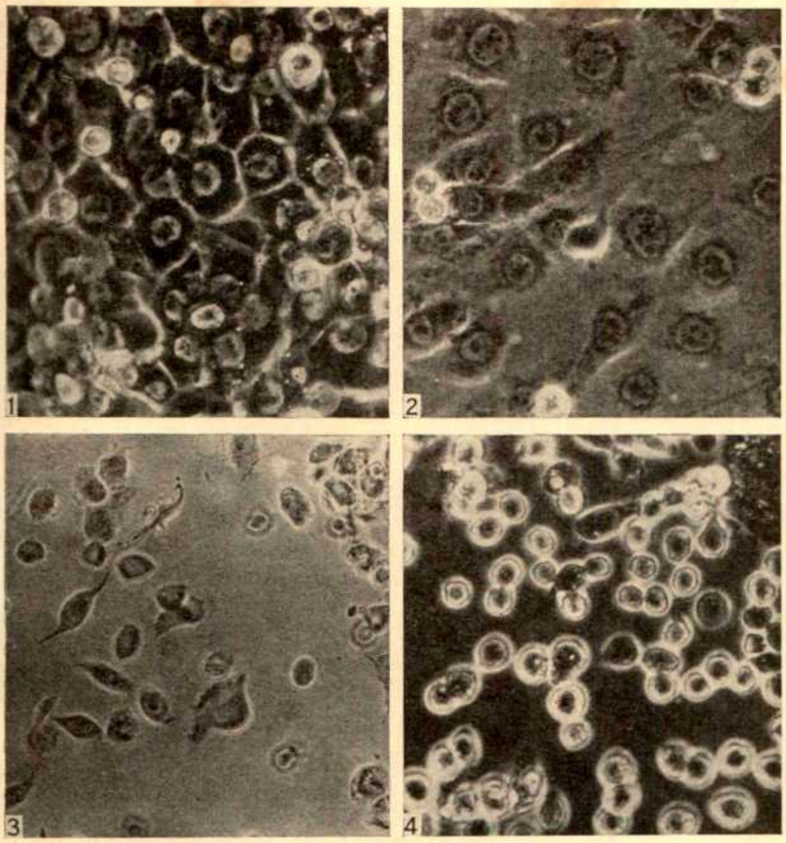
A was dissolved in 20 ml water, *B* in 30 ml water, *C* in 20 ml water and *D* in 20 ml water The calcium chloride was dissolved separately in a small amount of water and was added after *A*, *B*, *C* and *D* were thoroughly mixed The vitamins were then added The *p*H was adjusted to 6 5 with potassium hydroxide Streptomycin and penicillin were added just before the medium was sterilized through a sintered glass filter (porosity 5) After sterilization, 19 ml aliquots were taken and made up to 20 ml with 1 0 ml of heat-treated (60° C for 5 min) insect plasma from diapausing pupæ of *A eucalypti*

In October 1960 dissociated cells from ovaries of diapausing pupæ were placed in Porter flasks After 48 h the majority of cells had adhered to the surface of the culture vessel About 50 days after the cultures had been initiated, many cells began to show signs of degeneration (vacuoles and fat droplets in the cytoplasm) and by the seventieth day only a few cells were still healthy During the next four months very little cell-growth occurred Ten months (July–August 1961) after the cultures were started, the number of healthy cells increased rapidly, and the first sub-culture was made 12 days after the renewed cell-growth began Sub-cultures were made thereafter at about 14-day intervals for 2 months The early sub-cultures consisted of about 8,000 cells in 0 9 ml of medium on Porter flasks As the growth continued the cells were transferred to 2-oz medicine bottles Since November 1961 sub-cultures have been made at 8–10 day intervals The parent primary cultures of this strain were still growing after 15 months under *in vitro* conditions

The strain described has been established for 5 months and has been sub-cultured 29 times The two other strains which developed from dissociated

Table 1 INSECT TISSUE CULTURE MEDIUM (mg/100 ml)

	Salts	
	NaH_2PO_4 $2H_2O$	114
	$NaHCO_3$	35
A	KCl	224
	$CaCl_2$ (separate)	100
	$MgCl_2$ $6H_2O$	228
	$MgSO_4$ $7H_2O$	278
	Amino-acids	
	L-Arginine-hydrochloride	70
	L-Aspartic acid	35
	L-Asparagine	35
	L-Alanine	22 5
	B-Alanine	20
	L-Cystine hydrochloride	2 5
	L-Glutamic acid	60
	L-Glutamine	60
	L-Glycine	65
B	L-Histidine	250
	L-Isoleucine	5
	L-Leucine	7 5
	L-Lysine hydrochloride	62 5
	L-Methionine	5
	L-Proline	35
	L-Phenylalanine	15
	DL-Serine	110
	L-Tyrosine (dissolved in N/1 HCl)	5
	L-Tryptophan	10
	L-Threonine	17 5
	L-Valine	10
	Sugars	
	Sucrose (in g)	2 668
C	Fructose	40
	Glucose	70
	Organic acids	
	Malic acid	67
	α-Ketoglutaric acid	37
D	Succinic acid	6
	Fumaric acid	5 5
	(Neutralize org acids with potassium hydroxide)	
	Antibiotics	
	Penicillin 'G' (sod salt)	3
	Streptomycin sulphate	10
	Vitamins	
	Thiamine hydrochloride	0 002
	Riboflavin	0 002
	Calcium pantothenate	0 002
	Pyridoxine hydrochloride	0 002
	p-Aminobenzoic acid	0 002
	Folic acid	0 002
	Niacin	0 002
	Isoinositol	0 002
	Biotin	0 001
	Choline chloride	0 02



Figs. 1–4. The photographs were taken by phase contrast illumination with a Leica camera attached to a Unitron (model *BU*-13) inverted microscope. Figs. 1, 2 and 3 were taken from a thirty-second sub-culture of *A. eucalypti* ovarian cells and Fig. 4 from a twentieth sub-culture of *A. eucalypti* ovarian cells
Fig. 1, confluent sheet of cells; Fig. 2, type 1 cells 28–40μ diam.; Fig. 3, type 2 cells 16–18μ diam.; Fig. 4, type 3 cells 10–12μ diam.

cells have been growing for 3 months and have been sub-cultured 22 times. The fourth strain, which developed from cultures of undissociated ovaries, has been growing for 8 months and has been sub-cultured 44 times (36 sub-cultures have been made between September 1961 and January 1962).

After sub-culturing, the majority of cells remained floating for 2–4 h and then became attached to the surface of the culture vessel. After 4 days there was usually a confluent layer of cells on the surface of the culture vessel (Fig. 1). Mitotic divisions were numerous and many cells became detached and multiplied while floating in the medium.

The generation time of the cells at the beginning of the development of the strain was about 5–6 days, but since November 1961 the generation time has been 60–72 h. This is appreciably slower than the growth of vertebrate cells grown *in vitro* and may be partly accounted for by the lower temperature at which the cells are grown.

There are three distinct cell types present in the strains. One cell type is common to all four strains (Type 1). This cell (20–40μ in diameter) is polygonal in outline, rather flat and the cytoplasm is finely granulated. Usually, these cells form sheets which adhere strongly to the surface of the culture vessel (Fig. 2). The second type of cell (type 2), which is found in three of the strains, is round to fibroblastic in profile (15–20μ diameter) with a distinct outline and a finely granular cytoplasm. Many of these cells grow in small groups on the culture vessel, but they also multiply while floating free in the medium (Fig. 3). The third type of cell (type 3) is small and round (10–15μ in diameter) with a clear outline and a clear cytoplasm (Fig. 4). This cell type, which is predominant in only one strain, shows very little variation in size or shape and the cells tend to remain loosely attached to the surface of the culture vessel. All three types of cell multiply.

The establishment of the four strains has followed the course frequently observed in vertebrate tissue culture, where it is common for freshly explanted tissues or cells to undergo a period of growth followed by a period in which cell growth slows down or may stop altogether. This period may then be followed by a sudden renewal of growth, and in many instances the cells continue to proliferate for years.

The three other strains developed from cultures set up in August 1960, January 1961 and March 1961. The cultures set up in August 1960 contained undissociated ovaries; the other cultures consisted of dissociated cells. Contrary to results previously obtained[1] the action of trypsin had no adverse effect on the cells. A number of cell types was liberated on dissociation and it is probable that cells other than those from the ovariole sheath have continued to grow in culture[2,4,5].

In establishing these lines of cells certain principles have emerged. Whenever possible the medium should contain plasma from the insect the tissues of which are cultured, and that regard should be taken of the osmotic pressure, pH and ionic ratios of the blood, and these factors be altered in the medium where necessary. If it is not practical to obtain a sufficient supply of homologous plasma it is suggested that plasma from the silkworm (*Bombyx mori*) be used. In my experience, cells from several species of Lepidoptera have grown well and survived for many months when grown in media containing silkworm plasma. The incorporation into the medium of insect hormones (in the form of brain extracts, ring gland extracts, etc.) has not increased growth, but neither have these tissue extracts had any deleterious effect on the cells.

Cells from the strains have been deep-frozen in 10 per cent glycerol in tissue culture medium at −68° C and successfully regenerated.

A limited number of cultures of cells from the strains can be made available to interested workers.

I thank Dr. M. F. Day for his advice.

This investigation was supported in part by research grant *E*-2462 from the U.S. Public Health Service.

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LETTERS TO THE EDITOR

PHYSICS

An Isotope Effect in the Probability of ionizing a Molecule by Energy Transfer from a Metastable Noble-Gas Atom

Excitation of a molecule to an electronic state lying above the ionization potential need not result in ionization. The excitation energy can instead be degraded in a re-organization of the positions of the constituent atoms—by dissociation, predissociation, or internal conversion. This conclusion stems from the fact that the fraction of excitation acts leading to ionization; the 'efficiency of ionization', $\eta(E)$, is almost always substantially smaller than unity, at least when the excitation energy, E, is not more than 15–20 eV greater than the lowest ionization potential. Experimental values of $\eta(E)$ for many diverse molecules have been obtained, both for excitation by absorption of a photon[1] (η_p) and for excitation by collision of the second kind with an excited atom[2] (η_c). No information is available for the equally interesting case of excitation by electron impact.

Whether there is true competition in the transient excited state between pre-ionization and atomic re-organization is an important question which is raised but not resolved by these results. The two processes are not necessarily competitive : η might measure the probability of pathways that invariably lead to ionization, as distinguished from pathways that invariably do not. In a simple model, with only two alternative initial steps:

AB + excitation energy $E \rightarrow AB^+ + \varepsilon^-$ [direct ionization, probability δ] or

AB + excitation energy $E \rightarrow AB^*$ [excitation, probability $(1-\delta)$], the latter followed by:

$$AB^* \begin{array}{l} \nearrow AB^+ + \varepsilon^- \text{ [pre-ionization, rate } k_1\text{]} \qquad (1) \\ \searrow A \quad + B \text{ [atomic reorganization, rate } k_2\text{]} \qquad (2) \end{array}$$

it follows that:

$$\eta = \delta + (1-\delta)\, k_1\, (k_1 + k_2)^{-1}$$

(Here AB denotes the molecule; neither A nor B need be monatomic.) Thus it is conceivable that $k_1 \ll k_2$ and hence $\eta \approx \delta$, in which event processes (1) and (2) would not be competitive.

That the contrary is generally true is suggested strongly by arguments in a previous paper[2], which concludes that, indeed, $\delta \ll 1$ is the typical behaviour, and $\eta_c \approx \eta_p \approx (1 + k_2/k_1)^{-1}$ does measure a true competition.

The existence of these competitive pathways in the decomposition of AB^* can be tested by observing the effect on η of isotopic substitution in the molecule. Parameters determined by purely electronic re-organization—δ and k_1—should be unaffected thereby. But k_2 should be diminished, and η therefore increased, if heavier masses are substituted. Thus, $k_2 = k_2' M^{-1/2}$, and:

$$\eta = \delta + (1 - \delta)(1 + M^{-1/2} k_2'/k_1)^{-1} \qquad (3)$$

where M is an effective reduced-mass for such atomic motions as bring the molecule to a point in co-ordinate space subsequent to which pre-ionization is impossible. This experiment has been performed. The predicted isotope effect is found, and in the anticipated sense: in every case the isotopic molecule with heavier atoms has a greater value of η_c. Hence pre-ionization and 'atomic reorganization' are truly competitive.

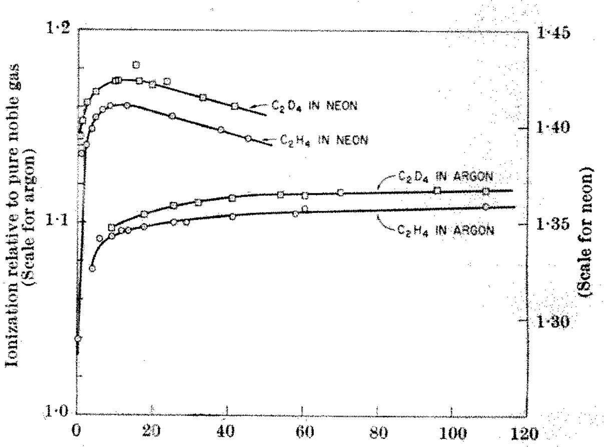

Fig. 1. Ionization current in the mixture, relative to that in the pure gas, plotted against contaminant concentration, for normal and for fully deuterated ethylene in neon and in argon

Measurement of η_c is accomplished by observing the total ionization produced by absorption of high-energy particles in a noble gas contaminated with the molecule under study[2,3]. The ionization exceeds that in the pure noble gas if the latter's lowest excitation energy, E_0, exceeds the ionization potential, I_x, of the contaminant, chiefly because of ionization of contaminant molecules in collisions with metastable noble-gas atoms[4-6]. For all monatomic contaminants this augmentation, ΔN_i, is about the same, although there are small differences which arise from the effect of sub-excitation electrons[7]. Molecular contaminants exhibit large differences in the collateral ionization, for η_c is peculiar to the individual contaminant molecule, and it may lie anywhere between 0 and 1. The value of η_c is given by[2] $\eta_c \approx \Delta N_i/\Delta N_i^{st}$, where ΔN_i is the augmentation observed (at the plateau, as in Fig. 1), and ΔN_i^{st} is the augmentation expected if the contaminant were monatomic and had an ionization potential equal to I_x. The value of ΔN_i^{st}, which is interpolated from experimental measurements of ΔN_i for monatomic contaminants[2], is larger, the smaller is I_x, and is given approximately by the empirical equation:

$$\Delta N_i^{st}/N_i \approx 0{\cdot}395 + 0{\cdot}34[1-(I_x/E_0)], \text{ with } I_x < E_0$$

In the experiments on the isotope effect, ionization currents produced by β-rays (mean kinetic-energy about 18 keV) from a source of nickel-63 deposited on a gridded electrode were determined in an ionization chamber, by methods already described[8], for various concentrations of the contaminant. Normal and fully deuterated ethylene and n-butane were studied, both in neon and in argon. In Fig. 1 the observed ionization in the mixture, relative always to the corresponding values, N_i, in the pure parent gas, is plotted against the measured concentration of contaminant introduced

The curve for each deuterated hydrocarbon is consistently higher than that for the corresponding normal one by the order of magnitude of 1 per cent —about ten times the estimated error of measurement In the plateau region the ratio of these ionization currents, $(N_i + \Delta N_i^D)/(N_i + \Delta N_i^H)$, is found to be remarkably independent of contaminant concentration Mean values for the four cases are presented in Table 1

Table 1 COMPARISON OF EXPERIMENTAL AND THEORETICAL ISOTOPIC IONIZATION RATIOS

Noble gas	Contaminant	Ionization ratio, $(N_i + \Delta N_i^D)/(N_i + \Delta N_i^H)$ Experimental	Theoretical	Assumed splitting
Argon	Ethylene	1 008$_3$	1 005	$CH_2 + CH_2$
			1 026	$H + C_2H_3$
Neon	Ethylene	1 010$_2$	1 004	$CH_2 + CH_2$
			1 017	$H + C_2H_3$
Argon	*n*-Butane	1 006$_9$	1 007	$C_2H_5 + C_2H_5$
			1 030	$H + C_4H_9$
Neon	*n*-Butane	1 008$_3$	1 002	$C_2H_5 + C_2H_5$
			1 009	$H + C_4H_9$

The magnitudes of the experimental results cannot be compared with theory immediately because none of the three constants in equation (3), δ, k_2'/k_1, and M, is known However, presuming that $\delta \ll 1$, one can calculate the ionization ratio on the basis of a specific assumption regarding M This is done in Table 1 for two extreme possibilities, symmetrical cleavage, and dissociation of a hydrogen atom The calculated isotope effects are of the same order of magnitude as those observed, comparison points to symmetrical splitting with Ar* ($\bar{E}$ = 11 6 eV), and dissociation of H (or of H_2) with Ne* ($\bar{E}$ = 16 7 eV) A suggestive fact is that with Ne*, but not with Ar*, there is ample energy to dissociate an excited hydrogen atom It should be emphasized, however, that the model embodied in equation (3) may be seriously oversimplified, for either process (1) or process (2) may encompass more than a single pathway (with the former, the ion may be left in various excited states, with the latter, there may be competitive 'dissociation' pathways)

Additional conclusions are that pre-ionization in these highly excited states is relatively slow, requiring times of the order of magnitude of 10^{-13} second or longer, and that pre-ionization in molecules is not a rare phenomenon, but a prevalent one, since[2] for most molecules the excited states with E somewhat greater than I possess a large part, and often the greater part, of the total oscillator strength of the valence electrons

The results also point to the importance of similar experiments with the absorption of light It is predicted that the same isotope effect will be found in η_p Moreover since $\eta_p = \sigma_{pi}/\sigma_p$, where σ_{pi} is the cross-section for photo-ionization and σ_p is the total absorption cross-section at the same value of $E = h\nu$, the entire isotope effect is predicted to lie in σ_{pi}, and none of it in σ_p

We thank Dr Leon M Dorfman (Argonne National Laboratory) for providing the deuterated hydrocarbons, and Dr Ugo Fano (National Bureau of Standards, Washington) for discussions One of us (R L P) also thanks Prof Michel Magat (Faculté des Sciences de Paris), in whose laboratory the theoretical basis for these experiments was developed

This work was performed under the auspices of the U S Atomic Energy Commission

WILLIAM P JESSE

St Procopius College,
Lisle,
Illinois

ROBERT L PLATZMAN

Argonne National Laboratory,
Argonne,
Illinois

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Search for an Effect of Magnetic Fields on Electromagnetic Radiation

IT has been suggested that some anomalies in astronomical observations may involve a photon–photon interaction of the type[1]

$$\frac{\Delta\nu}{\nu} = KlU \qquad (1)$$

where K is a constant equal to 2 6 × 10^{-15}, l is the path length in cm, and U is the energy density in erg/cm^3 It has further been suggested that the Mossbauer effect might be used to provide a test of this hypothesis if the radiation could be passed through a region of high photon density such as might be obtained in the vicinity of plasma[2] Since the form of this proposed interaction does not contain any frequency-dependence and since if it were to exist it would contradict accepted electromagnetic theory, it seemed that assuming U to be the energy density due to a static electromagnetic field was not an unallowable extension of an already radical hypothesis Since a static magnetic field is not quite analogous to the original circumstances discussed in ref 1, this experiment does not fully test the hypothesis stated in equation (1) However, a magnetic field produces by far the largest electromagnetic energy density obtainable in the laboratory (this was pointed out to us by J Heberle) and thus provides a conveniently easy experiment

Accepted electromagnetic theory does not predict any change in frequency for radiation passing through a magnetic field, some scattering effects are expected to take place at very high magnetic field strengths[3]

The measurements were carried out by allowing the 14-keV γ-rays emitted by iron-57 to pass through transverse and longitudinal magnetic fields produced by permanent magnets The velocity spectrometer used will be described elsewhere[4] The source used was cobalt-57 diffused into copper The absorber was $Na_4Fe(CN)_6 \, 10H_2O$ with the iron enriched with iron-57 This combination produced a single absorption line approximately 45 per cent deep (without background corrections) and 0 5 mm/sec wide

In the transverse case, the magnetic field was 5,500 œ at the centre of the gap traversed by the γ-radiation and fell off to half this value in about 0 5 in from the centre From equation (1), this would give a frequency shift of about 5 × 10^{-9} The measurements with the magnetic field along the direction of the photons were performed by providing a horseshoe magnet with pole pieces pierced by a hole 0 75 in in diameter This allowed radiation to go through one pole piece, cross the gap in a direction parallel to the magnetic field, and emerge through the hole in the other pole piece The field in the gap of this magnet was 1,700 œ and the calculated effect was about 5×10^{-10} Along this longitudinal path, the magnetic field in the gap was in the direction of propagation of the photons but was oppositely directed outside the magnet Hence the line integral of the field along the path was zero To allow for the possibility of a directional effect, the experiment was also repeated with the source placed in the field-free region inside the hole in one pole tip The effect calculated from equation (1) in this case was 5×10^{-11}

The results obtained from the velocity spectrometer were analysed by a least-squares programme The principal source of error in the measurement was from counting statistics This error was increased to allow for systematic errors such as temperature fluctuations within the instrument The results are shown in Table 1 It is clear there is no effect of a magnitude comparable with that expected from equation (1) This is not at all surprising The interpretation of the evidence supporting equation (1) has been seriously questioned by several authors[5], one of whom suggested that such an effect might be present but that K should be only a thousandth of the value given here[6] It is clear that we have not observed even this diminished effect It must also be made clear that our measurement does not really constitute a full test of equation (1) anyway since this experiment was done with a static magnetic field instead of a radiation field On the other hand, to our knowledge there have been no previous measurements in which the influence of magnetic fields on the frequency of photons has been tested to such accuracy (An experiment was performed by Banwell and Farr[7] to see if the velocity of light was unchanged in a transverse magnetic field They allowed one arm of an interferometer to pass through a region of 20,000 œ for 1 m If there had been a frequency shift of the type looked for in the present work, it would also have been observed in these measurements In terms of equation (1), the limit that can be set from these early results is about three orders of magnitude larger than that we can set from the first result in Table 1)

Table 1 SUMMARY OF RESULTS

Type of field	Measured effect $\left(\frac{\Delta\nu}{\nu}\right)$	Effect calculated from equation (1)
Transverse	$(2 \pm 4) \times 10^{-15}$	-5×10^{-9}
Longitudinal $\left(\int \vec{B}\ \vec{ds} = 0\right)$	$(1 \pm 5) \times 10^{-15}$	-5×10^{-10}
Longitudinal $\left(\int \vec{B}\ \vec{ds} \neq 0\right)$	$(1 \pm 4) \times 10^{-15}$	-5×10^{-11}

Our results can stand on this ground alone, without reference to any theoretical expectation The third result given in Table 1 may also be interpreted as showing that the net magnetic charge on the photon is less than 3×10^{-26} e m u The experiment could be repeated with at least 10-fold greater accuracy by using techniques developed for measuring the gravitational red shift Further increase in accuracy could be obtained by the use of electromagnets which can produce considerably stronger magnetic fields

We thank Dr M R Perlow for preparing the enriched absorber

This work was performed under the auspices of the U S Atomic Energy Commission

S S HANNA
G J PERLOW
J P SCHIFFER
J A. WEINMAN

Argonne National Laboratory,
Argonne, Illinois

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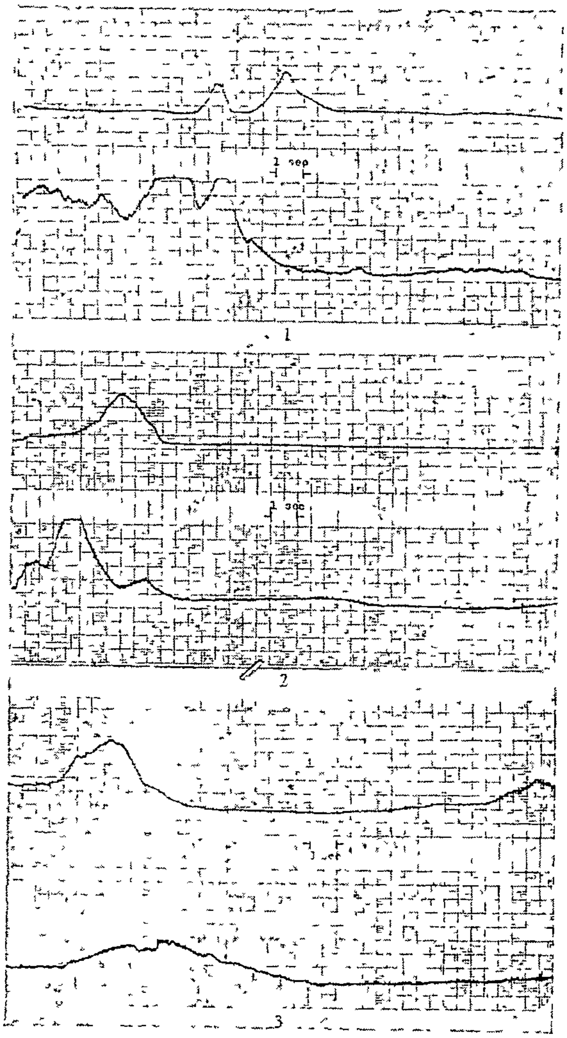
Photoelectric Tracking of Instantaneous Motions in Aurora

EXTENSIVE work done[1–6] on auroral drift motions is essentially based on the all-sky camera records The speeds so obtained range from 0 05 km /sec to 2 2 km /



1
2
3

Figs. 1–3 Recordings from intense and moving auroral bands on different nights Time increases from left to right and each big vertical division is one-half sec The time-delay between corresponding signal events is seen to vary sec Fast photographic techniques have also been used[7] to get short-term velocities of the order of 2 5 km /sec This communication is to present some cases of instantaneous motions recorded photoelectrically and confirmed by simultaneous visual observations The device is a double photometer system used earlier[8–9] collecting light from two fields each 0 55° in size and separated from each other by 3° Observations were made separately for two orientations of the field-pair, one at 90° to the other Signals were recorded using a two-channel Sanborn recorder at a chart speed of 10 mm /sec

Figs 1–3 show signals from bright moving bands as visually observed at the time of recording The intensity in each case was easily about 100 kilo rayleighs or more A time-lag is seen in one of the two channels between corresponding signal events This time-delay, which is indicative of motion of the auroral form across the two fields of view, seems to vary from 1 5 to 2 5 sec The delay may be seen in any one of the two channels This is perhaps a matter of speed and direction at transit across the two fields Assuming the auroral height as 100 km the speeds obtained range from 4 to 10 km /sec These are, however, components in the direction of motion Furthermore, only when the orientation of the field-pair is in the direction of motion of the auroral form could this kind of a time-delay in one channel be seen

The double-peak feature in Fig 1 with a drop in the signal level in between in each channel is intriguing Probably, this could be due to two independent auroral patches moving in succession The widths of the peaks in one channel seem to be reversed relative to those in the other The moving form may not be steady in speed, size or intensity during its passage across a field of view, particularly so across two fields of view

The high speeds mentioned here obtained over a few seconds could perhaps be distinguished from the usual drift motions observed from all-sky records Dzhordzhio in the U S S R has obtained[10] by photoelectric methods speeds of the order of 9 km /sec and mentions also speeds of 3–4 km /sec by visual estimates due to Galperin

I thank Dean B W Currie and Dr G G Shepherd for their help

R S Iyengar

Institute of Upper Atmospheric Physics,
University of Saskatchewan,
Saskatoon

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Temperature Coefficient of Electrical Resistance of High-Purity Rhodium

I SUPPORT Price and Taylor[1] in believing that the temperature coefficient of resistance of rhodium is appreciably higher than 0 00457, the maximum value hitherto recorded in the literature

As long ago as 1936, measurements made by the National Physical Laboratory on a sample of rhodium specially purified in our refineries indicated a value of 0 00463, confirming exactly the higher value now quoted by the above authors, and since then this value has been widely used in our own organization

It is to be hoped that the higher value will now be generally recognized

J C Chaston

Johnson, Matthey and Co , Ltd ,
Research Laboratories,
Wembley, Middlesex

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Sputtered III–V Intermetallic Films

In the course of investigating methods for the deposition of films of semi-conductive materials, it was found that the antimonides of indium and gallium can be readily deposited by means of cathodic sputtering Reactive sputtering has been widely used for several decades for formation of films of oxides and certain other compounds[1], but there seems to have been little interest in the direct sputtering of compound materials Kane and Wood attempted to produce a sputtered film of Ag_2Te, but obtained rather $Ag_{2-x}Te$ (ref 2) Recently, work concerned with sputtering yields for gallium antimonide[3] and directional patterns formed by sputtering from single crystal balls of indium antimonide has been reported[4], but in neither case were properties of the films discussed

The apparatus used was similar to that described by Belser and Hicklin[5] The substrate holder was adjustable in height relative to the suspended source and fitted with a small tantalum wire heater for controlling the temperature of the substrates Films were deposited on 1-in ^{2} substrates of fused silica or an aluminosilicate glass (Code 1723, Corning Glass Works) Prior to deposition, the substrates were thoroughly degassed in vacuum (about 10^{-5} mm mercury) at elevated temperatures The system was purged with dry argon (99 996 per cent minimum purity), which was also used as the bombarding gas for sputtering Pressures during sputtering were in the range of approximately 50–150μ mercury Sputtering was carried out at potentials of 2–4 kV

Table 1 PROPERTIES OF SPUTTERED INSB AND GASB FILMS

Sample No	Approximate substrate temperature (° C)	Film thickness (Å)	Resistivity (ohm–cm)	Carrier type	Hall mobility (cm^2/V-sec)
InSb					
(Source 1)*	—	—	1 9	n	65,000
2	175	72,000	2 0	n	28
5	175	12,000	0 26	n	110
6	225	1,300	0 002	n	21
10	160	11,000	0 016	n	160
(Source II)†	—	—	0 005	n	72,000
13	50	5,400	9 4	n	—
14	100	11,000	0 79	n	9 5
15	250	4,000	0 015	n	460
16	200	4,300	0 14	n	110
17	150	4,300	0 19	n	26
GaSb					
(Source I)‡	—	—	0 78	p	—
1	< 400	1,900	0 61	p	2 4
2	315	1,100	0 61	p	1 9
9	60	5,400	1,300	p	—
10	110	8,000	8,000	-	—
11	175	3,100	0 90	p	—
13	320	4,000	2 6	p	—
14	300	1,100	0 25	p	1 5
15	200	1,300	300	p	—
(Source II)§	—	—	0 10	p	—
16	350	4,300	0 24	p	7 5
18	300	4,300	5 3	p	—

* Supplied by Ohio Semiconductors, Inc
† Supplied by Cominco Products, Inc
‡ Supplied by Alpha Metals, Inc
§ Supplied by Micro State Electronics Corp

and for periods of up to about 1 h Following deposition, the films were normally annealed at the deposition temperatures for about 30 min Samples of source materials were in the form of undoped polycrystalline slices, with exposed surface areas of some 2–6 cm^2 Two samples of each material, obtained from different suppliers, were used

Results of the measurements of room temperature electrical properties of the indium antimonide and gallium antimonide films are presented in Table 1 Substrate temperature is seen to have an important effect on mobility Films deposited at the lower temperatures, below 100° for InSb and below about 300° for GaSb, were characterized by large values of resistivity and by mobilities below the measurable limit (approximately 1 cm^2/V-sec), GaSb 13, which was deposited at 320°, was anomalous in this respect, having an immeasurably small mobility Increasing temperature in general led to lower resistivities and higher mobilities, but film thickness also appears to have had an effect on these properties Higher values of the mobility can undoubtedly be obtained through investigations into the proper conditions for deposition and annealing The resistivities of the films deposited at the highest temperatures were frequently lower than those of the source materials In every case, the carrier type, as determined from Hall or thermoelectric probe measurements, was the same for the film as for the source

All the films reported here were metallic in appearance The compound characters of the higher temperature films were confirmed by means of X-ray diffraction Films deposited at the lowest temperatures (InSb 13, deposited at 50° C, and GaSb 9 and 10, deposited at 60° and 110°, respectively) gave no diffraction peaks, and were presumably amorphous

Diffraction patterns of the first films produced (InSb 2, 5, 6 and GaSb 1 and 2) showed the presence of oxides—Ga_2O_3, In_2O_3, and in certain instances possibly Sb_2O_3—in addition to the intermetallic compounds as major phases However, more careful purging of the chamber with argon, ion bombardment cleaning of the surface of the sources, and minor modifications to the chamber fittings have served to reduce the level of residual air in the system to the point where no indications of oxides were then discernible in the diffraction patterns

Study of the formation of the intermetallic compound films by this process will perhaps throw some light on the mechanism of sputtering If material transfer were atomic, that is, if antimony and indium or gallium sputter independently, combination of the deposited atoms to reform the intermetallic compounds on the substrates would not be expected at low substrate temperatures The diffraction evidence for the low-temperature, amorphous films is inconclusive, but it may be significant that there were no indications of the elemental materials Further, considerable fractionation of the dissociated compounds, as a result of the large differences in vapour pressures of antimony and the metallic constituents, would be expected if the older sputtering theory of localized heating and subsequent evaporation were valid No appreciable degree of fractionation occurred in the experiments reported here It might, therefore, be presumed that the intermetallic compounds sputter molecularly, or perhaps as polyatomic aggregates Extension of these investigations to other III–V compounds should help to clarify these points

I thank W A Gutierrez and R S Smith for the Hall effect measurements, Dr H deSchmertzing for the diffraction analyses and Dr C Feldman for advice

This work was sponsored by the Bureau of Naval Weapons, U S Navy

CHARLES MOULTON

Melpar, Inc,
Falls Church, Virginia

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OCEANOGRAPHY

Abnormal Hydrographical Conditions in the Menai Straits

RECORDS of the temperature and salinity of the water passing through the Menai Straits have been maintained in this laboratory for several years Samples for salinity determinations are taken regularly at the end of Menai Bridge pier All the evidence available suggests that samples taken at this point are truly representative of the main water mass in the Straits at the time Table 1 shows the monthly mean salinities recorded over a period of six years (1955–60) together with the records for the first eight months of 1961

The monthly means have been calculated from the results of regular samples taken at a fixed time at 7-day intervals It is apparent that although the mean salinity for January 1961 was quite typical of the values observed for this month during the previous six years the mean value for February 1961 was exceptionally high These unusually high salinities persisted until August although the magnitude of the anomaly tended to decrease from March onwards

In coastal waters exceptional conditions such as these might be very localized However, the total rainfall for the first eight months of 1961 as recorded at Valley Anglesey was 2 8 in above the mean total rainfall recorded at this Station for the corresponding periods during the years 1955–60 One would expect, therefore that any effect of local run-off would be to produce a negative salinity anomaly during the period in question

Furthermore, the sea-water temperature records for the Menai Straits and the air temperature records at Valley show a strikingly similar anomaly to the salinity records (Fig 1)

Table 1 MONTHLY MEAN SALINITY (PARTS PER THOUSAND) AT MENAI BRIDGE

	Mean	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
1955 to 1960	Mean of the Monthly means	32 44	32 37	32 46	32 64	32 74	32 84	32 73	32 69	32 53	32 47	32 31	32 44
	Min monthly mean	31 59	31 74	31 87	31 94	32 08	32 48	31 93	32 28	31 96	31 53	31 69	32 05
	Max monthly mean	32 87	32 83	32 90	33 16	33 54	33 54	33 19	33 19	33 03	32 80	32 73	32 80
1961	Monthly mean	32 45	33 78	34 01	33 64	33 82	33 80	33 63	33 19				

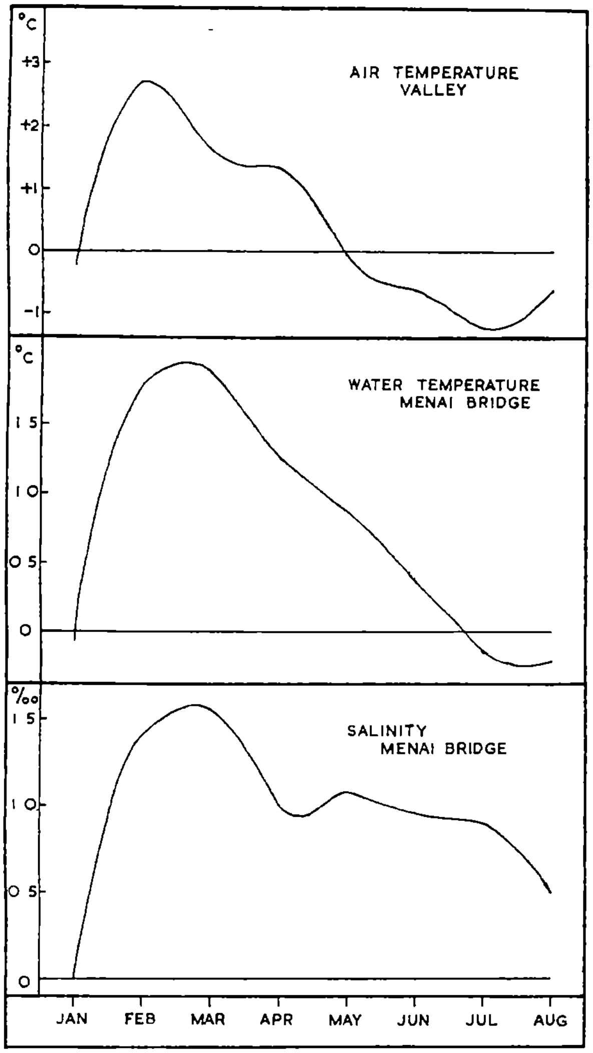

Fig 1 Monthly means for 1961 expressed as anomalies from the mean of the corresponding monthly means for the years 1955–60

It seems possible that the high salinities recorded at Menai Bridge during the early part of 1961 were in fact the result of an unusually strong incursion of Atlantic water into the Irish Sea The temperature records are suggestive of this incursion being the result of a sudden change in either or both the strength and direction of the Gulf Stream System A change in the oceanic circulation may have caused marked salinity and temperature anomalies only in limited parts of the Irish Sea such as the Menai Straits On the other hand it may have been much more widespread in its effects This will only become clear when the appropriate hydrographical data from a much wider area obtained by other laboratories become available In addition, it is to be expected that the faunistic records for the Irish Sea and associated waters for the period in question will provide additional evidence of any major change in the oceanic circulation which occurred

We thank the Director General of the Meteorological Office for supplying the meteorological data

J G Harvey
C P Spencer

Marine Biology Station,
Menai Bridge,
Anglesey

CHEMISTRY

Dynamic Contact Angles and Rates of Adsorption

Several published papers have indicated that the contact angle varies with the velocity with which a fluid/fluid interface moves, or is caused to move, along a solid surface[1–6] In particular, Ablett's results suggest that, under his experimental conditions, hysteresis is absent when the advancing angles, which increase with interfacial velocity, and receding angles which decrease, are extrapolated to zero velocity

We have devised a method whereby a bubble of one fluid can be caused to grow with a constant radial velocity between parallel plates, displacing a second fluid This process can be reversed, affording a means for measuring advancing and receding angles as a function of the interfacial velocity

A typical set of results is shown in Fig 1 for water displacing air, saturated with water vapour, between siliconed glass surfaces The receding angles, which are much more difficult to reproduce at the higher speeds, are also shown The values at zero velocity were obtained by the 'sliding drop' method[7]

It is clear that hysteresis is present, as is shown by the difference between the advancing and receding angles at interfacial velocities below 1 mm/min Below this speed, the angles are independent of velocity within the limits of experimental error (± 1°), confirming Yarnold and Mason's observations

Above this speed, the advancing contact angle increases linearly with the growth velocity up to about 4 mm/min At higher speeds, the rate of change diminishes until finally a limiting value is reached at about 8 mm/min The receding angle also varies linearly with the interfacial velocity above 1 mm/min This dependence is of the same form as that found by Ablett We believe that the absence of hysteresis in his system, and his ability to study receding angles at higher speeds than those reported here, were the results of water having penetrated the solid surface Our receding angles were always determined after the water had been in contact with the solid surface for 45 min

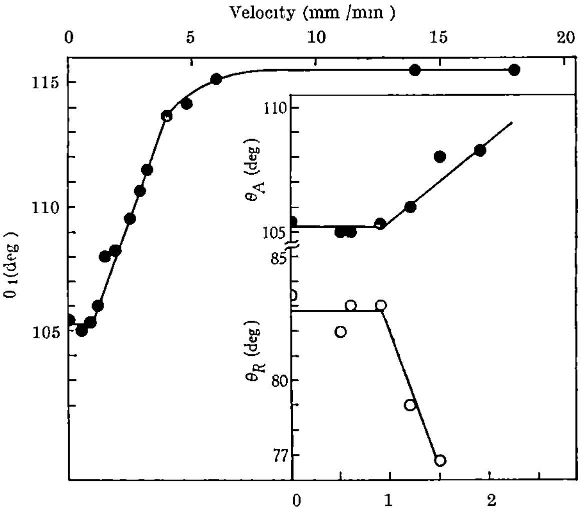

Fig 1 Advancing contact angles (θ_A, solid circles) as a function of the velocity of an air/water interface moving along a siliconed glass surface Inset Advancing and receding angles (θ_A, open circles) at low interfacial velocities Temperature, 22° C

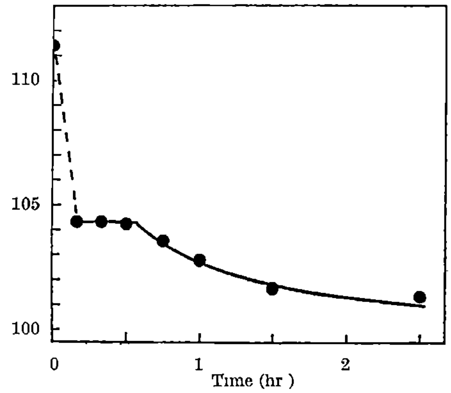

Fig 2 Relaxation of θ_A after halting an interface advancing at 3 mm /min Temperature, 22° C

Following Hansen and Miotto[8], we attribute the absence of a velocity effect at low speeds to the impressed velocity V being smaller than the natural displacement velocity V_N of the peripheral three-phase line of contact V_N is given by l/τ, where l is the peripheral thickness and τ is the relaxation time for water molecules in this periphery When $V \gg V_N$, the molecules are disorientated at the line of contact, and the corresponding boundary tensions will be displaced from their equilibrium values

Since the velocity effect is not observable for low radial growth velocities, we may set V_N as greater than 1 mm /min , or 2×10^{-3} cm /sec With l of molecular dimensions, τ is less than 10^{-5} sec Bockris, Mehl, Conway and Young[9] find the mean relaxation time for water molecules at the copper/water interface to be about 10^{-6} sec , intermediate between the values for bulk water (10^{-10} sec) and ice (10^{-3} sec) A shorter time would be expected at the siliconed glass water interface, and 10^{-5} sec is certainly an overestimate

If the interface is caused to advance over the surface with a velocity greater than 1 mm /min and the impressed drive is then cut off, the contact angle relaxes back rapidly to a value which is within 1° of the low velocity (or static) contact angle After differing periods, about 15 min in the case shown in Fig 2, the angle begins to decrease as the liquid penetrates the solid surface This process has been followed for periods up to 18 hr , and is roughly exponential

Similar results have been obtained when the siliconed glass plates were replaced by polyethylene plates, and when the air phase was replaced by a liquid hydrocarbon, suggesting that the technique could be used for following rates of adsorption at solid/fluid interfaces Fuller details will be reported on completion of these studies

These investigations were financially supported by the Iraq Petroleum Co , Ltd

G E P Elliott
A C Riddiford

Department of Chemistry,
University of Southampton

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Production of Vibrationally Excited Species from the Photolysis of Nitroso Compounds

There have been a number of recent investigations on the production of vibrationally excited species by the method of flash photolysis[1-5] with particular reference to two possible mechanisms, direct vibrational excitation or the initial formation of an electronically excited state the energy of which is then reorganized internally Basco and Norrish[1] have concluded that vibrationally excited nitric oxide was produced directly on photolysing nitrosyl chloride, whereas Symons and Yarwood[4,5] have interpreted their observation of the production of CCl radicals in the photolysis of a number of halogenated methanes in terms of the formation of an electronically excited substituted methyl radical the energy of which was internally converted into vibration Experiments have been carried out on the flash photolysis of trichloronitrosomethane (CCl_3NO) in an attempt to determine the fraction of energy appearing as vibration in each fragment produced on photolysis and to attempt to determine which of the two possible mechanisms is operative in this case A new transient spectrum resulting from the photolysis of this molecule is also reported

Absorption spectra were taken at known delays after the photoflash discharge by means of a second, spectroscopic flash lamp[6] controlled by an electronic delay unit Experiments utilizing a two-path vessel[7] indicated that the reciprocity relationship was found to hold for the absorption of the CCl radical and enabled the peak height resulting from the microdensitometer trace of its spectrum to be taken as a direct measure of relative concentration

The photolysis of CCl_3NO under adiabatic conditions in which no inert gas was added as coolant resulted in the production of CN and NO in high states of vibrational and rotational excitation, indicative of the high temperature reached A large number of mixtures of CCl_3NO and argon, suitably chosen for convenient temperature variation, were investigated for a series of delays throughout the complete spectral range (2150–5600 Å), no transient spectrum which might have been due to the production of a maximum free radical concentration at some optimum temperature was observed The experiments described here were carried out under isothermal conditions which were obtained by mixing the reactant with high pressures of inert gas, in this case either nitrogen or argon

The CCl radical concentration as observed by the transition ($^2\Delta - {}^2\pi$) (ref 8) was found to be dependent on the first power of the photoflash intensity and decayed by means of a first-order process, though measurements were made only in the duration of the flash The (0,0) transition of the γ system of nitric oxide was observed at the shortest delay (Fig 1), the (0,1) transition being extremely weak if present at all This provides good evidence for the first step as

$$CCl_3NO + h\nu = CCl_3 + NO$$

rather than

$$CCl_3NO + h\nu = CCl_2 + NOCl$$

as the nitrosyl chloride would have been further photolysed to nitric oxide in a highly vibrationally excited state[1] The position of the upper limit of the absorption peak in the visible (~ 6800 Å), which has been shown to be diffuse (Thrush, B A , private communication), corresponds to a bond dissocia-

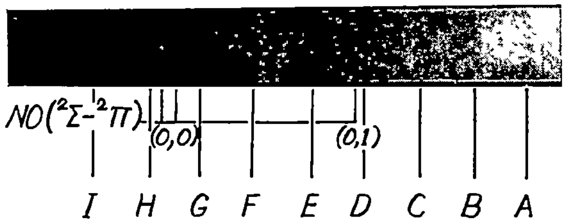

Fig 1 Flash photolysis of trichloronitrosomethane under isothermal conditions $P_{\mathrm{CCl_3NO}}$ = 0 5 mm , $P_{\mathrm{N_2}}$ = 500 mm , E = 2,500 Joules, t = 2 7 μsec

tion energy of $\leqslant$ 42 k cal mole^{-1}, giving rise to the decomposition of this molecule to a white solid The lower limit of the photoflash output (~ 1900 Å) corresponds to an energy of 150 k cal mole^{-1} It can be concluded that nearly all the excess energy above the dissociation energy must appear as vibration in the substituted methyl radical as the unimolecular reaction

$$CCl_3 = CCl + Cl_2$$

is endothermic to the extent of 107 k cal mole^{-1}. This result is similar to that observed on the photolysis of halogenated methanes[4,5] Preliminary experiments on the photolysis of CF_3NO resulted in the observation of the spectrum of nitric oxide in its electronic and vibrational ground state and is in agreement with the work of Hazeldine and co-workers[9], but CF was not observed This may be due to the fast reaction of any CF_3 produced with the parent molecule

Table 1 Spectrum *A–I*

Band	λ_{air} (Å)	v_{vac} (cm^{-1})	Δv_{vac} (cm^{-1})
A	2471 5	40,449	598
B	2435 5	41,047	572
C	2402 0	41,619	544
D	2371 0	42,163	596
E	2338 0	42,759	574
F	2307 0	43,333	532
G	2279 0	43,865	546
H	2251 0	44,411	559
I	2233 0	44,970	

A series of regularly spaced diffuse bands (Table 1) not previously reported were observed (*A–I*, Fig 1) The simplicity of the spectrum suggests that the carrier is diatomic The spectrum followed the CCl($^2\Delta$ − $^2\pi$) transition and the separation of the bands is comparable with, though significantly less than, the fundamental frequency of CCl in the ground state (846 cm $^{-1}$) (ref 10) By analogy with other iso-electronic systems such as CF and NO (ref 10), where the vibrational frequency of the excited $^2\Sigma$ state is significantly greater than that of the ground state, it is suggested that the upper state is a $^2\pi$ state rather than a $^2\Sigma$ state It is noted that the spectrum lies above the limit of dissociation to normal products.

The direct production of vibrationally excited substituted methyl radicals cannot be explained by treating the system as an impulse problem in terms of the proportion of the excess energy appearing in the radical, though there is no reason why the molecule can be so considered The initial formation of an electronically excited CCl_3 radical also appears unlikely as the lowest lying antibonding electronically excited state would require high energy to be reached[11] Walsh[11] has indicated that a 25 electron system of formula AB_3 would be tetrahedral, though the structure of no such system has, as yet, been reported and the part played by a possible change in shape is uncertain The group attached to the substituted methyl radical, whether it be a halogen atom, a nitro group[4,5] or a nitroso group, appears to have little effect on the fraction of energy appearing as vibration in the substituted methyl radical

I thank Prof R G W Norrish for his advice and encouragement throughout the course of this work

D Husain

Department of Physical Chemistry,
University of Cambridge

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A Reproducible Magnesium Oxide Standard for Reflectance Measurement from 0·3 to 2·6μ

The reflectance standard for colorimetric purposes adopted by the Commission Internationale de l'Eclairage in 1931 is a bed of magnesium oxide deposited from the smoke of magnesium ribbon or turnings burning in air As a standard it suffers from various defects and in spite of subsequent attempts to stipulate more closely the conditions to be observed in its preparation variations of 1 or 2 per cent can occur in the reflectance factor as measured in the visual spectral range[1] Alternative materials such as barium sulphate[2] have proved no better than magnesium oxide as reference standards The difficulties are enhanced if the standard is required to be applicable over a wider wave-length range, for example 0 3–2 6μ At some time in the future a theoretical perfect diffuser will be adopted as reference (C I E recommendations, 1959) but at present there is no practical alternative to magnesium oxide

The purpose of this note is (1) to describe briefly an improved electrostatic precipitation process which can be used to produce standards indistinguishable in reflectivity over the spectral range 0 3–2 6μ when they are examined in a Beckman *DK*2 ratio-recording spectrophotometer with an integrating sphere attachment, (2) to give some results on the effect of changes in the density of packing of magnesium oxide beds on their reflectance and to indicate their relevance to the choice of conditions for preparing standards from powder materials

Electrostatic deposition of magnesium oxide is carried out on each of a pair of flat silver disks previously polished to match in reflectance at 0 5μ and then fitted into aluminium cylinders to form cups 2 mm deep Each disk in turn is held facing downwards at an angle of 45° to the vertical and maintained at a voltage of between 6 and 10 kV with reference to an earthed electrode with a sharply pointed end 5–8 cm distant from the disk so that a brush discharge takes place without sparking Burning magnesium ribbon is held in an earthed holder below the pointed electrode so that the smoke from the uprushing convexion current is diverted by the electric field and deposited on the silver surface At intervals the beds of powder are compared with one another, and also separately with an opal glass tile, for reflectance over

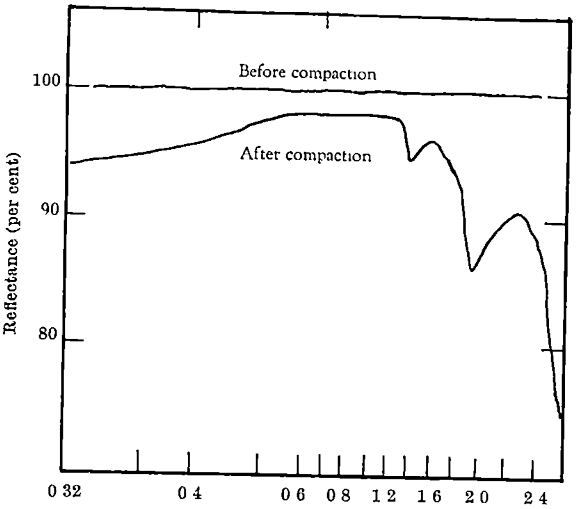

Fig 1 The effect of a change in apparent density from 8 7 to 30 per cent v/v on the reflectance of a standard prepared from magnesium oxide smoke

the whole wave-length range Further deposition is carried out until the beds show equal reflectance and the relative reflectance of the opal glass shows a minimum value at every point in the range The depth of bed required to attain these results lies between 1 7 and 2 0 mm Such standards can be used in dry dust-free conditions for about two weeks before replacements are necessary Beds prepared at different times over a long period have given the same values for a carefully preserved and cleaned opal glass tile, indicating that the method of preparation is highly reproducible

The effect of compacting such a bed under a pressure of 1,000 lb /in ^{2}, which changes the initial apparent density of 8 7 per cent v/v to about 30 per cent v/v, is shown in Fig 1 There is a marked loss of reflectance in the ultra-violet and near infra-red regions The specific absorption bands at 1 4 and 1 9μ, which are probably due to water[3] present as moisture in the original powder bed, become noticeable when the absorption to scattering ratio changes on compaction of the bed

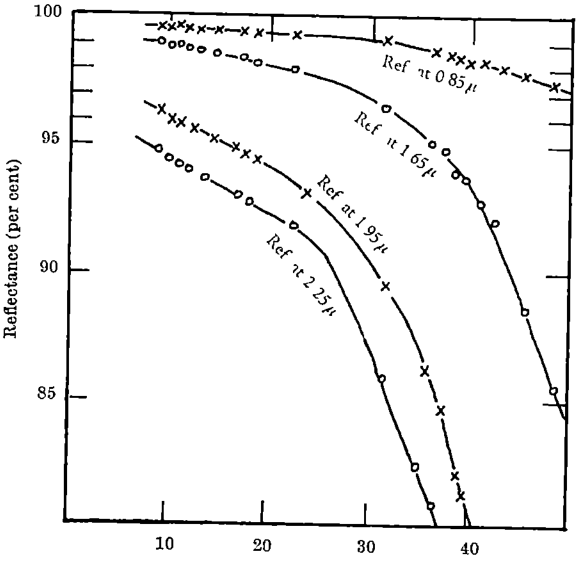

Fig 2 The effect of changes in apparent density on the reflectance at various wave-lengths of a thick bed of magnesium oxide powder having a maximum particle size of 1 0μ

Changes of reflectance with apparent density were studied with beds of light magnesium oxide (analytical reagent grade) of particle size under 1μ The rate of change of reflectance with density (Fig 2) is slow in the density range associated with the high bulkiness of small particle size powders[4] and increases rapidly as close-packing particle-to-void ratios are approached

With reflectance standards based on powder beds highest reflectance coupled with the slowest rate of change of reflectance with density is desirable The results given here show that this condition corresponds with low apparent density and can be obtained by the electrostatic technique described

J T McALOREN

The Chemical Inspectorate,
War Office,
Royal Arsenal,
Woolwich, London, S E 18

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Oxygen Effect as an Explanation of Differences between the Action of α-Particles and X- or γ-Rays on Aqueous Solutions of Amino-acids and Proteins

Alexander and Rosen, working with oxygen-free aqueous solutions of tryptophan and other substances, have discovered certain differences between the action of α-particles and of X-rays[1] They have also found such effects with proteins[2] In the case of aqueous solutions of benzene, the greater effectiveness of α-particles compared with X-rays in causing ring splitting (under oxygen-free conditions) is probably associated with the fact that the α-particles are producing relatively large amounts of oxygen simultaneously with free radicals, so that α-irradiation in the absence of added oxygen resembles X- or γ-irradiation in the presence of oxygen[3] This explanation superseded a previous view that the α-particles were producing some more specific effect The irradiation of tryptophan resembles that of benzene in that tryptophan undergoes ring splitting to formylkynurenine on X-irradiation in the presence of oxygen, but not in the absence of oxygen[4], suggesting that an oxygen effect (or perhaps a hydrogen peroxide effect) might explain Alexander and Rosen's results It would then be unnecessary to postulate the formation of activated hydrogen peroxide by α-particles In order to obtain further evidence on this question, we have studied further the effect of oxygen on the γ-irradiation of aqueous tryptophan

Fig 1 shows the difference in extinction coefficient between the irradiated and unirradiated solutions for 2 × 10^{-4} *M* tryptophan irradiated with γ-rays (66,400 rads) in the absence of air, in the presence of air, and in the presence of 10^{-3} *M* hydrogen peroxide (air absent), Our results with γ-rays in the absence of oxygen are in reasonable agreement with Alexander and Rosen's results with X-rays under nitrogen, considering the smallness of the change produced (the loss in absorption at 279 mμ is about 1 5 per cent)

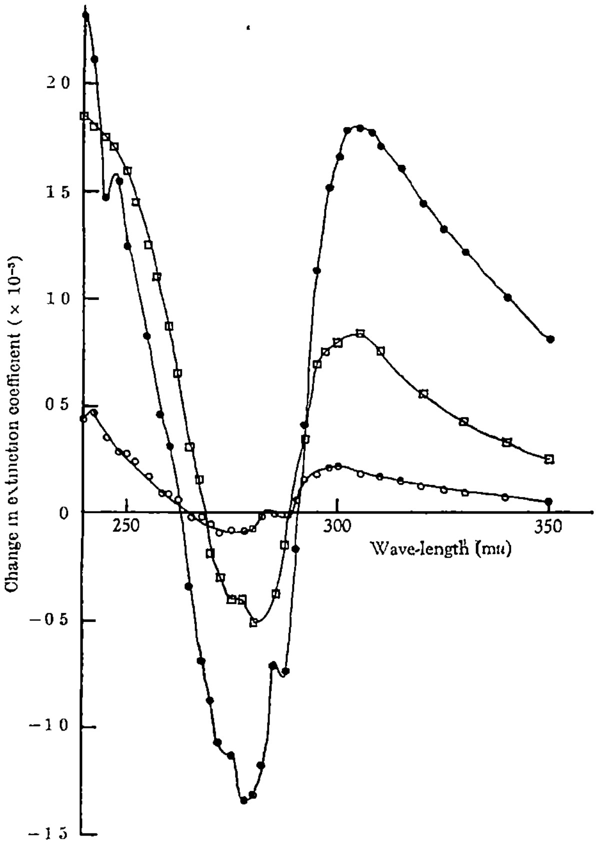

Fig. 1. Difference between extinction of γ-irradiated tryptophan (2×10^{-4} *M*) and unirradiated tryptophan. ○, irradiated in absence of air and hydrogen peroxide; ●, irradiated in presence of air; ☐, irradiated in presence of H_2O_2 (10^{-3} *M*)

Our results with γ-rays in the presence of oxygen or hydrogen peroxide show similar qualitative features to Alexander and Rosen's results with α-particles under nitrogen, that is, compared with the result of X- or γ-irradiation in the absence of oxygen, they show a larger drop in extinction coefficient near the tryptophan absorption peaks at 279 and 287 mμ, a larger rise below about 270 mμ, and an increased production of a substance or substances absorbing above 300 mμ. This observation suggests that the α-particles are indeed acting through the formation of oxygen and/or hydrogen peroxide from the water, simultaneously with free radicals. It should be noted also that previous work on the α-irradiation of aqueous solutions has also been interpreted in such terms[5,6].

We have measured the loss of tryptophan by a method described by Spies and Chambers[7]. Results are shown in Fig. 2. For γ-irradiation in the absence of air, the initial yield for loss of tryptophan was *G* = 1·2, in fair agreement with Alexander and Rosen's result for X-irradiation in the absence of air of *G* = 0·92[1]. Dissolved air increased the initial yield with γ-rays to *G* = 2·0. The yield with α-particles in the absence of oxygen was *G* = 1·8[1]. It is somewhat surprising that the α-particle yield should be so much more than the yield with X- or γ-rays in the absence of oxygen, since although the formation of oxygen and/or hydrogen peroxide would be expected to enhance the yield, radical production with α-particles is less than a fifth of that with X- or γ-rays. Perhaps a short chain reaction involving hydrogen peroxide occurs during α-irradiation. Ribonuclease (0·1 per cent) protects tryptophan (2×10^{-4} *M*) against radiation[1]. Alexander and Rosen found that ribonuclease reduced the yield for destruction of tryptophan by X-rays to 35 per cent of its value in the absence of ribonuclease, and the yield for destruction by α-particles to 63 per cent[2]. We have found a reduction to 50–60 per cent for γ-irradiation both in the absence and in the presence of air. The poor agreement between Alexander and Rosen's result with X-rays and our result with γ-rays makes it difficult to draw conclusions from these results.

The ammonia yield from γ-irradiated tryptophan (2×10^{-4} *M*) has been measured using Nessler's reagent. The yield is linear up to about 30,000 rads both in the absence and in the presence of air. The initial yield in the absence of air was *G* = 1·3 compared with *G* = 1·8 found for X-rays in the absence of air by Alexander and Rosen. Our initial yield for γ-rays in the presence of air was *G* = 6·8. Alexander and Rosen found *G* = 1·8 for α-irradiation in the absence of air. We suggest that this yield is qualitatively consistent with the decreased yield of radicals produced by α-particles compared with X- or γ-rays, coupled with the enhancing effect of the oxygen and/or hydrogen peroxide simultaneously produced.

Alexander and Rosen report that oxygen produced less than 5 per cent change in the α-irradiation of aqueous serum albumin, as determined by the change in ultra-violet absorption[2]. This would be expected if the α-particles produce their own oxygen and/or hydrogen peroxide. They state that oxygen produced less than 25 per cent change in the effect of X-irradiation. This would be consistent with a fairly large increase in true ultra-violet absorption due to an oxygen effect counterbalanced by a decrease in that part of the absorption which is due to light-scattering, the overall effect of X-irradiation in the presence of oxygen being qualitatively similar to, but quantitatively greater than, the effect of α-irradiation whether in the presence or absence of oxygen.

We conclude that the effects of α-particles on amino-acids and proteins in aqueous solutions can be interpreted in terms of the simultaneous production of free radicals and of oxygen and/or hydrogen peroxide from the water. If this view is correct, then oxygen or hydrogen peroxide would be expected to have a more important influence on details of the X- or

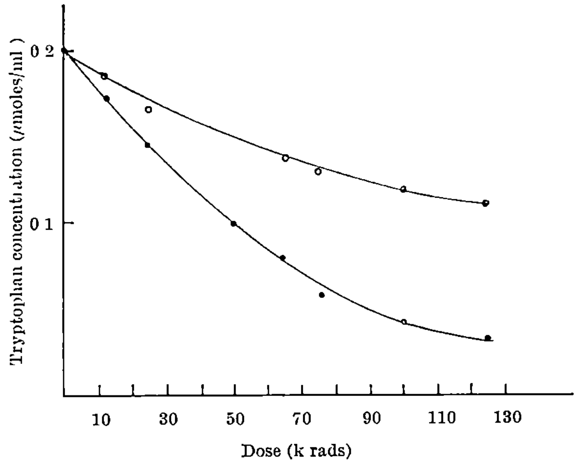

Fig. 2. Loss of tryptophan during γ-irradiation. ○, irradiated in absence of air; ●, irradiated in presence of air

γ-irradiation of aqueous solutions of proteins than is sometimes supposed.

A. J. Swallow
J. A. Velandia*

Nuclear Technology Laboratory,
Department of Chemical Engineering
and Chemical Technology,
Imperial College of Science and Technology,
London, S.W.7

* Present address: Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

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N-H Stretching Frequencies of Amines and Amides

Bellamy and Williams[1] found that for primary amines in which both N–H bonds are equivalent and mass and coupling effects negligible, the symmetric (ν_s) and asymmetric (ν_{as}) NH_2 stretching frequencies were related by equation (1) with a standard deviation of 4.8 cm⁻¹ and in the more restricted case of *meta* and *para* substituted anilines, Krueger and Thompson[2] found that equation (2) was obeyed with an average error of less than 1 cm⁻¹.

$$\nu_s = 0.876\,\nu_{as} + 345.5 \qquad (1)$$

$$\nu_s = 0.682\,\nu_{as} + 1{,}023 \qquad (2)$$

For primary amides, Puranik and Ramiah[3] deduced the following equation.

$$\nu_s = 1.1214\,\nu_{as} - 542.5 \qquad (3)$$

Krueger[4] has recently interpreted the lower slope of the aniline correlation (2) as opposed to the general amine correlation (1) and amide correlation (3) and the fact that the line relating ν_s and ν_{as} given by equation (3) is displaced from equation (2) as evidence for a systematic change of sp^3/sp^2 character of the amino-group in the aniline series as the electron withdrawing or donating power of the substituent alters. However, the displacement of line (3) is probably related to a dipolar field effect on the N–H bond which is *cis* to the carbonyl group[5]. For example, many secondary amides exhibit rotational isomerism and the N–H stretching bands are characteristically different[6] (*cis* amides, 3,420–3,440 cm⁻¹, *trans* amides, 3,440–3,460 cm⁻¹). Although steric effects may contribute to this shift in some compounds by a change in planarity and consequent change in resonance effect, this factor is not likely to be large where one of the groups is small. For example, formanilide[7] shows two N–H absorptions separated by 26 cm⁻¹, and for a typical value of ν_{as} (3,500 cm⁻¹) the displacement between ν_s in equations (2) and (3) is 27.6 cm⁻¹. Although the agreement may be to some extent fortuitous, unambiguous evidence[8] for the non-equivalence of the N–H bonds in primary amides is provided by the spectra of partially deuterated amides which show two N–H stretching bands 20–30 cm⁻¹ apart due to the NHD group, arising from the *cis* and *trans* relationship of the N–H bond to the carbonyl group. Steric effects could not account for this difference. A similar *cis-trans* isomerism of NHD groups has already been shown for *intra*molecularly hydrogen bonded amines[5,9]. Part of the change in slope for amides may arise from the variation of this interaction due to changes in polarity of the C = O bond.

The change in sp^2/sp^3 character in substituted anilines which Krueger deduces from the change in slope of equations (1) and (2) is more clearly evident from the N–H stretching frequencies of aliphatic and aromatic amines[10]. For example, pyrrole shows an N–H stretching band at 3,490 cm⁻¹ and in this case the nitrogen σ-bonds are close to planar (sp^2 hybridized). Secondary aliphatic amines show bands near 3,350 cm⁻¹ (in which the nitrogen is almost certainly sp^3 hybridized) and aromatic amines near 3,430 cm⁻¹ in which the nitrogen hybridization is intermediate. Similar values are obtained for primary amines if the means of ν_{as} and ν_s are taken[11]. The large shift in N–H stretching frequency between amines in which the nitrogen atom is sp^2 and sp^3 hybridized (about 140 cm⁻¹) is similar to that observed between the C–H stretching frequencies of saturated and unsaturated hydrocarbons (sp^2 and sp^3 hybridized carbon atoms, about 130 cm⁻¹)[12]. A systematic change in sp^2/sp^3 character in aniline derivatives cannot alone, therefore, account for the change in slope as the large changes in N–H stretching frequencies in the general amine correlation (1) are already due to a change in sp^2/sp^3 character.

Krueger also suggests that *ortho* substituents involving strong hydrogen bonds appear to lead to planar molecules in which the HNH bond angle may exceed 120°, for example, *ortho*-nitroaniline, 121.7°, and ethylanthranilate, 123.6°. Similar calculations for other hydrogen bonded systems such as 1-amino-anthraquinone[5] (138.2°) and 1-phenylazo-2-naphthylamine[9] (ν_{as} = 3,478 cm⁻¹, ν_s = 3,230 cm⁻¹, HNH bond angle insoluble) leads to the conclusion that the equations of Linnett[13] are inapplicable where the two N–H bonds are non-equivalent. Little reliance can, therefore, be placed on the calculations of bond angles where intramolecular hydrogen bonding occurs. It has already been shown, however, that good evidence for the planarity or near planarity of the amino group in many intramolecularly hydrogen bonded compounds is provided by the *trans* N–H stretching frequency of the NHD group in the monodeuterated derivative[5,9].

I thank the Royal Commissioners for the Exhibition of 1851 for the award of a scholarship.

A. G. Moritz*

Organic Chemistry Research Laboratories,
Imperial College,
London, S.W.7

* Present address: Defence Standards Laboratories, Maribyrnong, Melbourne, Australia.

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Evaluation of Gel-Filtration Media by Polymer Fractionation

GEL filtration is being examined as a possible method of fractionating polymers (for example, polystyrene) which dissolve in non-aqueous solvents. The gels used with water-soluble compounds (for example, starch[1] and cross-linked dextrans[2]) are not suitable for use in non-aqueous solvents, and a major part of the research has been concerned with the evaluation of other materials.

The exact mechanism of gel filtration is not clearly understood, but it appears to be a type of molecular sieving in which solute molecules penetrate the stationary phase (that is, the gel particles) and are retarded differentially. When a solution of molecules of different size is passed through such a column, some of the smaller molecules are retarded so that the average molecular weight of the first fraction to be eluted is higher than that of the original mixture while that of the last fraction is lower. It follows, therefore, that the difference between the two values for the highest and lowest fractions is an indication of the pore sizes in the gel (that is, the greater the differences, the greater the number of molecules retarded and therefore the larger the pore sizes). If no fractionation occurs it may mean that the pore sizes are too small (that is, no molecules retarded) or, on the other hand, it could mean that the pores are so large that all the molecules are retarded equally.

In these experiments a sample of a very heterodisperse polystyrene[3] (molecular weights ranging up to 10^6) was loaded on to the top of a column of the material under test and was eluted with benzene. The polymer fractions were recovered by freeze drying and characterized by single point viscosity measurements, the results being expressed as reduced viscosity η_{sp}/C, a quantity related to the viscosity average molecular weight.

Initially swollen cross-linked polystyrene beads[4] and polyethylene gels were investigated since it was thought that in benzene these materials would have structures similar to the aqueous gels, cross-linked dextrans and starch. Good fractionation results were obtained (Table 1) but the most highly swollen gels tended to pack down and/or block the supporting glass sinters.

Table 1

Media used to pack the column	Fractions Highest $\eta sp/C$	 Lowest $\eta sp/C$
References		
Whole polymer	1.08	1.08
Solvent volatilization method (ref. 3)	3.13	0.17
Gels		
Polyvinyl alcohol	1.25	0.72
Cellulose acetate	1.65	0.69
Polyethylene (high density)	1.28	0.85
Polyethylene (low density)	1.88	0.53
Cellulose	2.35	0.55
Cross-linked polystyrene beads	2.53	0.24
Silica gel 'Santocel *A*'	3.78	0.13
Other materials		
Crushed sintered glass porosity, 40μ	1.02	1.05
Corning porous glass No. 7930 porosity 40 Å	0.99	1.08
Porous pot	1.21	0.92
Fire Brick Johns Manville *C*22	1.82	0.80
Molecular sieve type 5*A*	1.59	0.90
Porous cement	1.63	0.84
Activated charcoal	1.33	0.99

Cellulose gels (Table 1) formed by the regeneration of gelled viscose solutions are firmer but the most porous ones have the disadvantage of tending to contract in the presence of benzene. It was decided, therefore, to look at even more rigid structures.

Porous glass would be ideal for gel filtration since presumably it could be used in a wide variety of conditions. Unfortunately, no fractionation was observed (Table 1) with (1) the Corning porous glass No. 7930 (porosity 40 Å) or (2) crushed sintered glass (porosity 40μ). However, since in one case the pores may be too small and in the other case too large, it seems likely that a glass with the appropriate porosity can be prepared. Meanwhile other porous materials have been examined.

Best results have been obtained with highly expanded silica gels which have a stability approaching that of glass showing little tendency to contract in a large number of solvents over a wide temperature range. With the gel 'Santocel *A*' the efficiency of fractionation was of the same order as that found with a conventional precipitation method[3] (Fig. 1), and it is suggested that columns of this material could be used for routine polymer fractionations. However, the use of these materials is not restricted to polymers, for silica gels can be prepared with a very wide range of porosities suitable for almost any molecular weight range.

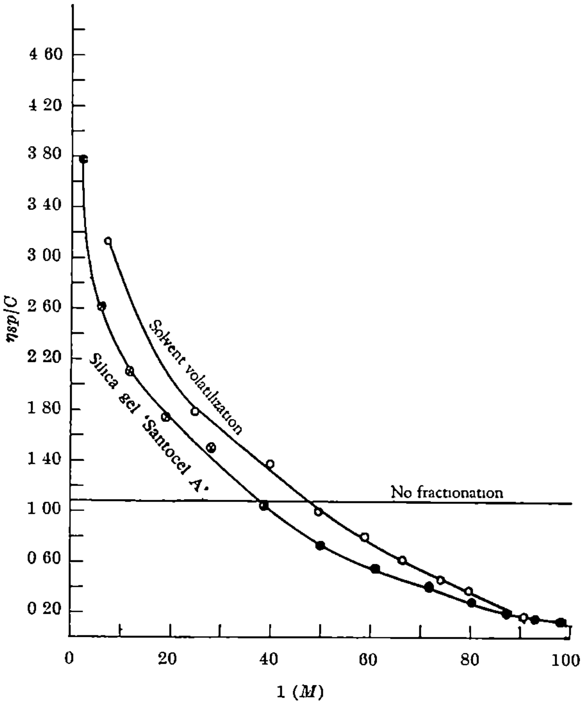

Fig. 1. Fractionation with silica aerogel 'Santocel *A*'. I (*M*), Cumulative weight fraction calculated for each fraction as total weight of all preceding fractions plus half the weight of the fraction, and then divided by total weight of all the fractions

Most of the other materials tested were very stable but the degree of fractionation obtained was poor. However, in some cases (for example, the Linde type molecular sieves, Johns Manville firebrick, etc.) the differences between the highest and lowest values of $\eta sp/C$ were large enough to indicate that these materials might be useful for the separation of lower molecular weight materials.

I thank Miss S. A. Monksfield and Mrs. H. M. Aplin, who carried out the fractionation experiments, Mr. R. G. Goldsmith, who prepared the gels and some of the other materials, and Monsanto Chemicals, Ltd., for the gift of the 'Santocel *A*'. This work is pub-

lished by permission of the Director of the National Chemical Laboratory.

M. F. Vaughan

National Chemical Laboratory,
Department of Scientific and Industrial Research,
Teddington, Middlesex

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RADIATION CHEMISTRY

Action of Ionizing Radiation on Rat Liver Cell Nuclei

The site of attack by OH radicals on the various components of some cell nuclei has been explored using the radiation-induced carboxylation technique recently described[1]. This involves irradiation of oxygen-free suspensions of the nuclei in the presence of sodium bicarbonate, under conditions where the radicals can react with the nuclear constituents. The negative polarons[2], $(H_2O)^-$, produced from the water react with the bicarbonate according to

$$(H_2O)^- + HCO_3^- \rightarrow CO_2^- + OH^- + H_2O \qquad (1)$$

In general, carboxylation depends on the formation of a free radical from an organic molecule (RH), for example, by the process

$$RH + OH \rightarrow R + H_2O \qquad (2)$$

and association of the free radical with the CO_2^- radical ion according to

$$R + CO_2^- \rightarrow RCO_2^- \qquad (3a)$$

$$RCO_2^- + H^+ \rightleftarrows RCO_2H \qquad (3b)$$

In order to assay the carboxylic acids produced in the various components of the nuclei, bicarbonate labelled with carbon-14 was used.

Nuclei were prepared from rat-liver by Dounce's method[3], involving homogenization with dilute citric acid at pH 4.0. The isolated nuclei were then suspended in phosphate buffer (4×10^{-3} M, pH 8.0) containing labelled sodium bicarbonate (6×10^{-3} M, 5 μc./ml.). The suspensions of the nuclei (0.2 mg/ml.) were evacuated and then irradiated with cobalt-60 γ-rays. After irradiation the nuclei were precipitated with perchloric acid (final concentration 0.4 M). Aliquots were assayed for activity after taking to dryness on planchets. The remaining nuclei were treated as follows. Lipids were extracted with ethanol and ethanol/ether (3:1) at 55° C. Nucleic acids (DNA and RNA) were then extracted by heating twice with 10 per cent sodium chloride at pH 8.0, and precipitated with ethanol. RNA was removed by treatment with 0.2 N sodium hydroxide at 80° C and the DNA reprecipitated. Removal of small amounts of contaminating protein from the DNA was achieved by incubation with pronase. Aliquots of each component were counted.

Table 1 records some of the results obtained. The actual experimental figures are given in terms of counts/min per mg of each component. The percentage of total activity in each fraction is also shown in Table 1. It can be seen that, of the total extent of carboxylation, only 3–4 per cent go into the DNA fraction, by far the major reaction occurs in the protein and lipids.

Table 1. Irradiation (cobalt-60 γ-rays) of suspensions of rat liver nuclei (0.2 mg/ml.) in phosphate buffer (4×10^{-3} M, pH 8.0) and in the presence of ^{14}C-labelled sodium bicarbonate (6×10^{-3} M, 5 μc./ml.), dose-rate 1.15×10^4 rads/min. (The figures in the parentheses give the percentage of total incorporation in each fraction)

Dose (rads $\times 10^{-4}$)	Total* (c.p.m./mg)	Lipids (c.p.m./mg)	Protein (c.p.m./mg)	RNA (c.p.m./mg)	DNA (c.p.m./mg)
3.45	342 (100%)	3,544 (31.2%)	216 (53.6%)	183 (1.1%)	110 (3.2%)
11.5	1,095 (100%)	9,688 (26.7%)	748 (57.8%)	398 (0.8%)	401 (3.6%)

* Recovery was about 90 per cent of the total activity.

The composition (per cent) of these nuclei was approximately. protein 85, DNA 10, RNA 2, lipid 3. Thus, even though the lipid fraction makes up only about 3 per cent of the bulk of the nuclei, it accounts for 30 per cent of the total incorporation, this is demonstrated by the high values of the counts/min per mg for this particular fraction. Such a high reactivity of the lipid material may be associated with its chemical nature as well as with the fact that it is predominantly in the nuclear membrane, and may thus 'trap' OH radicals generated in the ambient medium.

If one assumes that the different proteins present in the cell nuclei have undergone about the same degree of carboxylation, it would follow that in the protein, which is possibly more specifically associated with the DNA about 7 per cent of the incorporation has occurred, that is, the relative extent of carboxylation of the DNA and of the protein in the complex is in the ratio 1:2.

It should be pointed out that there may be certain qualifications on any absolute interpretation of the results obtained by the carboxylation technique. In particular, carboxylic acids produced at certain sites may be unstable and, furthermore, steric hindrance and other chemical factors may affect the reaction of the CO_2^- radical ion. The extent of such interference is, at present, unknown. Nevertheless, this appears to be a useful technique for a preliminary survey of certain features of the effects of ionizing radiations on more complex systems.

We thank the Rockefeller Foundation, the Northern Council of the British Empire Cancer Campaign and the Medical Research Council for financial support.

G. Scholes
J. J. Weiss
C. M. Wheeler

Department of Chemistry,
King's College,
University of Durham,
Newcastle upon Tyne, 1

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BIOCHEMISTRY

Appearance of New *N*-Terminal Groups by the Action of Rennin on Casein

Wissmann and Nitschmann[1], studying the action of rennin on α-casein by the method of Sanger[2], found, after the action of rennin for 30 min., not only the original *N*-terminal amino-acids arginine and lysine but also a new *N*-terminal amino-acid,

Table 1 NUMBER OF *N*-TERMINAL GROUPS (MOLE/10^5 GM DNP-PROTEIN) IN CASEIN AND PARACASEIN, DETECTED BY THE FLUORO-DINITROBENZENE METHOD

	Transformation casein→paracasein				Proteolysis of paracasein		
	DNP-casein	DNP-paracasein			DNP-paracasein		
Incubation time	0 min	10 min	30 min	90 min	1 day	3 days	7 days
Arginine	2 4	3 5*	4 5*	4 2*	4 7*	4 6*	3 7*
Lysine	0 30	0 37	0 28	0 30	0 25	0 64*	0 90*
Glutamic acid	0 11	0 16	0 11	0 10	0 12	0 24*	0 32*
Serine	(+)	(+)	(+)	0 20	0 12	0 13	0 23*
Methionine	(+)	(+)	(+)	(+)	0 14	0 60*	0 83*
Phenylalanine	(+)	(+)	(+)	0 16*	0 57*	0 99*	1 06*
Alanine	(+)	(+)	(+)	(+)	(+)	0 11*	0 25*
Aspartic acid	(+)	0 13	(+)	(+)	(+)	(+)	0 10
Leucine	(+)	(+)	(+)	(+)	—	—	—
Threonine	(+)	(+)	(+)	(+)	(+)	—	0 15*
Glycine	(+)	(+)	(+)	(+)	(+)	(+)	0 11
Cystine	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Valine	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Total	2 81	4 16	4 89	4 96	5 90	7 31	7 65

Amounts smaller than 0 1 mole/10^5 gm DNP-protein are indicated by (+)

Correction factors for losses of DNP-amino-acids during hydrolysis were determined for DNP-casein All values were corrected with these factors

The increase of end groups as a whole after 10 min of rennin action is significant at the 5 per cent level, even if arginine is not considered

* Values which in the *t*-test differ significantly at the 5 per cent level from the corresponding values at zero minutes

namely, phenylalanine Thus these workers concluded that the non-protein nitrogen which is formed by the transformation α-casein → para-α-casein + non-protein nitrogen, results from the rupture of a peptide bond

By comparing the amounts of non-protein nitrogen which are set free after the action of rennin on different casein components, Wake[3] showed that the non-protein nitrogen originates from the κ-fraction This author also used Sanger's method to determine whether or not the release of non-protein nitrogen resulted from rupture of a specific peptide bond Observations made before and after rennin treatment of the κ-casein gave no indication of any difference in *N*-terminal groups Thus it was worth while re-investigating the problem of whether or not the splitting reaction is actually a specific proteolysis

We describe here the proteolytic activity of rennin, if any, during the formation of paracasein, and also our investigation of the effect of rennin on paracasein during a prolonged period of incubation The latter was done in order to obtain a decisive answer to the suggestion of Wake that the results of Wissmann and Nitschmann were due to proteolysis by rennin as the *p*H was lowered nearer to its point of optimum during the process of isolating the para-α-casein

The sodium-caseinate used in this investigation was prepared by the method of McMeekin *et al* [4], which avoids inclusion of milk protease For the incubation with rennin a 10 per cent solution of whole casein was prepared by dissolving the dry protein in distilled water by addition of 0 1 *N* sodium hydroxide and adjusting the solution to *p*H 6 6 The rennin was purified by zone electrophoresis to be described by Payens[5] Before incubation the rennin was dissolved in acetate buffer of *p*H 5 6 In these experiments 100 mgm /ml of casein solution was incubated with 0 004 mgm /ml of purified rennin at 30° C The activity of the rennin was established at 2,910,000 units by comparison with a standard preparation The rennin units used in this investigation are defined as the number of grams of normal milk which can be clotted at 35° C within 40 min by 1 gm of rennin

After the incubation the rennin was inactivated by heating at 70° C for 15 min Thereafter, each solution was dialysed for 48 hr against distilled water at 4° C and freeze-dried During incubation care was taken to prevent growth of micro-organisms

In order to follow the hydrolysis of paracasein, end group analysis was carried out according to the method of Fraenkel-Conrat *et al* [6], except that the determination of dinitrophenyl (DNP)-arginine was estimated by Biserte's method[7] The release of non-protein nitrogen from casein immediately after addition of rennin was followed by the method of Alais *et al* [8] The maximum yield of non-protein nitrogen estimated by this method was reached after 10 min It is generally assumed that within the same time the formation of paracasein from casein is completed Thus if the splitting reaction is based on the rupture of a peptide bond the appearance of a new *N*-terminal group might be expected within this time It is emphasized that even in the case that only one peptide bond per protein molecule is split, the method for end group analysis is sensitive enough to detect this extent of hydrolysis

As shown in Table 1, a significant increase occurred only in *N*-terminal arginine during the first 30 min An explanation of these results can be given in two different ways First, it may be suggested that during the splitting reaction peptide bonds are broken and new *N*-terminal arginine groups are set free It is also possible that by the action of rennin the *N*-terminal arginine groups become more easily available The latter would be in accordance with a recent suggestion of Alais and Jollès[9] that the liberation of non-protein nitrogen results from the splitting of an ether group between *N*-acetylneuraminic acid and a galactose group of the casein If this is true, no new end groups would be expected when rennin acts on κ-casein, as found also by Wake Considering the data in the literature and our own work, we came to the following interpretation

κ-casein can be considered as the factor which is responsible for the micelle stabilization of the casein complex[10] Through the action of rennin κ-casein is transformed and can no longer act as a protective colloid which encloses the calcium-sensitive groups of the casein complex As a result of this action the end groups of the casein components become more susceptible to attack by fluoro-dinitrobenzene and an increasing amount of arginine, which is the original end group, can be detected

Following the action of rennin with prolonged incubation a specific proteolysis of the paracasein is observed Especially those peptide bonds are split where methionine and phenylalanine are enclosed (see

Table 1) However, this specific protease activity could be detected only after an incubation time which was much longer than the minimum time for rennet clotting

On the basis of these results it is apparent that if the amount of phenylalanine found by Wissmann and Nitschmann was liberated by an increase of the proteolytic activity of the rennin, an increasing amount of methionine should also have been found Since Wissmann and Nitschmann did not find such a liberation of methionine, Wake's suggestion seems to be invalid

We thank Prof R Jenness, of the University of Minnesota, for his help, Dr T A J Payens for supplying purified rennin samples, and Mr H P Wijnand for technical assistance and statistical analysis of the results

P J de Koning
J Stadhouders

Netherlands Institute for Dairy Research,
Ede, Holland

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Determination of Polygalacturonase in Fruits

The action of polygalacturonase[1] is most commonly investigated either by viscometry[2] or by the Willstatter-Schudel hypoiodite procedure[3] Recently, Foda[4] showed that the enzyme action was more conveniently and rapidly measured by using a colorimetric method based on that of Willaman and Davison[5]

A highly efficient extraction of polygalacturonase from a number of fruits has been made by the following method 30 gm of the macerated tissue were thoroughly stirred with 2 25 gm of a 10 1 (w/w) mixture of sodium chloride and the disodium salt of ethylenediamine tetraacetic acid, and then kept at 3° C for 15 min For macerates with a low dry matter content, the mixture was then centrifuged and the supernatant liquid filtered off To samples with a higher content of total solids, 20 ml of a 7 5 per cent solution of the salt mixture was stirred in first, left for a further 15 min and then centrifuged and filtered In all cases the remaining slurry was extracted with additional 20 ml portions of the salt solution until at least 60 ml had been collected All extracts were kept below 3° C The procedures used by Foda[4] for precipitating, washing and dissolving the enzyme were not altered

In the determination of polygalacturonase in plant extracts it is usual for the aliquots taken from the reaction mixture at various time intervals to be made either strongly acid[4] or alkaline[6], in order to stop further enzyme activity But exposure of the partially purified enzyme to *p*H 0 6 and to *p*H 10 0 has been shown to reduce but not eliminate the activity[7] The surface active agent 'Teepol', added to the sample to a concentration of 1 0 per cent (v/v), is, however, completely effective in preventing continued enzyme action

The substrates of Jansen acid[6], Ehrlich acid *C*[8], Wichmann pectic acid[8], McCready acid[9] and partially neutralized McCready acid[10], in comparable concentrations, were reacted with standard amounts of polygalacturonase from tomato fruit The five acids showed a decreasing rate of reaction in the above order, with the first three substrates mentioned being almost equally acceptable to the enzyme Hence the utilization of Wichmann pectic acid, the most usual substrate for polygalacturonase, was continued

In the method of Foda[4] the reducing groups in 1 ml of the samples of the reaction mixtures were estimated by heating them for 1 hr at 100° C with 2 ml of saturated picric acid and 1 ml of 20 per cent (w/v) sodium carbonate in a stoppered graduated test-tube After cooling, the reactants were diluted to 10 ml with water But the excess substrate formed a precipitate at this stage and the turbidity thus produced affected the subsequent absorption readings By diluting with absolute ethanol instead of water, however, it was found that the pectic acid could be coagulated The tubes were then shaken, left for 1 hr at 3° C with occasional stirring, centrifuged, decanted and centrifuged again, resulting in an optically clear solution A series of standard solutions containing 0–0 80 mgm of α-D-galacturonic acid monohydrate per ml and 1 0 per cent (v/v) 'Teepol' gave a linear increase in optical density There was also a straight line relation between the enzymatic production of reducing end groups and the time of reaction, provided that the increase in reducing power from a 0 25 per cent (w/v) solution of Wichmann pectic acid was kept within the above limits

Table 1 Activity of Polygalacturonase in Various Fruits

Common name	Scientific name	Percentage of dry matter in the macerated fruit §	Activity
Tomato (variety 'Potentate')*	*Lycopersicon esculenteum*	6 1	3 68
Avocado†	*Persea gratissima*	22 3	0 24
Medlar†	*Mespilus germanica*	24 4	0 10
Pear (variety 'Doyenne du Comice')*	*Pyrus communis*	15 8	0 06
Pineapple†	*Ananas comosus*	19 6	0 09
Cranberry*	*Vaccinium macrocarpum*	12 9	0
Persimmon*	*Diospyros virginiana*	20 7	0
Cucumber (variety 'Butcher's Disease Resister')*	*Cucumis sativus*	4 4	0
Tangerine†	*Citrus nobilis*	12 1	0
Carrot*	*Dorcus carota*	9 8	0
Melon†	*Cucumis melo*	15 5	0
Grapes‡	*Vitis vinifera*	21 3	0

* Whole fruit
† Flesh only
‡ Whole fruit with seeds removed
§ Dried for 48 hr at 80° C in a forced draught oven

The activity is given as increased reducing power, expressed as gm galacturonic acid monohydrate per 100 gm fresh weight of ripe fruit per hour

A measure of the activity of polygalacturonase to be found in a number of species, using these modifications, is given in Table 1 Although claims for the presence of the enzyme in both cucumbers[11] and carrots[12] have been made, these were not confirmed The activities previously shown were obtained only through the use of long reaction periods in conjunction with viscometry in the former and chromatography in the latter case No attempt has yet been made to find how the activity of the enzyme is affected by the inhibitors which are undoubtedly present in some fruits[13]

G E Hobson

Glasshouse Crops Research Institute,
Rustington, Littlehampton,
Sussex

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β-Glucuronidase in the Rat Placenta

Histochemical investigations, using the ferric hydroxyquinoline method of Fishman and Baker[1], indicate that the β-glucuronidase of the fully established rat placenta is predominantly fœtal As early as the 9-day stage there is moderate activity in the embryonic structures At 11 days an intense reaction is obtained in the endoderm of the visceral yolk sac, in the trophoblast and giant cells of the central zone and ectoplacental cone and in the endovascular plasmodium growing into the decidual arteries The activity of the visceral yolk sac remains high, with a terminal drop in the last two or three days of pregnancy The giant cells, the junctional zone trophoblast and the endovascular plasmodium, with the exception of the glycogen cells, retain a high activity until about the 19th day, but the labyrinthine activity begins to fall off at about 15 days By the 22-day stage the reaction in all the trophoblastic structures is greatly reduced

In the early stages of implantation there is considerable activity in the uterus, with a moderate reaction in the cells of the antimesometrial decidua except for the implantation zone[2] and the peripheral fibrinoid capsule[3] The reaction in the mesometrial decidua is principally confined to the endothelial linings of the blood vessels, particularly in the decidual cone[4], and to a few decidual cells The uterine muscle, the uterine epithelium and the blood vessel walls of the mesometrium react strongly throughout pregnancy, but by the 11-day stage the reaction in the decidua basalis is minimal and is restricted to the blood-vessel walls and to a few metrial gland cells The anti-mesometrial decidual cells continue to react as long as the capsularis persists, but the decidua basalis is unreactive during the whole period of its degeneration and disappearance

In view of our uncertainty of the functions of β-glucuronidase *in vivo* these findings are of some interest A possible explanation of the trophoblastic enzyme is that it is concerned with the conjugation of œstrogenic hormones reaching it from the maternal blood, thus limiting interference by maternal œstrogens in the genital development of the fœtus The total of the several placentæ of a litter must represent a significant enzyme activity and may play an important part in the conjugation of pregnancy œstrogens The reduction in the trophoblastic activity during the last few days may be related to a changing proportion of conjugated and free œstrogens in the maternal blood in the immediate *pre-partum* period, such as Cohen, Marrian and Watson[5] described in the human The enzyme in the visceral yolk sac could have a similar function to that suggested for the trophoblast, conjugating œstrogens diffusing through from the uterine lumen It does in any event seem more reasonable to suppose that the β-glucuronidase of the placenta is concerned with the formation rather than with the hydrolysis of glucuronides It may even be that the trophoblast is a 'target organ' for œstrogenic hormones and that the trophoblastic enzyme is concerned with the fixation, metabolism and physiological action of œstrogens, as Fishman[6] has suggested for sex tissues generally, instead of with their inactivation

The relatively low activity of the maternal placenta is probably due to the high level of circulating corpus luteum hormone, since there is evidence that in some situations the administration of progesterone prevents the rise in β-glucuronidase activity associated with œstrogens[6] On the other hand, the considerable reaction in the antimesometrial decidua is difficult to correlate with this It is interesting, in view of the inverse relationship reported by Harris and Cohen[7] between esterase activity and β-glucuronidase activity in the uterus, that with the exception of the visceral yolk sac, which is rich in both enzymes, there is an approximate inverse relationship between the placental distributions of naphthyl acetate-esterase and β-glucuronidase For example, esterase activity only appears in the trophoblast during the last few days of pregnancy, when the β-glucuronidase activity is falling off

D Bulmer

Department of Anatomy,
University of Manchester

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Possible Production of Several Exopenicillinases by *Bacillus cereus*

During the course of an investigation of the specificity of protein synthesis in *Bacillus cereus*, the proteins excreted by this micro-organism in exponential growth have been isolated and examined with the help of a variety of techniques Among these proteins, the enzyme penicillinase is found

Bacillus cereus, penicillinase constitutive strain derived from N C T C 569 [1], was grown aerobically in Roux flasks using the medium described by Kogut *et al* [1] and including 0 02 M citrate[2] At the end of the culture, the *p*H was lowered to 6 0 with a few ml of 3 M citric acid, and, after centrifugation in the cold, the supernatant was lyophilized immediately The powder was afterwards redissolved in a minimum amount of distilled water, and the concentrated solution so obtained submitted to a column treatment on 'Sephadex *G*-50', allowing a 73 per cent recovery of the proteins in the form of a solution at *p*H 6 10

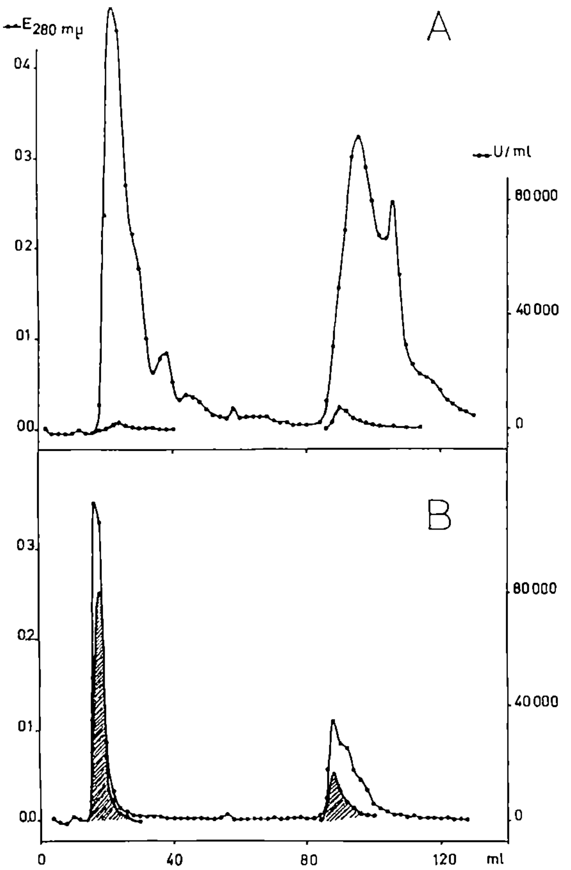

Fig 1 Chromatography on DEAE-cellulose, Cl⁻ form (1 × 32 cm), of proteins from the supernatant fluid of *Bacillus cereus* cultures (*A*) total exterior proteins, (*B*) purified penicillinase The gradient (linear change from 0 to 3 M sodium chloride over 200 ml) started at effluent volume 80 ml protein recoveries, based on the value of $E_{280\ m\mu}^{1\ cm}$ for the different fractions as compared with the corresponding value for the sample applied to the column, were quantitative in both cases Penicillinase activities, also quantitatively recovered, were determined by a microtitrimetric procedure[8], at *p*H 6 50, the units being the same as used by Perret[9] The scales are the same in both parts of the diagram, and those for ultra-violet extinction and activities were adjusted so as to conform to the data of Kogut *et al* [1] on the extinction coefficient of crystalline penicillinase at 280 mμ

in 0 005 M acetic acid–pyridine buffer, and free of other contaminants After lyophilization of the pooled protein fractions, the powder obtained was examined by column chromatography on DEAE-cellulose previously equilibrated with 0 005 M piperazine-hydrochloride buffer[3] *p*H 6 10, and using a linear sodium chloride gradient The result obtained is depicted in Fig 1*A*

It is apparent from this diagram that, under these conditions, penicillinase activity is distributed in two well-separated peaks The existence of two protein fractions exhibiting penicillinase activity can also be demonstrated qualitatively in the same material as here on agar-gel electrophoresis plates, excluding the possibility of a chromatographic artefact, and this observation is substantiated on the total exterior proteins from the constitutive strain of *Bacillus cereus* as well as on similar extracts prepared from the inducible strain grown in presence of penicillin It is, furthermore, noteworthy that purified penicillinase prepared according to Kogut *et al* [1] up to (and including) the precipitation in saturated ammonium sulphate also exhibits the same two enzymatically active fractions as here when chromatographed under the same conditions as the total exterior proteins (Fig 1*B*) , the active components, however, appear in reverse proportions It might be mentioned finally that both protein fractions are similar on the basis of their resistance to iodine[4,5], and correspond well to α-penicillinase

The possibility therefore seems to exist that penicillinase is excreted by *Bacillus cereus* in the form of two distinct protein species, at least , one of these, being positively charged at *p*H 6 10, must have an isoelectric point fairly different from that of the crystalline preparation of Kogut *et al* [1] It is not clear at present whether the two penicillinases are produced simultaneously, or if a phenomenon of limited proteolysis[6] is involved It is known, indeed, that the supernatants of *Bacillus cereus* contain a protease[2], which might resemble the subtilisin of *Bacillus subtilis* When, on the other hand, the proteins excreted by *Bacillus cereus* are isolated by successive dialyses and lyophilizations at neutral *p*H, a large number of protein species can be separated by agar-gel electrophoresis which react specifically with penicillinase-antibody, the production of which has been induced in rabbit serum by injections of the same purified penicillinase already mentioned, and several of these components seem to be still enzymatically active This suggests that penicillinase is susceptible to discrete proteolytic degradation without loosing its activity The conditions used in the present work (0 02 M citrate, *p*H 6 10), however, do not favour a proteolytic action of the subtilisin type[2,7], at least after the growth period

Further work is in progress in order to clarify this point, to characterize the two penicillinase components immunologically and to refine the chromatographic purification of penicillinase as suggested by the results of Fig 1

We thank Prof H Chantrenne for his advice The financial assistance of the Rockefeller Foundation is gratefully acknowledged

J -F Pechère*
J Zanen†

Laboratoire de Chimie Biologique,
Université Libre de Bruxelles,
Bruxelles 16

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*Chargé de Recherches du Fonds National de la Recherche Scientifique Present address Laboratoire de Biologie Générale, Université de Liege, 22 Quai Van Beneden, Liege

†Titulaire d'une bourse de l'Institut Interuniversitaire des Sciences Nucléaires

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Synthesis of 'Bacterial' Protein by Cultured Chick Cells

Primary isolates of chick embryo fibroblasts cultivated as monolayers were found to resume protein synthesis only after a delay of 24–48 hr The extent of the lag period was dependent in part on the composition of the medium with which the cells were nourished following release from the embryo by trypsinization A marked shortening of the lag was

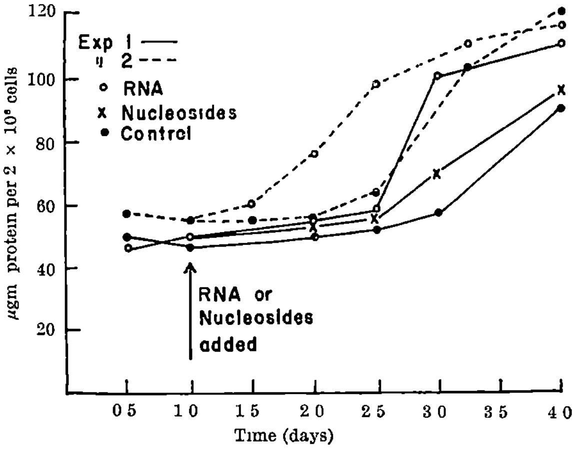

Fig 1 Protein synthesis in RNA-treated cells In both experiments 300 μgm RNA from the soluble fraction of *E coli B* was added at day 1 and remained in contact with the cells throughout Exp 1, medium contained 1 5 per cent horse serum, nucleosides 100 μgm each of four major RNA components, Exp 2, medium contained 3 per cent horse serum

observed when phenol-extracted ribonucleic acid (RNA)[1,3] was added to the medium shortly after the cells became attached to the glass (Fig 1) The effect was abolished by pretreatment of the RNA with ribonuclease and was not duplicated by combinations of nucleosides RNA preparations obtained from chick embryo, rabbit liver, or *Escherichia coli B* proved equally effective

The distribution of 'active' RNA in bacteria resembled that of the so-called 'messenger' fraction of other investigators[4,5] This similarity suggested that cells responding to heterologous RNA might be synthesizing protein characteristic of the donor cell

The protein of choice would be an enzyme produced by the donor of RNA, but not ordinarily by the recipient cell However chick cells exposed to *E coli* RNA could not be shown to synthesize β-galactosidase or ornithine transcarbamylase

A second choice was protein with the antigenic characteristics of bacterial proteins, which might be detected with antiserum directed against *E coli* protein Each RNA messenger molecule is a potential contributor of antigenic information as opposed to a small minority bearing information for the synthesis of a particular enzyme Moreover, full functional integrity of the messenger is perhaps not necessary for the replication of antigenic determinants

When protein released from chick cells by grinding or sonic treatment followed by ammonium sulphate precipitation was reacted in a complement fixation test with such an antiserum duly absorbed with chick embryo tissue, reactive material was detectable only in cells exposed to *E coli* RNA, and increased in quantity with time (Table 1) Untreated cells had no detectable amount of bacterial' protein

Table 1 COMPLEMENT FIXATION WITH PROTEIN FROM CHICK CELLS VERSUS ANTI-*coli B* ANTIBODY

		Exp. 1	Exp 2	Exp 3	Exp 4
Control cells	day 0	<1*	<1	<1	<1
	day 1	<1	<1	<1	<1
	day 2	<1	<1	<1	<1
RNA-treated cells	day 0	4	<1	4	2
	day 1	2	2	128	32
	day 2	16	16	64	32
	day 3	—	32	—	—

* Units of complement-fixing antigen per 0 5 mgm of cellular protein Values expressed as the reciprocal of the highest dilution of the protein sample showing at least 2+ complement fixation The system contained eight units of anti-*coli B* protein antiserum (absorbed with chick cell homogenate)

It remained, however, to demonstrate conclusively that the reactive protein was synthesized subsequent to the addition of RNA and did not arise from the modification of preformed protein resident in the cells or from the release of protein introduced with the RNA Convincing evidence of *de novo* synthesis might be obtained by labelling with L-leucine-^{14}C protein synthesized following contact with RNA 'Bacterial' protein isolated from other cellular proteins by precipitation with specific antiserum should be labelled if synthesized *de novo* For this purpose, uniformly labelled L-leucine-^{14}C was added to the culture medium immediately after the RNA At intervals cells were collected and the protein was liberated by sonic treatment or grinding Following separation from the cell debris by centrifugation, the protein was precipitated with ammonium sulphate and redissolved after dialysis, in veronal buffer at *p*H 7

The specific radioactivity of all the newly synthesized protein was determined, and specific precipitates were formed by reacting the cell protein with rabbit antiserum to *E coli B* protein, followed by goat or horse anti-rabbit γ-globulin The proportions of the rabbit γ-globulin–anti-rabbit γ-globulin reactants were selected for precipitin equivalence When only rabbit serum specific for *E coli* protein was added, no precipitate was formed

Whatever the source of the cellular protein, the precipitating system (rabbit serum + anti-rabbit γ-globulin) gave precipitates of similar total protein However, significant radioactivity was associated with the washed precipitate only when protein synthesis occurred in cells exposed to *E coli* RNA (Table 2)

Table 2 BACTERIAL PROTEIN SYNTHESIS DETERMINED BY SPECIFIC IMMUNOLOGIC PRECIPITATION

Treatment	Time after ^{14}C-leucine (hr)	Total counts in protein (c p m /5 × 10^7 cells)	Counts precipitated	Total counts in precipitate (per cent)
Exp 1				
None	24	8,300	250	3
RNA*	24	14,650	1,800	12
None	36	12,500	300	2 4
RNA	36	22,700	1,600	7 2
None	48	17,300	600	3 5
RNA	48	28,500	1,500	5 2
Exp 2				
None	12	800	40	5
RNA*	12	1,250	70	5 6
None	24	6,300	300	4 8
RNA	24	6,800	280	4 1
None	36	12,400	250	2
RNA	36	11,300	1,500	13
None	48	15,200	400	2 6
RNA	48	21,400	1,700	7 8

* *E coli* RNA from soluble fraction, 300 μgm /10 ml of culture remained in contact with cells throughout the experiment

RNA from chick embryos stimulated protein synthesis just as did its bacterial counterpart, but elicited no synthesis of material precipitable by the antisera utilized When the antiserum to *E coli* protein was replaced by normal rabbit serum or serum from rabbits immunized against influenza virus, the precipitates contained no significant radioactivity In response to bacterial RNA, some 5–15 per cent of newly synthesized protein could be shown to possess the antigenic character of *E coli* protein A tentative interpretation of the time-course suggests a limited period of effectiveness of the RNA

These results indicate that some molecular species of RNA obtained from *E coli B* penetrate into chick embryo fibroblasts and influence the synthesis of protein This RNA not only accelerates protein synthesis but also provides specific information for the antigenic character of the protein The latter function in intact cells resembles that observed for messenger RNA in sub-cellular protein-synthesizing systems[4,5] It also resembles the role of the RNA of plant and animal viruses in the infected cell

This function of bacterial RNA in a chick cell lends support, however modest, to the concept of a universal RNA code in biological systems The fact that the protein formed was not precipitable by specific antiserum alone and proved but weakly reactive in the fixation of complement suggests that it is not identical to *E coli* protein The evidence could be interpreted to point toward small univalent molecules (haptens) or hybrids with a minor bacterial component as the products

This work was supported by grants *E*–957 and *SF*–124–*C*3 from the National Institutes of Health, U S Public Health Service, and a grant from the Milton Fund of Harvard University

HAROLD AMOS *
KATHERINE E KEARNS
Department of Bacteriology and Immunology,
Harvard Medical School, Boston, Mass

* Senior Research Fellow of U S Public Health Service

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Effects of Standing on the Protein Extracts of Barley Grain

DURING investigations on the water-soluble proteins of barley grain we have found that a few days standing has pronounced favourable effects on the extracts While standing a small precipitate is formed This precipitate contains only about 3 per cent of the total protein in the extract Paper-electrophoreses of fresh extracts after 1–2 h extraction time are very blurred After 2–7 days standing electrophoresis shows a good separation of eight fractions[1] The fresh extracts also yield irreversible precipitates on ammonium sulphate fractionation, dialysis against water or freeze drying After some days standing no irreversible precipitation occurs

A survey of other work on the water-soluble proteins of plant seeds indicates a similar tendency In work on barley[2–5], wheat[6], *Ricinus*[7] and *Jatropha*[8] proteins, one of the two equivalents of standing—very long extraction time (12–48 h) or prolonged dialysis against buffer—have been used

It is generally believed that most of the irreversible precipitates which form on fractionation of seed proteins are protein–phytate complexes[9] We have, however, also found large amounts of carbohydrates in some precipitates (quantitative anthrone test) Furthermore, the strange blurring patterns of the paper-electrophoreses are probably caused by the high viscosity of fresh extracts due to polysaccharides Accordingly, we tested whether the water-soluble polysaccharides and phytate are hydrolysed while the extract was standing

100 g of finely ground acetone-treated barley was extracted for 1 h at 4° C with 200 ml water with occasional stirring The slurry was centrifuged at 16,000 r p m for 10 min at 4° C The clear extract was incubated at 4° C and the hydrolyses of phytate and polysaccharide were followed by measuring the inorganic phosphate and viscosity of the extract The results are shown in Fig 1 It is clear that the two reactions occur and are completed within 5 and 2 days respectively The increase in phosphate must be due to hydrolysis of phytate as there are not sufficient amounts of other hydrolysable phosphoric acid esters in barley grain When phytate was added to the extract it was also hydrolysed at a rate corresponding to the increase of inorganic phosphate during the incubation of the extract

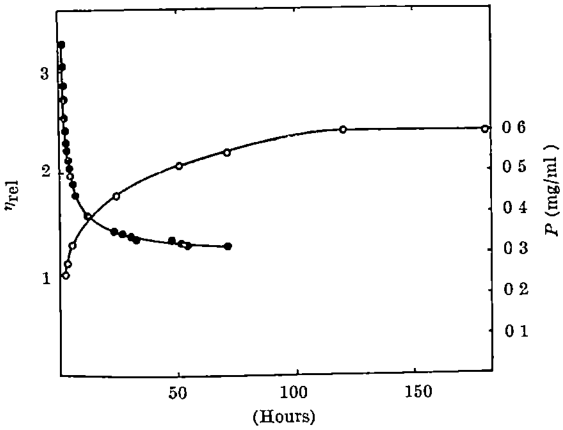

Fig 1 Increase of inorganic phosphate (○) and decrease of viscosity (●) of water extract of barley grains while standing at 4° C

The high viscosity of the extracts caused by polysaccharides and the formation of protein–phytate complexes interfere in protein fractionation of fresh extracts After some days standing of the extracts, fractionation is possible This is, we believe, due to the two reactions demonstrated in this work

This work was supported by Valtion luonnontieteellinen toimikunta (Finnish State Commission of Natural Sciences)

J MIKOLA
M NUMMI
T-M ENARI
Laboratory of Brewing,
Helsinki, Finland

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PHYSIOLOGY

Inverse Caloric Nystagmus obtained by Unilateral Stimulation of the Cat

FOR descriptive purposes, two types of inverse nystagmus may be differentiated (1) secondary nystagmus, wherein responses opposed in direction to a primary reaction occur shortly after the primary has declined , (2) homolateral nystagmus, wherein the first (the primary) response to the stimulus has a direction opposite to the normal primary reaction The occurrence of a secondary reaction to rotatory

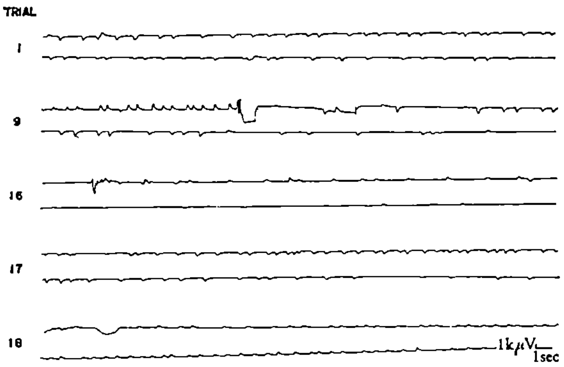

Fig 1 Nystagmus recordings from a cat stimulated in total darkness with a series of unilateral calorizations The nystagmus tracings cover a 66-sec period immediately after stimulus cessation Upward deflexions of the recording pen indicate eye movements to the right, downward deflexions indicate eye movement to the left Examples of secondary nystagmus (trial 9) and of homolateral nystagmus (trials 9, 16, and 18) are clearly evident Stimulus water 3–5° C applied to right ear for 15 sec

stimuli has been frequently observed in both man and animals[1–5] However, with unilateral caloric stimulation of man under usual testing conditions, the secondary apparently does not occur[1]

Although very little information is available with respect to inverse nystagmus and aural irrigation of animals, Riskaer and Permin have noted some occurrences of both secondary and homolateral nystagmus obtained by unilateral calorization of rabbits[6] Kristensen, however, reported uniform and appropriate nystagmus from rabbits unless the head was lowered 15°–20° from normal[7] Under the latter condition, inverse nystagmus appeared

The results reported here were obtained from one of several cats which demonstrated both secondary and homolateral nystagmus to unilateral caloric irrigation during previous testing sessions The animal was restrained, and its head held in the position of normal carriage, according to the casting method of Crampton, Schwam and Warburton[8] Needle electrodes were inserted into the skin at the outer canthi and a ground electrode was located near the mid-line of the skull Recordings of the horizontal components of eye movement were made on an Offner type *T* electroencephalograph using a 1 4 sec RC time constant in amplification

The stimulating apparatus consisted of a 2-qt water-bath with attached tubing and a glass nozzle, an adjustable stand to provide a 2-ft head of water, and a pan to collect the return flow from the ear Water temperature immediately prior to each trial was maintained at 3 0°–5 0° C

The animal was stimulated for 15-sec periods Nystagmus was recorded in total darkness during, and for several minutes following, irrigation Trials were spaced by rest periods of 10–12 min in full-room illumination

Records obtained from this animal appear in Fig 1 During the first 6 trials, a brisk response in the appropriate direction was obtained on termination of stimulation This was followed by a secondary reaction in the next 2 trials A clear homolateral response occurred during trials 9, 10, 11, 16, and 18 Intervening and following trials yielded an appropriate response

These results indicate that inverse nystagmus may occur in cats as a result of unilateral caloric stimulation It apparently does not occur in all such animals However, the sequence of events seems more orderly than that reported for rabbits by Riskaer and Permin[6] In the cats exhibiting the reaction an appropriate response was obtained in early trials, and was followed by a reverse reaction—the secondary Homolateral nystagmus appeared only after a number of calorizations, and was not observed without a secondary having appeared in earlier trials Further information on the occurrence of this reaction under different stimulus conditions is in preparation[9]

The results were collected at the U S Army Medical Research Laboratory, Fort Knox, Kentucky

WILLIAM E. COLLINS

Civil Aeromedical Research Institute,
Federal Aviation Agency,
Oklahoma City, Oklahoma

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An Alveolar-Arterial P_{CO_2} Discrepancy

REPORTS in the literature have indicated that the infusion of *tris* (hydroxy-methyl) amino methane (*tris*) results in increased blood *p*H and carbon dioxide content, with no change or a lowering of P_{CO_2} (ref 1) Pulmonary ventilation has been reported to decrease, while large amounts of 'bicarbonate' are excreted through the kidneys[2] These results have been interpreted in part as showing a respiratory centre responsiveness to *p*H in the absence of a change in P_{CO_2} (ref 2)

In an attempt to measure the effect of *tris* and another amine, arginine (free base), on acid-base balance and excretion of carbon dioxide by the lungs and kidneys, it soon became apparent that, with fixed ventilation a discrepancy existed between arterial P_{CO_2} as calculated from the Henderson–Hasselbalch equation and alveolar P_{CO_2}, found from measurements of expired gas, following the administration of these amines The experiments to be reported do not provide an explanation for this discrepancy However, caution is suggested in using these calculated values until this discrepancy is satisfactorily explained

Mongrel dogs were anæsthetized with 'Nembutal' (intravenous), tracheal intubation performed, and ventilation controlled throughout the entire experiment at a constant rate and volume by means of a mechanical respirator After 30–60 min of controlled ventilation, arterial blood and expired gas were collected Blood *p*H was immediately measured anaerobically at 38° C with glass electrode *p*H meter, plasma carbon dioxide content measured by the manometric method of Van Slyke, and blood P_{CO_2} (Pa_{CO_2}) calculated from the Henderson–Hasselbalch equation, using a pK' of 6 10 The volume of expired gas was measured in a Tissot gasometer, and its oxygen and carbon dioxide content measured by the method of Scholander Alveolar ventilation was calculated from the equation alveolar ventilation in l /min *BTPS* = 0 8658 times carbon dioxide expired in c c /min *STPD* divided by Pa_{CO_2} in mm mercury[3] Following two such measurement periods *tris* or arginine was given intravenously at the rate of 0 33 m mole/kgm /min 45–60 min after the beginning of one of the experiments, arterial blood and expired gas were collected and analysed as before At this time the alveolar ventilation formula was used in the form $Pa_{CO_2} = 0\ 8658\ X\ V_{ECO_2}/V_A$, on the assumption that the V_A computed during the control periods still obtained (It is probable that experimental V_A was less than control since, despite constant V_I, experimental V_E averaged 92 6 per cent of control V_E due to reduced V_{CO_2} To assure calculation of the maximum possible Pa_{CO_2}, the entire measured decrease in V_E was subtracted from control V_A for this calculation Thus the actual P_{CO_2} discrepancy may be greater than reported here) The value of Pa_{CO_2} so obtained was compared with that calculated from the Henderson–Hasselbalch equation

For comparison, experiments are included which were carried out in the same way, but in which acid-base balance was varied by either hyperventilation or hypoventilation throughout the experiment, or by the administration of sodium bicarbonate or various organic acids following the control periods

The high degree of accuracy with which arterial P_{CO_2} may be computed by the technique used here is indicated by the results of the control series In 39 such experiments the Henderson–Hasselbalch P_{CO_2} differed from that derived from the expired gas by a mean of − 0 2 mm mercury ± 1 5 (*S D*) This close agreement was found despite the unsteady state present during hyperventilation and acid or bicarbonate infusion, in experiments where *p*H ranged from 6 68 to 7 81, carbon dioxide from 3 2 to 61 1, and P_{CO_2} from 3 3 to 69 However, it is apparent from Table 1 that during the infusion of *tris* or arginine, blood P_{CO_2} calculated from the Henderson–Hasselbalch equation is substantially higher than that derived from expired air in every instance (range + 6 to + 104 mm mercury, mean + 22 4)

The explanation for this highly significant discrepancy is not given by these experiments If, at equilibrium, there were significant amounts of a compound other than the bicarbonate salt of *tris* or arginine present (such as the carbamate), the *pK'* of the system might be expected to differ from that of the usual $(BHCO_3^-)$—(H_2CO_3) system However, tonometry experiments have shown that, at equilibrium, the commonly used *pK'* of about 6 10 for the Henderson–Hasselbalch equation properly describes the relationships among *p*H, P_{CO_2}, and 'total carbon dioxide'

These findings are also similar to those we and others have found following the administration of carbonic anhydrase inhibitors[4] Experiments on dogs with 'Diamox(*R*)', performed exactly as described here, have also shown a higher P_{CO_2} on the basis of the Henderson–Hasselbalch equation A similar effect has been noted with carbonic anhydrase inhibitors in man with ventilation fixed by maximal exercise[5] The probable cause of these findings has been well discussed by Berliner and Orloff[4], who point out that equilibrium between carbon dioxide and bicarbonate is not so rapidly attained in the pulmonary capillary in the absence of carbonic anhydrase and that this could lead to a difference between alveolar carbon dioxide tension and that found in arterial blood *Tris* is known to affect certain enzyme systems[6] The possibility exists that *tris* and other basic amines might also have an effect on carbonic anhydrase Though the possibility may be entertained that a true diffusion gradient for carbon dioxide occurs, this seems most unlikely in view of the high solubility of this gas Thus it may be that these findings result from a state of disequilibrium, due either to formation of an intermediate compound or to interference with the enzymatic release of carbon dioxide in the lungs

Table 1 PLASMA CHEMISTRIES DURING CONTROL AND EXPERIMENTAL PERIODS

		Control			During infusion			
		*p*H	(CO_2)s m mole/l	P_{CO_2} (*H–H*) mm Hg	*p*H	(CO_2)s m mole/l	P_{CO_2} (*H–H*) mm Hg	P_{CO_2} (V_A) mm Hg
Tris	1	7 23	23 6	54	7 83	43 1	26	16
	2	7 39	21 8	35	7 64	33 1	31	23
	3*a*	7 36	24 8	43	7 66	40 2	36	21
	3*b*				7 73	45 4	34	26
Arginine	1	7 26	22 9	49	7 67	42 9	37	18
	2	7 07	27 6	89	7 23	66 9	153	49
	3	7 33	22 8	42	7 55	33 3	38	29
	4	7 32	23 1	44	7 35	26 6	47	41

(*H–H*) indicates P_{CO_2} derived from the Henderson–Hasselbalch and (V_A) that from the expired gas calculations

Whatever the reason, it seems clear that calculation of any of the parameters of the Henderson–Hasselbalch equation from measured values of the others during infusion of *tris* or arginine-free base may be subject to considerable error Hence any attempt to correlate such calculated values with changes in respiration must be made with due caution

This work was supported by a U S Public Health Service grant No *H*–1641

J WILLIAM POPPELL
PARKER VANAMEE

Department of Medicine and the
Division of Experimental Surgery and Physiology
and the Andre and Bella Meyer
Physiology Laboratories,
Memorial Sloan-Kettering Cancer Center,
New York

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Effect of Œstradiol on Phosphorylase Activity of Intrasplenic Ovarian Graft in C57*BL* Mice

NON-TUMORAL intrasplenic ovarian tissue of mice utilizes appreciable amounts of endogenous glycogen[1] Œstradiol, an inhibitor of the ovarian intrasplenic development[2], increases the glycogen content of ovarian graft when parenterally given to mice, while the weight of the graft is greatly reduced[3] This would suggest that hormonal repression at the hypophysis-level somehow interfered with metabolic reactions involved in the synthesis and/or degradation of glycogen Glycogen utilization is mediated through phosphorylase while it is synthesized by glucose transfer from uridine diphosphoglucose[4] It is a well-established fact that phosphorylase activity is

Table 1 PHOSPHORYLASE ACTIVITY IN NORMAL AND INTRASPLENIC OVARIES
(All values in μmoles/100 mg fresh tissue/10 min)

Conditions	No of ovaries	Average weight of the ovaries (mg)	No of experiments	With 5′AMP	Without 5′AMP	Ratio of activity $\frac{\text{without 5′AMP}}{\text{with 5′AMP}}$
Normal ovaries	101	3 3 ± 0 10*	5	7 0 ± 0 77	5 5 ± 0 46	78
Intrasplenic ovaries	22	17 7 ± 1 2	5	5 9 ± 1 7	6 4 ± 1 7	110
Intrasplenic ovaries plus œstradiol	33	9 1 ± 0 6	6	4 7 ± 0 44	1 9 ± 0 79	41

* *S E*

under hormonal control Thus muscle and liver phosphorylase *b* are activated to phosphorylase *a* by epinephrine[5] and glucagon[6] Furthermore, adrenocorticotrophic hormone specifically activates adrenal gland phosphorylase[7]

This communication indicates that œstradiol treatment decreases the ratio phosphorylase *a* to *b* in intrasplenic ovarian tissue possibly by depression of the follicle-stimulating hormone The method of Cori and Illingworth[5] for phosphorylase activity determination was adopted Castrated adult female mice of the *C57BL* strain from our colony, bearing one ovary into the spleen for 60 days, were utilized As previously[3], 10 μg daily of an aqueous suspension of 17β-œstradiol were injected intraperitoneally for 10 days Non-injected ovarian grafted females of the same age as well as non-injected normal females were used as controls The ovarian graft was recovered from the spleen by careful dissection of any splenic parenchyma macroscopically visible After weighing an adequate number of ovaries, they were pooled and homogenized in cold 0 15 M potassium fluoride An aliquot of the homogenate containing 6–12 mg (fresh weight) of tissue was used for the enzymatic assay The reaction mixture in a final volume of 0 6 ml contained 12 μmoles glucose-1-phosphate, 6 μmoles adenosine monophosphate (5′AMP), 0 6 μg glycogen, and 40 μmoles glycylglycine buffer, *p*H 6 0 The temperature was 30° and the incubation period 10 min When indicated, adenosine monophosphate was replaced by distilled water Each determination was carried out in duplicate The reaction was stopped by the addition of 1 8 ml of 10 per cent trichloroacetic acid Inorganic phosphorus in the acid supernatant was measured by the method of Fiske and Subbarow[8]

From Table 1 it is evident that œstradiol decreases the phosphorylase activity of intrasplenic ovary, that is, from 6 4 ± 1 7 μmoles of inorganic phosphorus/100 mg fresh tissue, to 1 9 ± 0 79 μmoles of inorganic phosphorus/100 mg fresh tissue, provided that 5′AMP is omitted When the latter is added, no significant effect of œstradiol is observed In normal as well as in non-injected intrasplenic ovaries, the ratio-activity without 5′AMP/activity with 5′AMP is above 65, which according to Cori *et al* [5] indicates that phosphorylase predominantly is in the *a* form On the other hand, the same ratio for intrasplenic ovaries under the influence of œstradiol is only 41, which indicates that phosphorylase is in the *b* form in this case

Purves and Griesbach[9] have shown that in spayed females rats the glycoprotein content of the gonadotroph cells of the hypophysis disappear under œstradiol treatment concomitantly with the gonadotropic potency Furthermore, Miller and Pfeiffer[10] observed that mice bearing intrasplenic ovarian tissue maintain a high level of blood follicle-stimulating hormone On the basis of these findings, one is tempted to correlate the suppression of the follicle-stimulating hormone by œstradiol treatment to the changes in activity of the intrasplenic ovary phosphorylase Thus, the follicle-stimulating hormone would be specifically involved in the activation of intrasplenic ovarian phosphorylase

Work to establish whether this mechanism applies to intrasplenic ovary or in addition to normal ovary is now being carried out

This work was aided by a grant from the Commission for Scientific Research of the University of Chile Œstradiol was obtained through the courtesy of Dr Carlos Dratschmidt from Schering and Co , Ltd , Santiago, Chile

SILVIO BRUZZONE
ANA BRANCATELLI

Instituto de Química Fisiológica y Patológica,
Escuela de Medicina,
Universidad de Chile,
Santiago

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Fœtal Synthesis and Transplacental Passage of Homologous Serum Proteins in the Rabbit

BRAMBELL *et al* [1] have demonstrated the passage of various antibodies from the mother to the fœtal rabbit It may now be considered that such passage is not a special property of antibodies, since it has been shown[2,3] that heterologous serum proteins, when injected into the mother, appear intact in various compartments of 19–21 and 24–26 day rabbit fœtuses If transplacental passage of large molecules is indeed a general phenomenon of normal development, it is clearly of importance to ascertain the source(s) of serum proteins in the fœtal circulation *vis-à-vis* such passage Since the serum proteins of the mother may be considered to be in a steady state while their amounts in the fœtus are continuously changing, some measure of the synthesis by the fœtus is desirable and even necessary for an investigation of the passage of similar proteins from the mother into the fœtus The present investigation was undertaken to measure fœtal synthesis of serum proteins and to examine the passage with the aid of isotopically labelled serum proteins , some preliminary results are reported here

Fœtal synthesis Dutch rabbits were opened under anæsthesia at the end of 24 days of gestation and each fœtus was given an intraperitoneal injection of 50 μc of lysine labelled with carbon-14 (specific activity 1 mc /mmole) through the uterine wall At various intervals after injection, one or two fœtuses were removed and fœtal blood was collected from the heart Maternal blood was also sampled at various times The serum samples were divided into 50-μl aliquots, one of which was planchetted and counted on an end-window Geiger counter Another aliquot was precipitated with an equal volume of 10 per cent trichloroacetic acid (TCA) and a known volume of the supernatant was counted In some cases the precipitate was washed with 5 per cent TCA, dissolved in 0 1 N sodium hydroxide and counted on a planchette Whenever possible, two other aliquots were electrophoresed on paper (Whatman No 1, barbiturate buffer of *p*H 8 6 and ionic strength 0 1, and 30 m amp of constant current for 16 h) One paper strip was stained in bromophenol blue after the run and the other was cut across into 5-mm wide pieces These pieces were placed in planchettes and counted in a modified windowless Geiger counter There was good agreement between counts in the TCA precipitate above and the total counts on paper

Data on the incorporation of lysine into serum proteins, for 24 h after injection, were thus obtained and curves were fitted manually The maternal curve rises very slowly with time and reaches a plateau at about 14 h after injection, but the fœtal curve rises very steeply, reaches a peak at about 3 h and declines gradually to less than a fourth of the peak value by about 12 h after injection

The rate of incorporation, obtained from the initial part of the fœtal curve, is 350 c p s per ml of serum per hour The specific activity of fœtal serum proteins at the peak of incorporation is 840 c p s per ml of serum The total protein concentration of 24-day fœtal sera, estimated by the Biuret method[3], is 22 1 mg per ml of serum Assuming that the incorporation of lysine labelled with carbon-14 indicates synthesis of proteins $\left(\frac{840}{22\ 1 \times 350} = 0\ 11 \text{ mg/h}\right)$, 2 64 mg of total serum proteins are synthesized by the fœtus during the 24 h, that is, the 25th day of gestation This is clearly a minimum figure, underestimated by possibly as much as 20 per cent, since the specific activity at peak incorporation only approximates the maximum value attained by serum proteins It is noteworthy that this peak value is achieved in the fœtus in less than a quarter the time taken by the mother

Fig 1 shows the distribution of the incorporation of labelled lysine into the various serum proteins Duplicate electropherograms, one stained and the other estimated for radioactivity, show counts corresponding to albumin and α-, β- and γ-globulins It is interesting to find that the relative distribution of counts corresponds closely to the relative percentages of these protein fractions in 24-day fœtal serum and are different from those of maternal serum

Passage from the mother into the fœtus Dutch rabbits (2 3 to 2 7 kg) were given 0 25 – 1 0 mc of cystine (specific activity 1 mc /mmole) and bled 8–12 h later About 6 per cent of the administered activity was incorporated in the total serum proteins of the animal and 69–73 per cent of the total serum activity was in the TCA-precipitable fraction

About 30 ml of this labelled serum was injected intravenously into a 19 day pregnant rabbit and the fœtuses were sampled 3½–4 h later Less than 1 per cent of the maternal serum concentration of protein-bound activity was found in fœtal serum proteins and about 0 2 per cent in the protein of amniotic and allantoic fluids

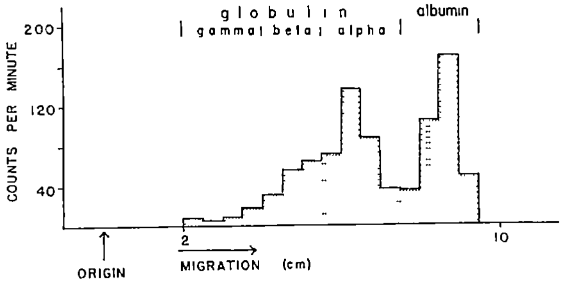

Fig 1 Distribution of radioactivity among serum proteins synthesized by the 24–25 day old rabbit fœtus The fœtus was given ^{14}C-lysine intraperitoneally Forty μl of fœtal serum was electrophoresced in duplicate on paper, one strip was stained for proteins and the other counted

In order to determine whether maternal serum proteins pass to a greater extent into older fœtuses, 10–12 ml of labelled serum were given intravenously to 24-day pregnant rabbits Samples obtained 11 and 22 h after injection showed that the protein-bound activity in fœtal serum was 2 per cent of that of the maternal serum at 11 h and 17 per cent at 22 h The amniotic fluid and stomach contents of the fœtus showed about 1–2 per cent of the protein-bound activity present in an equal volume of the maternal serum

There seems to be no doubt that increasing amounts of protein are passed into the fœtus with advance in time after injection and at advanced stages of gestation[3]

Winkler *et al* [4] used iodinated rabbit serum albumin to study passage in albino rabbits in the last week of gestation The activity of iodine-131 in fœtal serum at 16–24 h after injection appeared to indicate passage, however, the activity of fœtal samples was considerably reduced by dialysis or on electrophoresis of the serum

In our passage experiments, electrophoresis of fœtal serum followed by localization of radioactivity on the paper strip, indicated at least three peaks of activity coincident with the albumin and α- and β-globulin bands The fœtal stomach contents showed only two peaks of activity, in the albumin and γ-globulin regions.

This investigation was begun in 1957 and was supported by grants from the Office of Naval Research *Nonr* 233 (40) and the Atomic Energy Commission, No *At*-04-1-*Gen*-12

ABRAHAM C KULANGARA*
Department of Zoology
OLE A SCHJEIDE
Laboratory of Nuclear Medicine
and Radiation Biology,
Department of Biophysics and
Nuclear Medicine,
Department of Radiology,
School of Medicine,
University of California,
Los Angeles 24

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* Previous name K C Abraham

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Suppression of Tuberculin Reaction in the Scorbutic Guinea Pig

In a recent publication from this laboratory it was noted that vitamin C deficiency in guinea pigs prevented induction of experimental allergic encephalomyelitis as well as development of the tuberculin reaction[1]. Replacement of the scorbutic diet with a normal stock diet four weeks after deprivation began and three weeks after inoculation of the challenge vaccine containing brain and killed *Mycobacterium butyricum* in water-in-oil emulsion permitted subsequent development of tuberculin sensitivity although the guinea pigs failed to develop clinical or histological signs of experimental allergic encephalomyelitis. It was important to determine the exact basis for the animals' failure to respond to tuberculin by development of a skin reaction because of the bearing this information might have on the induction of experimental allergic encephalomyelitis. Did absence of vitamin C prevent the end organ response (development of a positive skin reaction to tuberculin or, in the case of experimental allergic encephalomyelitis, the inflammatory lesions in the brain), or was the vitamin essential to the primary immunological response to the injected antigen(s)?

It is known that a generalized state of delayed hypersensitivity can exist in animals which fail to give a positive skin reaction, for example, tuberculous rabbits may lose their cutaneous hypersensitivity without decrease or loss of cellular hypersensitivity[2]. The present communication shows that the skin of the scorbutic animal is capable of responding to tuberculin if the delayed hypersensitivity has been induced prior to deprivation of vitamin C. Thus we can assume that the inhibition of the response comes from interference with the actual induction of delayed hypersensitivity rather than interference with the skin's reactivity.

Disease-free, mixed-colour, male guinea pigs, bred in a closed colony in the Animal Production Section at the National Institutes of Health, were used for all experiments described. Their weights were 400–500 gm. They were obtained from a stock colony one week prior to inoculation of tuberculin and maintained on the diet specified. All animals were maintained in large general-purpose cages, six to a cage, on a bed of sawdust and cedar shavings.

Tuberculin sensitivity was induced in the guinea pigs by one intracutaneous injection of 0.1 mgm *Mycobacterium butyricum* (Difco) in 0.1 ml. of an emulsion made up of one part phenol–saline (0.5 per cent phenol–0.9 per cent sodium chloride), one part melted 'Aquaphor' (Duke Laboratories), and two parts mineral oil (Fisher Scientific Co.).

The 'normal diet' consisted of stock diet for guinea pigs (N.I.H. Formula *A*) plus a daily supplement of greens, cabbage and kale. Vitamin C-deficient diet was supplied by Nutritional Biochemical Corporation. The guinea pigs on the 'scorbutic diet plus vitamin C' received 50 mgm. ascorbic acid (Lederle) daily in 0.1 ml. intraperitoneal injection. All animals received tap water *ad lib*.

Tuberculin sensitivity was measured as mm. induration 24 hr. after intradermal injection of 0.1 ml. of second-strength tuberculin (0.05 mgm./ml.).

All animals were placed on their respective diets, as indicated under diet I in Table 1, for a week prior to inoculation with *M. butyricum*. The skins were tested two weeks after inoculation and, after these tests, the guinea pigs were placed on their second diet. The second skin test was performed three weeks after institution of the second diet.

Table 1. SUPPRESSION OF TUBERCULIN RESPONSE IN THE GUINEA PIG BY VITAMIN C DEFICIENCY

Diet I	Diet II	No. of animals	First tuberculin response mm. induration (mean ± S.D.)	Second tuberculin response mm. induration (mean ± S.D.)
Normal	Normal	6	10.7 ± 2.1	12.8 ± 2.1
Normal	Scorbutic	8	10.3 ± 2.3	11.9 ± 1.4
Scorbutic plus vitamin C	Scorbutic	6	11.0 ± 4.4	9.3 ± 2.9
Scorbutic	Scorbutic plus vitamin C	5	0.4 ± 0.2	7.6 ± 2.0

Guinea pigs were put on diet I one week prior to injection with Freund's complete adjuvant (0.1 mgm. *Mycobacterium butyricum* ID). Two weeks after the injection they were challenged with 5 μgm. tuberculin and the skin reaction observed 24 hr. later (first response to tuberculin). Each group was then placed on diet II, and after three more weeks again challenged with 5 μgm. tuberculin (second response to tuberculin).

As shown in Table 1, the animals on the vitamin C-deficient diet, when inoculated with *M. butyricum*, failed to respond to tuberculin as in the previous investigation[1]. Also, as had been observed previously, restoration of vitamin C to these animals resulted in subsequent development of sensitivity to tuberculin. In the present series of animals, the second tuberculin test was carried out only three weeks after the vitamin C supplementation was instituted. This was possibly too short a time for the animals to recover completely from their scorbutic state, since the skin reactions observed were somewhat less pronounced than the reactions of animals on the normal diet. There was no difference in the first tuberculin response of animals on a normal diet and the response of animals on the scorbutic diet plus vitamin C injections. Removal of vitamin C from the diet after the hypersensitive state had been induced did not alter significantly the tuberculin response (line 2 in Table 1).

From these results it would appear that the vitamin C-deficient guinea pig is able to respond dermatologically to tuberculin once tuberculin hypersensitivity has been induced. Failure of the induction of the delayed hypersensitivity phenomenon itself would appear to be the best explanation for the absence of response to tuberculin in those animals which were on the vitamin C-deficient diet at the time they were inoculated with *M. butyricum*.

PETER S. MUELLER
MARIAN W. KIES

Laboratory of Clinical Science,
National Institute of Mental Health,
U.S. Department of Health, Education and Welfare,
Bethesda, Maryland.

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Age Differences in Cyclic Motor Reaction

DIFFERENT manifestations of time judgment in man, becoming still more important with the advent of flights into space, have been examined by a vast number of psychologists as well as psychiatrists[1], and last but not least by physiologists, including those of the Pavlovian school[2]. They used methods of estimation of presented intervals, production and reproduction of intervals, and finally methods aiming at revealing the mechanism of time judgment itself I examined the motor reproduction (tapping) of intervals of 5 sec during 120 sec after a training period of 60 sec in each experiment The results are given in Table 1

Table 1 ACCURACY AFTER 120 SEC OF REPRODUCING 5 SEC INTERVAL

	Women (13) (aged 20–30)	Men (4)	Girls (18) (aged 10–14)	Boys (26)
Accurate (within ± 2 sec) (%)	10 56	6 67	2 63	2 44
Shortened ('overestimated') (%)	23 94	20 00	52 63	58 54
Prolonged ('underestimated') (%)	65 50	73 33	44 74	39 02
No of experiments in group	286		120	

Sex differences are as yet not considered with regard to numeral inhomogeneity of the groups reported

Frolov[3] claims that in adults shortening of intervals prevails and he assumes that this 'overestimation' represents the prevalence of inhibition in the equilibrium of the two main neural processes My results do not support this hypothesis unless it is accepted that in groups of children inhibition prevails, which is not probable On the contrary my results are in accordance with the findings of Hormia[1], who also quotes experiences that tendency to 'underestimation' gradually rises with age In some other way my point of view is supported by the finding of lengthening intervals, reproduced verbally, in narcosis (Robson *et al* [4]) Holubář[5] observed a preceding reaction during conditioning of time-reflex, I observed the same, but only in children, during 60-sec training of the tested interval

To explain his findings, Frolov considers the trace reflex to be the underlying pattern of the motor reproduction of intervals, Hull[6], on the contrary, considers this category of performance as conditioned reflex to cyclic phase My own explanation agrees more clearly with Hull the command for the motor reaction might cause, as usually accepted, a focus of excitation in the brain, and in the case of preliminary set-up delay motor realization is inhibited for the time necessary Lack of inhibition would then result in a preceding motor reaction, its dominance over excitation would cause delayed reaction

JAROSLAV TEJMAR

Institute of Hygiene,
Prague, 10

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Lipid Metabolism in *Astacus astacus* (L.)

ALTHOUGH not all vertebrate classes have been examined with regard to lipid metabolism, it has been established that vertebrates are able to synthesize cholesterol from acetate by way of squalene Sometimes this phenomenon has been generalized for invertebrates However, this view has proved to be incorrect A wide variety of insects have been found to require cholesterol as an indispensable component in their food[1–3], and it has therefore been suggested that insects cannot synthesize their own sterols, but rather modify the steroid nuclei present in their food It has been shown that the carnivorous beetle *Dermestes vulpinus*[4] is incapable of incorporating carbon-14 of fed 1-^{14}C-acetate into squalene or cholesterol The same has been found for the bluebottle *Calliphora erythrocephala* (Meig)[3] In Annelida, it has been found that mevalonic acid is converted into squalene by *Lumbricus terrestris*[5], but the cyclizing of this hydrocarbon to a sterol could not be demonstrated The work recorded here is concerned with the possible biosynthesis of cholesterol in *Astacus astacus* (L)

Table 1 ACTIVITY OF LIPIDS IN *Astacus* INJECTED WITH 1-^{14}C-ACETATE

	Counts/min
Crude lipids	286 7±5 4*
Fatty acids	262 0±6 0
Saturated fatty acids	397 6±5 1
Unsaturated fatty acids	205 9±2 8
Unsaponifiable fraction	145 7±2 0
Wax-like substances from the unsaponifiable fraction	135 0±0 8
Crude squalene	4 7±1 0
Squalene hexahydrochloride	0 1±0 0
Cholesterol-digitonide	0 4±0 0
Cholesterol	–0 02±0 02

* *S E*

During autumn, ten animals (in the intermoult stage) were injected four times at intervals of 48 h Each dosage consisted of a volume of 0 4 ml containing 4 mg of 1-^{14}C-sodium-acetate (activity 2 5 μc /mg)

After a period of eight days the animals were killed by freezing at −20° C They were dehydrated with ethanol, dried and exhaustively extracted with ether in a Soxhlet apparatus The ether extract was evaporated to dryness in a stream of nitrogen and yielded 3 0313 g of lipids

In order to remove the unsaponifiable fraction from the residue the lipids were saponified with alcoholic potassium hydroxide The non-saponifiable fraction was extracted with ether and yielded 0 5309 g of a solid mass after evaporation in a stream of nitrogen

The fatty acids were isolated from the saponified fraction and separated into saturated and unsaturated fatty acids by lead salt fractionation The unsaponifiable fraction was extracted many times with portions of petroleum ether (b p 60–70° C) From the extracted substances 0 2350 g (probably wax-like compounds) appeared to be insoluble in petroleum ether

The petroleum ether solution was chromatographed on alumina After 150 ml petroleum ether had passed through the column, most of the squalene proved to have been eluted The elution was continued with 100 ml of benzene and finally with 150 ml of ether to remove the sterols[6] The petroleum ether fraction yielded 27 5 mg of crude squalene A sample was used for isotope analysis To the remaining 15 6 mg, 15 1 mg carrier squalene was added and the hexahydrochloride was prepared according to Heilbron's method as modified by Loud and Bucher[7]

The ether contained 220 6 mg of crude sterols from which the cholesterol was isolated with digitonin It was removed from the digitonide with pyridine

From the various fractions samples were burned to carbon dioxide, which was precipitated as barium carbonate The activity was determined with a Geiger–Muller tube, under the conditions of infinite thickness According to Calvin *et al* [8] these activities are proportional to the specific activities and therefore comparable

The results given in Table 1 show that *Astacus astacus* (L) can convert acetate into fatty acids but not into squalene and cholesterol

The labelling of the saturated fatty acids is about twice as high as that of the unsaturated fatty acids This is in agreement with the results obtained in vertebrates and points to the formation of the unsaturated from the saturated fatty acids

I thank Prof H J Vonk for advice

D I Zandee

Laboratory of Comparative Physiology,
University of Utrecht

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PHARMACOLOGY

Catecholamine Metabolism in Morphine Withdrawal in the Dog

I have noted[1] a decrease in the catecholamine content of the rat brain after withdrawal of chronic administration of morphine This finding, however, seemed to be confined to a certain strain of rats with very conspicuous abstinence symptoms, while an increased urinary excretion of adrenaline and noradrenaline[2] was observed in three different rat strains For studies of abstinence phenomena of the excitatory type, comparable with those found in man, higher species must be studied In 1932 Tachikawa[3] reported an increased amount of 'adrenaline' in the blood of chronically morphine-treated dogs when the drug was withheld In 1960 and 1961 Maynert and Klingman[4,5] reported a decrease of brain and adrenal catecholamines in morphine-tolerant dogs and rabbits when an abstinence syndrome was produced by nalorphine No numerical data were given for the decrease

The present work deals with the effects on catecholamines in brain, adrenals and urine of dogs, during induced morphine tolerance and subsequent abstinence

After an acclimatization period, the 24-hr urines of 4 saline-injected dogs weighing 3–5 kgm were collected daily in separate metabolism cages during 5 days, before the morphine injections were started (control-levels) The effect of one subcutaneous injection of nalorphine was then recorded The excretion of catecholamines was also recorded in the beginning of the daily morphine injections 2 months later when morphine had been given in doses increasing from 3 to 100 mgm /kgm /day the effect of nalorphine (10 mgm /kgm) was studied on the morphine-tolerant animals Finally the effect of abrupt withdrawal was followed for 3 weeks

Urinary catecholamines were estimated according to Euler and Lishajko[6] Recovery experiments yielded 64–72 per cent (mean 68 per cent) for added amounts of adrenaline and 65–75 per cent (mean 71 per cent) for noradrenaline No corrections were made for these losses

Twelve young dogs out of three litters, each weighing 4–10 kgm, were used Ten were treated with daily injections of morphine hydrochloride for about 2 months and two dogs were untreated controls 24 hr after a final morphine dose of 100 mgm /kgm the ten animals were divided in two groups Five dogs received saline injections and the other five received two doses of nalorphine 10 mgm /kgm 2 hr. apart Two hours after the last injection the animals were killed by a shot through the heart The brains were dissected out immediately and the adrenals 15 min later The brains were divided into 3 portions (1) telencephalon, (2) cerebellum, (3) brain stem (excluding the central nuclei belonging to 1) They were treated by the method of Bertler *et al* [7], but the final determination of catecholamines was made on a Coleman fluorimeter. The telencephalon was also analysed for the content of dopamine according to Carlsson and Waldeck[8] Recoveries (means of 6 experiments) were: adrenaline 85 per cent, noradrenaline 92 per cent and dopamine 87 per cent The adrenals were extracted with 5 per cent trichloroacetic acid and, after dilution in appropriate amounts of *N* sodium acetate buffer at *p*H 6 5, determined for catecholamine content like the urine eluates Recovery adrenaline 98 per cent, noradrenaline 95 per cent

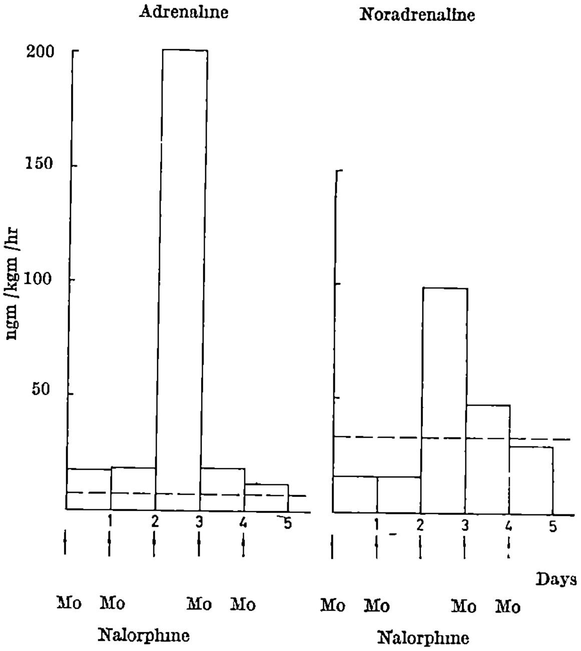

Fig 1 Daily urinary excretion (means of four animals) of adrenaline (left) and noradrenaline (right) in dogs made tolerant to 90 mgm./kgm of morphine hydrochloride daily On the third day the morphine injections were replaced by nalorphine 10 mgm / kgm Broken horizontal lines represent mean levels of the same animals when untreated during a preceding control period

Arrows indicate daily injections

During the control period the urinary excretion of noradrenaline was 31 6 ± 1 9 ngm /kgm /hr and adrenaline 7 8 ± 0·8 ngm /kgm /hr (means ± $S E$ of 20 observations) Administration of 10 mgm /kgm of nalorphine hydrochloride did not influence the noradrenaline-level, but increased the adrenaline excretion on the day of injection to 37 5 ± 10 0 ngm /kgm /hr. ($P < 0 01$) The urinary excretion of adrenaline and noradrenaline rose as a result of injection of 3 mgm /kgm of morphine hydrochloride daily and returned to the normal range within ten days on the same dose The same pattern reappeared when the dose was increased to 6 mgm /kgm in accordance with the findings in rats[2] Two months later when the animals were receiving 90 mgm /kgm of morphine daily the substitution of morphine by

Table 1 NORADRENALINE (NA) AND DOPAMINE (DA) IN THE BRAIN, ADRENALINE (A) AND NA IN THE ADRENALS (MEANS $\pm S$ E) OF DOGS TREATED WITH MORPHINE (Mo) IN DOSES INCREASING FROM 3 TO 100 MGM./KGM. FOR TWO MONTHS AND THE EFFECT OF NALORPHINE-INDUCED ABSTINENCE

		Telencephalon		Brain stem	Cerebellum (ngm /gm)	Adrenals (μgm /gm)	
No of dogs	Treatment	NA	DA	NA	NA	A	NA
2	None	128	206	332	97	1250	150
5	Mo 2 months. Saline 1 ml × 2	160±16	184±20	339±12 6	111±14	1381±119	307±87
5	Mo 2 months Nalorphine 10 mgm /kgm × 2	88±9 8**	191±33	219±26 7**	52±6 4**	552±137**	160±32

** Significance-level for difference between saline- and nalorphine-treated animals $P < 0$ 01

one injection of 10 mgm /kgm nalorphine resulted in a very marked increase of adrenaline for one day, while the accompanying increase of urinary noradrenaline persisted for a few days when the morphine injections had been reinstituted (Fig 1) When the morphine injections were abruptly withdrawn there was a sharp rise of the adrenaline excretion (mean of the maximal levels was 119 ± 20 ngm /kgm /hr reached on the second and third days) followed by a more delayed and moderate increase of noradrenaline (mean 87 ± 11 ngm /kgm /hr , the highest levels reached on the 3rd–6th day after withdrawal) This is in good accordance with the findings in rats[2]

Table 1 summarizes the effects of nalorphine on the catecholamine content of brain and adrenals There was a significant decrease of catecholamines in both organs and in all parts of the brain, and there was noticed an especially pronounced decrease in the animals which had the severest abstinence symptoms

This work was supported in part by a grant from the Alcohol Research Committee of the Swedish Medical Research Council

LARS-M GUNNE

Department of Physiology and
Department of Alcohol Research,
Karolinska Institutet,
Stockholm 60

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Effect of 'Busulphan' on the Fœtal Gonad

BOLLAG[1] first reported that pregnant rats given an injection of 'Busulphan' 5–7 days before parturition produced sterile offspring Later, Galton and Till[2] stated that "earlier in pregnancy quite large doses do not interfere with its course and the F[1] and F[2] generations are apparently healthy" In view of these studies and our knowledge of the effect of 'Busulphan' on the adult rat testis[3–5], it was decided to re-investigate the sensitivity of the proliferating cell systems of the fœtal gonad during development

Rats were given a similar injection of 'Busulphan' in arachis oil (10 mg/kg, intraperitoneally) at selected times between the 5th and 20th day of pregnancy, the day sperm were found being regarded as day 0 A proportion of each litter was killed on days 1 and 15 *post partum* and the remainder at 6 months In the 1-day-old male, the drug effect on the gonocyte population of the testis was assessed and in the 15-day and 6-month-old rats the subsequent effect on spermatogenesis

After an injection on day 5 or 7 *post coitum* the 1-day-old neonatal gonad appeared normal A marked increase in the number of seminiferous tubules containing no sex cells ('sterile' tubules) was observed in animals treated on day 9 , it approached 100 per cent following injection between days 11–14 inclusive (Fig 1) After administration of 'Busulphan' on day 18 or 20 of pregnancy the testis appeared normal at 1 day *post partum* Nevertheless, the 15-day gonad demonstrated that the drug had in fact prevented the subsequent development of the germinal epithelium This observation implies that there is a lag of several days between administration of 'Busulphan' and its destructive effect on the gonocyte population

An attempt is being made to determine (*a*) the extent of the regenerative capacity of the testis , (*b*) to what extent histological damage can be correlated with fertility Histological studies on the testes of 6-months-old rats are at present incomplete The adult material examined so far shows that the percentage of 'sterile' tubules rises from approximately 15 per cent after exposure *in utero* on the 11th day to nearly 100 per cent after exposure between the 14th to 16th days inclusive Following treatment on the 11th day of gestation 65 per cent of tubules were 'sterile' in the 15-day *post partum* testis, and it is

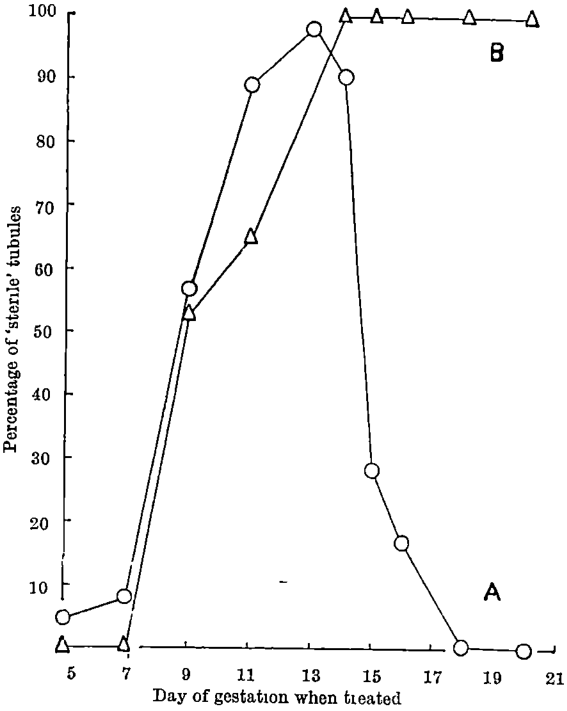

Fig 1 Curve *A* indicates the results found in the 1-day *post partum* testis and curve *B* the results at 15 days *post partum* Each point on the curves is a mean value from 3 offspring, and is based on a count of 1,800 seminiferous tubules counted in transverse section Tubules were classified as normal or 'sterile', the latter containing only supporting cells

suggested that the diminution to 15 per cent in the adult testis is the result of the regenerative capacity of the organ. So far it appears that animals are fertile following treatment on or before the 11th day, but sterility is the rule following treatment later in gestation.

These investigations demonstrate that the sensitivity of the gonocyte population to 'Busulphan' increases rapidly in pregnancy from about implantation. There is practically total destruction of these cells by injection of 'Busulphan' on or after 13 days *post coitum*. The failure of the gonocyte population to regenerate contrasts with the response of spermatogonia type *A* to the same treatment in the adult testis. Certain of these latter cells are known to be selectively suppressed for several weeks by 'Busulphan' at the same dose-level, after which recovery occurs[4,5]. We have previously emphasized the sensitivity to this substance of the early spermatogonial cell in the adult male rat and this clearly applies to its precursor, the gonocyte. At the same time, it is interesting that the number of other actively dividing cells within the tubules, the Sertoli cell precursors, appears to remain unaffected. As in the adult testis there is a differential sensitivity of proliferating cells within the fœtal gonad.

Work on day-old female offspring has shown no subsequent effect on the oocyte population after previous exposure to 'Busulphan' on either day 5 or 7 of gestation. A decrease in the number of oocytes occurred in the 1 day *post partum* ovary following drug administration between 9 and 16 days of gestation. Primordial oocytes were not seen in 15 day *post partum* ovaries from animals treated between days 13 and 16 of pregnancy, both inclusive. The effect was less marked after injection on day 9 while the drug appeared to have no action when administered on days 5, 7, 18 or 20 of gestation, that is, the 15-day *post partum* ovary was histologically normal.

The action of 'Busulphan' in the ovary therefore seems limited to the gonocytes, that is, precursors of the oocytes, and that when the gonocytes enter meiotic prophase on the 17th day of fœtal life, a notable decrease occurs in sensitivity to the drug. In spite of the homogeneous appearance of the gonocyte population during the 13th to 16th day of gestation, the results of treatment with 'Busulphan' during this time suggest that the cells exhibit a differential sensitivity to this compound. Some are able to continue development into oocytes and become associated with follicular development in the pre-pubertal animal. These follicles become atretic. Other gonocytes, the precursors of oocytes which will undergo development in the adult animal, are damaged by the drug. This will account for the absence of oocytes in the adult ovary.

The work is being extended to other related alkylating agents which have been shown to produce different effects on spermatogenesis.

B. N. HEMSWORTH
H. JACKSON

Department of Experimental Chemotherapy,
Christie Hospital,
Manchester, 20

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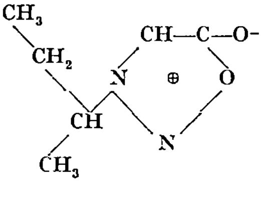
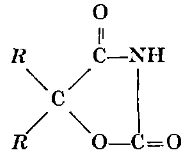
A New Class of Central Nervous System Stimulants

ALTHOUGH the mesoionic compounds called sydnones have been known and studied for some time[1], there have been no pharmacological studies reported on the ring system. A closer examination of the sydnone ring reveals a rough similarity to the rings of 2,4-oxazolidone (II) and 2-oxazolidone (III).



(I)



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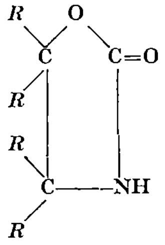

(III)

These are anticonvulsants, however, if the nitrogen atoms of both are alkylated with fairly long chains, the action is often reversed, leading to a convulsant compound[2]. Accordingly, a series of new *N*-alkyl sydnones have been prepared and the pharmacology studied.

Compound (I) was synthesized as follows: secondary butylamine in dry ether in the cold was treated with one half equivalent of ethyl bromoacetate in dry ether by slow addition. After standing overnight, a 35 per cent yield of ethyl (*sec*-butylamino) acetate was distilled at 57°/0.4 mm (found: N, 8.90). The amino-ester was then hydrolysed to the amino-acid in refluxing base for 5 h. This compound was then treated *in situ* with acid followed by a 30 per cent solution of sodium nitrite, slowly introduced at 0°. After standing overnight, a 65 per cent yield of *N*-nitroso-*sec*-butylamino-acetic acid was filtered off, m.p. 79°–80° (found: N, 17.26). The nitrosoamino-acid was then refluxed 3 h with acetic anhydride and then allowed to stand in the dark for three days. The excess anhydride was removed under vacuum and a 67 per cent yield of a straw-coloured oily 3-*sec*-butyl sydnone was distilled at 160°/3 mm (found: N, 19.96), refractive index at 20°, 1.4928, λ_{max} (ethanol) 290 (E-6300).

The LD_{50} of (I) injected subcutaneously in male brown mice ($C57B \times 1101$ hybrids) was calculated by the methods of Litchfield and Wilcoxon[3] as 135 ± 9 mg/kg and the convulsive dose as 117 ± 13 mg/kg. Pentylenetetrazole under the same experimental conditions was calculated as LD_{50} 75 ± 3 mg/kg. The convulsions beginning in less than 1 min after administration of compound (I) produced ticking in the facial musculature and fore-limbs. This was followed by clonic movements which frequently developed an extensor component. Almost all the animals died in tonic convulsions. Although a few deaths were recorded on the fifth day, these calculations were based on mortality-rate in 72 h

A spinal cat, transected at *C*-1, showed no convulsive activity in dosage to 100 mg/kg A decerebrate cat showed a definite convulsive pattern at 200 mg/kg

When 41 mg/kg of either (I) or pentylenetetrazole was administered intravenously to a dog given sufficient pentobarbital sodium intravenously to cause apnœa, a more favourable respiratory response and rise in blood pressure, following the transient fall after administration of either, was obtained with (I)

The respiratory stimulation of (I) appeared to be predominantly central as there was little decrease of activity after vagotomy or denervation of the carotid sinus

The compound did not potentiate acetylcholine and produced no acetylcholine esterase inhibition[4] There was no antispasmodic activity against acetylcholine or histamine on the guinea pig ileum[4] No spasmodic activity was noted in isolated tissue although a positive Straub test[5] was observed in mice injected with (I)

Chemically, compound (I) differs importantly from (II) and (III) in that the ring is aromatic in character, possessing a sextet of electrons in association with all of the atoms comprising the ring[6] Further experiments in this laboratory have indicated that other compounds of this type possessing alkyl or aromatic-alkyl groups at position 3 and alkyl groups at position 4, on the carbon atom, are equally if not more active and less toxic We are at present synthesizing and pharmacologically evaluating an extensive series of these compounds

Lemont B Kier
Lauretta E Fox
D Dhawan
I W Waters

College of Pharmacy,
University of Florida,
Gainesville

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HÆMATOLOGY

Absorption-Elution Method of Grouping Blood-Stains

Based on an examination of eleven blood-stains, Schleyer has published a critique of the absorption-elution test[1] Since my own experience is so completely at variance with Schleyer's, this note is written with the purpose (*a*) of recording statistics based on the examination of approximately 600 blood-stains of both experimental and actual case material (*b*) of pointing out where Schleyer's procedure is, on his own description, markedly different from that recommended by Kind[2,3]

Testing experimental blood-stains of known group Some 400 blood-stains of known group have been tested in this Laboratory, mostly in duplicate, since the introduction of Kind's method These experimental groupings (in which anti-A, anti-B and anti-H sera have been used) were carried out on blood-stains made on filter paper and cotton cloth, or as dried aqueous extracts[4] The age varied from a few minutes to several years In every instance the proper and expected reaction has taken place agglutinations were unequivocal and easily visible to the unaided eye These tests on blood-stains of known group were performed on every occasion when unknown case material has been grouped in order to provide controls

Testing case material of unknown group These total more than 200 (again, mostly in duplicate), and are recorded as in Table 1

Table 1

	1959	1960	1961
No of groupings attempted	35	64	125
No of successful groupings	10	35	98
Percentage successful	29	55	78

The method was adopted in early 1959 and used for selected cases Since 1960 I have used it exclusively for antigen testing The improvement in the percentage of successful results stems from many causes, but a major factor was, I feel sure, the increasing experience of the operators The causes of failure are only too well known to the serologist with practical experience, for example, inadequacy of sample, the presence of grease, dirt and absorbing substrata Although the test is basically very simple, it will not tolerate too much arbitrary variation The substratum has, in the main, been cotton and woollen cloths, but has included paper, wood, leather, steel, soil and vegetation 'Successful grouping' in Table 1 is intended to mean a clear-cut macroscopic result which has been confirmed in most cases with the results obtained from agglutinin tests[5]

Variations in the technique introduced by Schleyer

(1) The indicator cells used by Schleyer in testing for anti-A elution are A_2, whereas Kind recommends A_1 A_1 cells are more sensitive than A_2 cells as an indicator of anti-A elution

(2) Glass slides with 12-mm cavities are recommended for the test Schleyer uses spotting tiles The greater thermal capacity of the tiles will tend to retard the heating of the test suspension in the moist chamber and consequently a longer elution time will be required

(3) The original paper[3] specifies that 30 μl of 2 per cent red cell suspension should be added to individual cavities before elution Schleyer uses "a few drops" Undue dilution of antibody will diminish the final agglutination intensity

(4) Although gorse (*Ulex europeus*) is suggested as a source of anti-H, Schleyer uses *Laburnum alpinum Watereri* The lack of result using this reagent suggests a low titre of the extract Anti-H extracts should be discarded if the titre is less than 1 in 128 (ref 6) As the final agglutination intensity is, in part, dependent on the amount of antibody elution, it is of importance to use an antiserum of adequate strength

(5) Schleyer uses a lens to read all his results, after transferring the agglutinates to slides Kind mentions that a microscopic examination may be necessary for thinner smears, but this procedure is exceptional In practice I find recourse to the microscope unnecessary and the tedium of constant pipetting is avoided Only clear-cut results are accepted, all others are rejected

(6) I experience no difficulty in distinguishing between A_1 and A_2 stains, nor do I note any practical

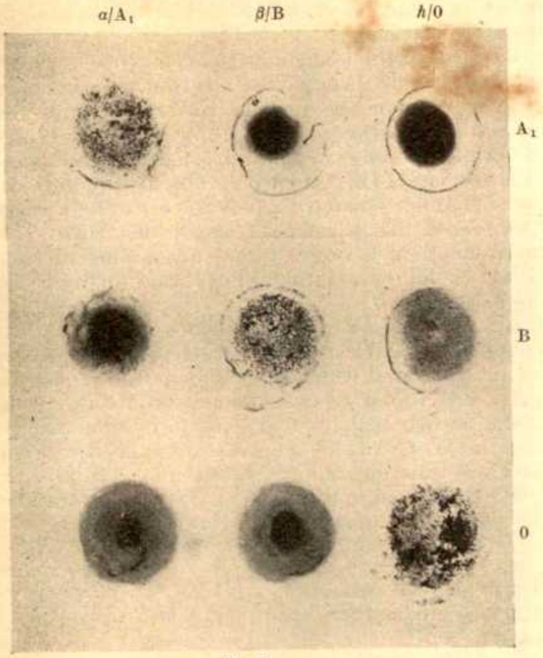

Fig. 1

differences between grouping fresh and old stains, although, of course, blood-stains gradually become insoluble and more difficult to extract.

Table 2 presents reactions for the various groups, and Fig. 1 shows typical test results.

Table 2

	α/A₁	β/B	h/0
A_1	+	−	−
A_2	+	−	+
B	−	+	(+)
A_1B	+	+	−
A_2B	+	+	+
0	−	−	+

I thank Dr. H. J. Walls (Northern Forensic Science Laboratory) for providing a translation of Schleyer's paper.

R. A. OUTTERIDGE

Home Office Forensic Science Laboratory,
Rutland Drive,
Harrogate, Yorks.

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PATHOLOGY

Virus-like Behaviour of Human Carcinoma

A THOROUGH study of 20 autopsy cases of metastatic carcinoma to liver[1,2] and kidney and of 72 cases of primary carcinoma of the liver (hepatoma)[3,4] has revealed the following facts:

(1) Continuity of structurally normal parenchyma with contiguous cancer parenchyma (Figs. 1 and 2). This was observed in 30 per cent of hepatoma cases in the liver and in 100 per cent of metastases to the liver and kidney. This higher percentage of positive findings in the material which promised less probability for this finding is ascribed to more careful sampling of blocks for sectioning.

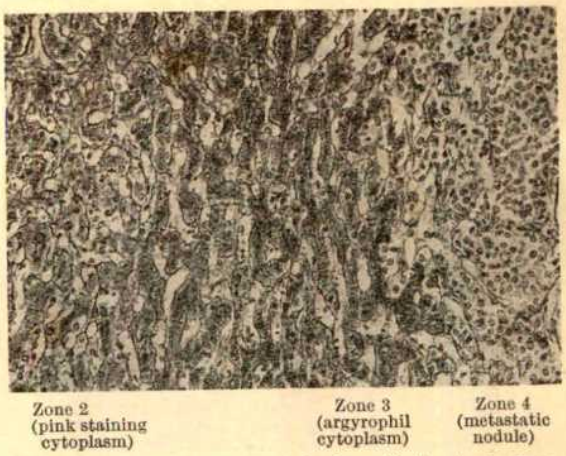

Fig. 1. Metastasis of a carcinoma of vulva epithelium to the liver

(2) Presence of single cancer cells in the vicinity of tumours as integral components of the host parenchyma, under conditions where invasion could be excluded (Fig. 3).

(3) Morphological evidence for transformation of normal cells into cancer cells.

(4) Transformation is preceded by cytochemical changes not visible with routine stains, but recognizable if special stains are applied.

A typical tumour nodule is surrounded by roughly concentric zones of structurally normal parenchyma. From outside in, one can distinguish four zones characterized by typical staining reactions of their cytoplasm. In Table 1, the colours of the zones are

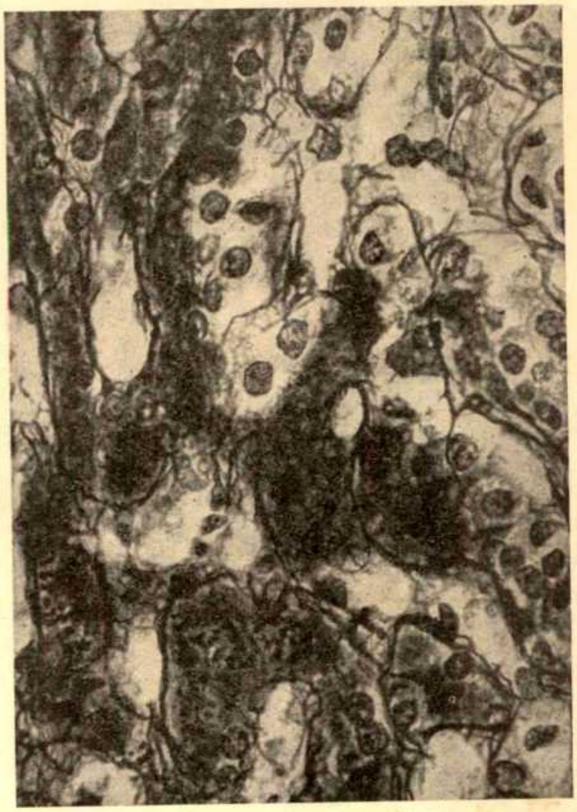

Fig. 2. Detail from Fig. 1 showing continuity of cancer parenchyma with liver

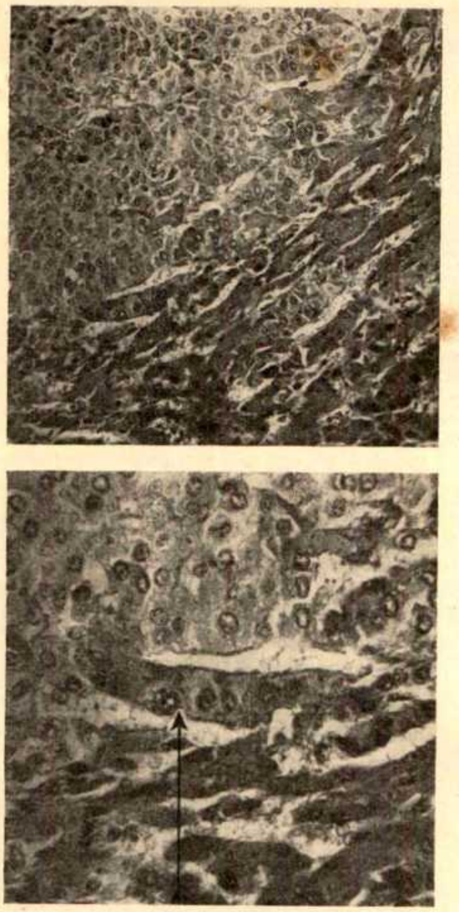

Fig. 3. Marginal area of a hepatoma (Bantu). Below, details from above figure. Arrow indicates cancer cells within a liver plate

described as they appear: (*a*) when stained with hæmatoxylin and eosin; (*b*) when impregnated with uranium and silver according to Wilder[5]; (*c*) when stained with acridine orange and observed with the fluorescence microscope according to Schuemmelfeder's method[6], as modified for paraffin sections by Abrams and Elias[7]. Zones 2, 3 and 4 are easily seen in Fig. 1.

With the acridine orange method the nuclei of all cells, whether malignant or normal, known to contain DNA, fluoresce red-orange, a fact which led to the following virological explanation of malignant transformation.

Let us assume hypothetically that the genome of a certain chromosome in every cancer cell contains one abnormal gene (a DNA molecule) which causes the cell to act as a cancer cell, and which also causes its own mass replication.

Table 1. COLOURS OF CYTOPLASM

	Hæmatoxylin and eosin	Wilder's uranium and silver impregnation	Acridine orange fluorescence
Zone 1 (zona sana)	red	pink	green
Zone 2 (zona affecta)			deep yellow
Zone 3 (zona determinata)		black or dark grey	
Zone 4 (zona maligna)	blueish purple	colourless clear or light grey	red orange

Replicas of the malignant gene leave the nucleus of the cancer cell through the electron microscopically visible pores in the nuclear membrane and, while present in the cancer cell cytoplasm, cause both its red-orange fluorescence and its slight basophilia.

These large DNA molecules break up into components small enough to penetrate through the cell membrane. These relatively small constituents of the malignant gene fluoresce yellow but are invisible after hæmatoxylin-eosin staining or uranium and silver impregnation.

However, close to the tumour, where the concentration of these particles is greater, they form aggregates within or around which silver precipitates.

The argyrophil particles penetrate into the nucleus of the normal cell. A few of them become incorporated into one of the chromosomes in the original position, thus imparting 'genetic information' to the invaded nucleus. As soon as the incorporation is completed, the normal cell has become a cancer cell.

HANS ELIAS

Department of Anatomy,
Chicago Medical School.

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Cytotoxicity of Human Malignant Effusions

A COMMON objection to the more widespread use of human ascitic fluid as a growth medium is its frequent cytotoxicity, the nature of which is not well understood. Several investigators have described an antibody-complement system lethal to mouse cells, which is often present in normal human serum[1-3] and also in sera of some other species[3]. It is not demonstrable in human cerebrospinal fluids though[4].

The objective of this investigation was to determine whether a similar antibody system could be found in human malignant effusions, and if so, to compare its effect on mouse cells and human cells. In addition, the influence of various means of inactivation on the growth-promoting capacity of the fluids was examined.

Six samples of ascitic fluid were obtained from patients with malignant effusions, who had not had any medication for at least 4 weeks. Each sample was divided into 4 parts which were processed as follows: (1) no treatment; (2) heated at 52° C for 30 min to inactivate C′2 of complement; (3) incubated for 1 h at 37° C with 'Zymosan' (1·5 – 2·0 mg/ml. of ascitic fluid) to inactivate C′3 of complement; (4) incubated with mouse fibroblasts (Earle's strain *L*) for 1 h at 4° C (10^6 cells/ml. of ascitic fluid) to remove *L*-cell specific antibody while complement remains active.

After treatment, all four types of ascitic fluid were centrifuged at 2,000 rev/min for 20 min, and stored at −20° C up to the day of use.

Experiments were performed on strain *L* cells, strain *C*-4 human carcinoma cells[5] and a 3-months-old culture of human fibroblasts, which had been established in this laboratory. All cells were maintained on Waymouth's medium *MB*752/1, supple-

Table 1. STRAIN *L* : CELL GROWTH AFTER ONE WEEK IN DIFFERENT MEDIA
Total cell mass expressed as optical density of crystal violet ×100

Ascitic fluid	Control maintenance medium	40% Ascitic fluid + 60% Waymouth's medium			
		Untreated	Heated	'Zymosan'-treated	Adsorbed on *L*-cells
Mac	16·5±1·5	1·5±0·81	12·8±0·95	17·8±1·70	17·8±2·0
Ran	21·5±1·1	22·0±1·34	22·3±1·18	24·0±0·84	20·3±1·22
Lep	41·6±2·0	0	12·7±0·35	2·8±0·62	3·5±0·26
Haw	44·8±1·1	0	9·6±0·89	9·6±0·77	14·6±0·63
Hop	41·6±2·0	1·5±0·22	15·3±0·55	10·1±0·77	—
Sig	12·2±0·51	0·7±0·06	7·3±0·57	6·6±0·28	7·6±0·35

Each value represents the mean ± *S.E.* of 6–8 cultures.

Table 2. STRAIN *C*-4 : CELL GROWTH AFTER ONE WEEK IN DIFFERENT MEDIA
Total cell mass expressed as optical density of crystal violet ×100

Ascitic fluid	Control maintenance medium	40% Ascitic fluid + 60% Waymouth's medium			
		Untreated	Heated	'Zymosan'-treated	Adsorbed on *L*-cells
Mac	19·3±1·30	13·8±1·60	10·5±0·8	15·3±0·2	14·0±0·17
Ran	22·3±1·51	10·4±0·05	6·5±0·55	9·8±0·71	8·8±0·32
Lep	28·0±1·05	16·5±1·22	13·6±0·71	15·6±1·0	16·3±1·45
Haw	11·1±1·14	4·8±0·55	1·0±0·32	6·6±0·89	5·0±0·32
Hop	10·0±0·80	6·1±1·80	0	5·1±0·95	6·3±0·45
Sig	23·5±0·71	12·4±0·63	13·4±0·30	14·8±0·71	12·8±0·65

Each value represents the mean ± *S.E.* of 6–8 cultures.

Table 3. HUMAN FIBROBLASTS : CELL GROWTH AFTER ONE WEEK IN DIFFERENT MEDIA
Total cell mass expressed as optical density of crystal violet ×100

Ascitic fluid	Control maintenance medium	40% Ascitic fluid + 60% Waymouth's medium			
		Untreated	Heated	'Zymosan'-treated	Adsorbed on *L*-cells
Mac	20·3±0·75	8·3±0·71	9·5±0·66	10·5±0·40	12·7±0·94
Ran	9·5±0·17	1·0±0	1·0±0	5·5±0·32	2·7±0·55
Lep	45·8±2·04	12·9±0·68	9·4±0·44	13·9±0·71	12·1±0·65
Haw	25·3±1·05	10·4±0·73	11·0±0·78	11·0±0·92	14·1±0·99
Sig	20·3±1·83	10·0±1·21	13·5±1·38	11·8±0·71	14·0±0·65

Each value represents the mean ± *S.E.* of 6–8 cultures.

mented as follows: for strain *L*, 5 per cent calf serum; for *C*-4 cells, 10 per cent fœtal calf serum, 10 per cent umbilical cord extract and occasionally 5 per cent ascitic fluid; for human fibroblasts, 10 per cent fœtal calf serum, 2 per cent umbilical cord extract and 0·05 per cent lactalbumin hydrolysate.

Trypsinized cell suspensions were divided into 5 parts, centrifuged, and resuspended in the following five media: (*A*) Control: maintenance medium. (*B*) Four ascitic fluid media: 60 per cent Waymouth's medium and 40 per cent of ascitic fluid treated by the 4 methods described.

Inocula of 50,000–70,000 cells in 1·0 ml. of medium were distributed among screwcap tubes. The cells were allowed to grow for one week, and then the total cell mass per tube was determined by the colorimetric method of Grady *et al.*[6]. In addition, cell growth in each tube was graded microscopically on a + to ++++ basis; this grading always confirmed the colorimetric results. Also, cell clumping was noted and graded + to +++.

Five of the six samples of ascitic fluid were toxic to *L*-cells (Table 1), and in each case both inactivation of the complement and adsorption on *L*-cells resulted in a highly significant increase in cell growth ($P < 0·01$). Cell death and lysis took place in untreated fluids. Clumping was fairly marked in heated and 'Zymosan'-treated fluids but negligible after adsorption. No similar pattern was found in experiments on homologous cells (Tables 2 and 3). However, two of the procedures used for inactivation repeatedly altered the quality of the growth media to a statistically significant degree: preheating impaired subsequent growth of *C*-4 cells in three instances (Ran, Haw, Hop) and of human fibroblasts in one case (Lep). This was associated with clumping and poor adhesion to glass of the *C*-4 cells, but not the fibroblasts. Also, the use of *L*-cell adsorbed fluid resulted in significantly improved human fibroblast growth in four cases; no such effect was noted in *C*-4 cultures.

It may be concluded that an antibody-complement system lethal to mouse cells occurs in human malignant effusions, but that this system had no effect on the homologous cultures tested, of which one was fibroblastic and one epithelial. The prevention of clumping of *L*-cells by preceding adsorption suggests polyvalency of the antibody involved. Similarly, the cytotoxic antibody in human serum is not bound by human cells, and seems to be at least divalent[7].

The detrimental effect of heating on homologous cell growth may be of a similar nature to that described for serum by Weiss[8], namely, the impairment of adhesive activity due to destruction of heat-labile α-globulin. This would explain the absence of this effect on *L*-cells, since they can adhere to glass even in protein-free media. There is no explanation at present for the enhancement of human fibroblast growth by adsorbed fluids.

The striking difference in response among all 3 cell types to modifications of malignant effusions indicates the extent to which experimental results can be influenced by such factors, and the importance of using natural growth media in their optimal form for specific cell populations.

I thank Dr. S. Fedoroff for advice.

This work was supported by a grant from the National Cancer Institute of Canada.

N. AUERSPERG

Cytological Diagnostic Laboratory,
2656, Heather Street,
Vancouver 9,
British Columbia.

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RADIOBIOLOGY

Protective Effect of Nitrous Oxide against Total-Body Radiation in the Mouse

IT has been shown that nitrous oxide and certain inert gases reduce oxygen-dependent radiosensitivity in broad bean roots and mouse Ehrlich ascites tumour cells *in vitro*[1]. Irradiation in one atmosphere of 80 per cent nitrous oxide with 20 per cent oxygen markedly reduced the radiosensitivity of mouse ascites tumour cells, as judged by chromosomal damage at anaphase. In a later investigation[2], using a different criterion of tumour growth after a standard inoculum of cells irradiated *in vitro* into normal animals, protection was not afforded by this nitrous oxide–oxygen mixture at 1 atmosphere pressure. Data have not yet been presented on a protective effect at elevated pressure. They demonstrated that xenon, the most effective of the inert gases in reducing oxygen-dependent radiosensitivity, was protective only at pressures in excess of 1 atmosphere in these *in vitro* systems.

A few investigations have been reported on the use of nitrous oxide–oxygen mixtures at 1 atmosphere on irradiation damage *in vivo*. Various unpublished results have been summarized[3] which suggest that no protection, as measured by lethality in the mouse from total-body irradiation, could be shown by these gas mixtures, and that the protective effect was due solely to the experimental conditions of the *in vitro* studies.

We have been investigating the protective effects of nitrous oxide and the inert gases in mixture with oxygen as measured by lethality in mice from whole-body irradiation and find that nitrous oxide does give significant protection at elevated pressures.

As shown in Table 1, two different strains of mice were used. In the initial study *CBA* mice from our own colony, both males and females, were irradiated at 8 weeks of age. Prior to irradiation, these mice were randomly distributed among the various experimental groups when removed from their litter; following irradiation, mice from the different experimental groups were mixed to eliminate 'caging effect'. The animals were exposed in an aluminium pressure chamber at 2 atmospheres pressure. The mice were exposed in groups of six while confined within plastic cylinders through which the test gas entered the chamber. By thus limiting movement, the dose variation from head to tail of the mice was diminished to ± 4 per cent. The irradiation was with 200 kVp at a quality sufficient to give a dose variation of ± 20 per cent dorso-ventrally. A commercially mixed tank of nitrous oxide and oxygen requested to contain 20 per cent oxygen, but on analysis in our laboratory by gas chromatography found to contain only 17 per cent oxygen (pO_2 130 mm mercury), was used. This mixture at 2 atmospheres pressure gave a highly significant reduction in 30-day lethality, as compared with groups in air at 2 atmospheres. Note in group *A* that a similar 30-day lethality is produced by 15 per cent lower radiation exposure when the air pressure is raised from one to two atmospheres. At least part of the protection in this group may be due to the slight reduction in oxygen tension from air at 2 atmospheres.

Two additional groups of mice have now been run on a mixture with guaranteed analysis of more than 20 per cent oxygen with 80 per cent nitrous oxide, which by gas chromatography in our laboratory was found to contain 20·36 per cent oxygen (measurement made by Dr. H. Zauder). To eliminate the possible influence of œstrus on radiation lethality, only male mice of the *C57L/J* strain were used in this experiment. Group *B* in Table 1 were approximately 10 weeks of age and were exposed to 750 r. measured at the centre of the mouse chamber, which was an LD_{50} (30 days), in air (at 2 atmospheres pressure). Group *C* were closer in age to the *CBA* group; approximately

Table 1

Strain (group)	Age	Sex	Gas	Pressure (atm)	pO_2	Radiation exposure	Lethality (31 days)	Significance	Anæsthetic deaths*
CBA (*A*)	8 weeks	M and F	Air	1	160	825 r.	37/4 (90%)†		
			N_2O—83% O_2—17%	1	130	825 r.	40/42 (95%)	N.S.	
			Air (P)‡	1	160	825 r.	11/31 (35%)	$P < 0·001$	1
CBA (*A*)	8 weeks	M and F	Air	2	320	700 r.	35/45 (78%)		
			N_2O—83% O_2—17%	2	260	700 r.	17/46 (37%)	$P < 0·001$	
			Air (P)‡	2	320	700 r.	38/42 (90%)	N.S.	1
C57L/J (*B*)	10 weeks	M	Air	2	320	750 r.	15/30 (50%)		
			N_2O—80% O_2—20%	2	320	750 r.	13/30 (43%)	N.S.	2
			Air (P)‡	2	320	750 r	23/30 (77%)	$P < 0·05$	
C57L/J (*C*)	8 weeks	M	Air	2	320	775 r.	41/42 (98%)		
			N_2O—80% O_2—20%	2	320	775 r.	23/36 (64%)	$P < 0·001$	3

* Included in 30-day lethality.

† Includes one death during exposure from asphyxia due to twisted position in chamber.

‡ 1·2 mgm. pentobarbital intraperitoneally 15 min. before irradiation.

8 weeks, and were exposed to 775 r. approaching an LD_{100} (30 days), if exposed in air at 2 atmospheres. In groups *B* and *C*, since we were unable to standardize age accurately in these mice from a commercial supplier, the mice were weighed on the day preceding irradiation and randomized in the experimental groups according to weight. There again is clear-cut evidence of protection from the lethality of total-body irradiation, which is more clearly demonstrated in the higher dose group.

We have made one attempt to rule out the possibility that this protection is due only to narcosis, with diminished tissue oxygenation, by subjecting groups of mice to a comparable level of anæsthesia with pentobarbital during irradiation in air at 2 atmospheres pressure. The pentobarbital was given at a dose of 1·2 mgm. to mice with an average weight of 24 gm. In air at one atmosphere pressure there was protection from the lethal effect of total-body irradiation. This is contrary to the findings of Paterson[4]. In air at two atmospheres pressure, there not only was no protection in either *CBA* or *C57L* strain mice, but actually a higher lethality in the group anæsthetized with pentobarbital. This was only of borderline statistical significance in the *C57L* strain. In all these groups, those animals dying during the radiation interval have been scored as deaths during the first 30 days following irradiation. These deaths were presumably due to respiratory arrest from the anæsthetic agent and were less than 4 per cent of the experimental group in both the nitrous oxide and pentobarbital series.

The mode of action of the anæsthetic gas in reducing oxygen-dependent radiosensitivity is unknown. Our investigation shows, however, that nitrous oxide does diminish radiation sensitivity *in vivo*, in the presence of adequate oxygen supply, if the gas mixture is at elevated pressure. The diminution of oxygen-dependent radiosensitivity by anæsthetic gases thus appears not restricted to particular *in vitro* systems and might be applicable to clinical situations by altering an adverse oxygen effect in tumour versus normal tissue sensitivity.

We thank Dr. B. N. Drolette for statistical calculations. This investigation was supported by a research grant No. *C*4685 from the National Cancer Institute, U.S. Public Health Service.

J. C. Evans
Louis R. Orkin

Department of Radiology and Anesthiology,
Albert Einstein College of Medicine,
Yeshiva University,
New York, 61.

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State of Binding of Natural Manganese in Human Cerebrospinal Fluid, Blood and Plasma

It was suggested earlier[1,2] that the physiological behaviour of microconstituents of the body might not be reflected in nutritional, enzymological or even 'physiological' experiments conducted exclusively with pure or synthetic forms of these agents. More specifically, investigations using manganese additives to diets or to enzyme systems *in vitro* had been quoted as an illustration of experiments that might not bear directly on physiological parameters[3]. The basis for the reservation lay with the suspicion that these manœuvres might not result in quantitative duplication of the chelates, postulated to be characteristic of individual tissues and foodstuffs, and that, on the contrary, they might create artefactual complexes as well. It was conceded, however, that the natural state of binding of this element had not been investigated and that its natural chelates remained largely putative.

Tentative support for the existence of natural manganese ligands was derived from experiments in which artificial isotopes of this metal were observed to become bound to intracellular organelles[4,5], to porphyrins[6,7] or to plasma protein fractions[8] of animals or man. However, artificial isotopes might be suspected to reveal unnatural metabolic patterns, since their chemical form, the time allowed for equilibration, and many other factors are variables in the evolution of the observed pattern. Therefore, investigations of the native, natural manganese isotope (^{55}Mn) were assigned exclusive pertinence to the study of the natural state of binding of this microconstituent.

The sensitivity and the high precision necessary for such work are met by neutron activation analysis. The method used here has been published separately[9].

At the outset we searched for manganese-containing pools of the body, the metal of which might be readily separable into micro- and macro-molecular fractions. Spinal fluid was intriguing because its minimal protein concentration might favour a non-protein-bound form of this element, provided of course, that it did contain measurable quantities of manganese. Since blood, plasma and serum are instrumental in the transport of this metal, they were also of interest to the present investigation.

All reagents tested during initial investigations contained significant levels of manganese, with the possible exception of an optical crystal of sodium chloride[8]. Also, contact with some metallic instruments increased the manganese contents of biological samples significantly[9,10]. Hence, reagents were avoided and, instead, a high gravitational field was applied for the sedimentation of macromolecular manganese under an ambient temperature of + 5° C. Concordantly, either platinum or non-metallic materials were used in the construction of needles, instruments and vessels. These awkward steps appear to be indispensable until contamination becomes controlled by the simpler methodology now under investigation.

Spinal fluid samples from seven humans contained manganese concentrations varying between 0·83 and 1·50 μgm./l. A given sample of spinal fluid generally contained about half the concentration of the corresponding plasma sample. In the one patient who was fed manganous sulphate, the spinal fluid concentration was 3·0 μgm./l., indicating that this concentration increased concordantly with that of the red cells, in contradistinction to the steady plasma-levels (*vide infra*). Centrifugation of spinal fluid at $11 \times 10^4 g$ (ref. 10) for 48 hr. yielded a supernatant fraction which was protein-free by nephelometry[11]. This fraction contained 93–94 per cent of the metal while the protein sediment contained the balance. The whole venous blood of 19 donors contained 8·6 to 14·5 μgm. manganese per litre and their plasma contained 1·83–3·10 μgm./l. Three samples of hæmolysed normal human red cells and four of fresh human plasma were subjected to the same centrifugation for periods of time up to 60 hr. In the case of the hæmolysates, the protein-free supernatant layers contained 3·9, 6·7, and 10·5 per cent respectively of the total manganese. Hæmolysates from the person who had been fed manganese sulphate for many months (blood manganese being 42·35 μgm./l. and plasma manganese 2·82 μgm./l.) contained 4 per cent of the total metal in the protein-free supernatant layers. In the cases of the four plasma samples, the protein-free fraction contained 4, 7, 15, and 25 per cent of the total plasma manganese on repeated duplicate analysis.

It is known that alkaline, oxygenated solutions of manganese yield slowly a sedimentable fraction, probably a hydroxide[13]. This was confirmed by means of high-speed centrifugation of solutions of manganese salts in 0·9 per cent sodium chloride, brought to *p*H 7·2 by means of sodium hydroxide: about 14 per cent of the metal could be sedimented after manipulations identical to those referred to here. Since the *p*H of spinal fluid becomes alkaline on standing (*p*H 7·8), and thus favours formation of hydroxide, the manganese in this fluid might have remained non-sedimentable by virtue of having become stabilized as one or more micromolecular chelates. As opposed to this deduction, it is also fair to assume that the manganese of blood and of plasma was sedimentable by virtue of being bound to the proteins rather than simply as a consequence of the formation of hydroxides, since the sedimentable portion was high indeed. This latter view is compatible with the experiments which showed $^{54}MnCl_2$ to become bound to specific receptors in blood and in plasma[6-8]. Hence, it is safe to assume that the protein-poor spinal fluid contains primarily micromolecular manganese chelates while the protein-rich plasma and blood contain primarily macromolecular complexes of this element.

As has already been pointed out, manganese-54 was found to become bound to different species of receptor sites in plasma as opposed to red cells: in the first a globulin[7] and in the second a hæm[5,6] incorporated the tracer. It therefore became germane to ascertain whether the receptor sites of the native, natural element were as dissimilar as those of the artificial one. To demonstrate a dissimilarity between receptor sites, proton competition experiments were performed with samples of plasma and of hæmolysate. Acidification of plasma with distilled hydrochloric acid (*p*H 4·3–4·5), followed by high-speed centrifugation, yielded protein-free supernatant fractions containing 85–100 per cent of the total manganese concentration in these samples. In striking contrast, acidified hæmolysates yielded protein-free supernatant fractions containing only 17–24 per cent of the concentration of the metal in the hæmolysates. These tests showed that plasma manganese is more readily replaceable by protons than is red cell manganese.

The result of the present experiments illustrates the existence of more than one chemical species of manganese in the body. Therefore, it is highly problematical whether *in vitro* additives of manganese (or of other co-factors for that matter) can duplicate exclusively natural chemical forms of as high a divergence as that revealed here. On the contrary, the natural manganese compounds themselves emerge as a promising subject for study.

This work was supported by the U.S. Atomic Energy Commission. It was presented in part at the forty-fifth annual meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, during April 10–14, 1961.

GEORGE C. COTZIAS
PAUL S. PAPAVASILIOU *

Medical Research Center,
Brookhaven National Laboratory,
Upton, Long Island, N.Y.

* ICA participant from the University of Athens, Greece.

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BIOLOGY

Estimation of Storage Life of Fruits

As long ago as 1924 Kidd[1] produced a "progress curve of fungal invasion" which was used by her and co-workers[2,3] for assessing the storage life of apples. Similar curves have been obtained by workers in the Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, for the development of physiological disorders in cool stored fruits (Fig. 1). We have found this relationship may be made linear, in each case, by transforming percentage disorder to a probit[4] and storage time to a logarithm (Fig. 2). In addition to the advantage of linearity for calculating estimates, this transformation shows storage life is log normally distributed. Since such a distribution has a wide occurrence it is probable that it will apply to other disorders.

The above observation leads to convenient methods of estimating mean storage life and its standard error. There are two approaches in storage experiments, namely: (1) use of one sample when each fruit may be assessed repeatedly during storage; (2) use of several separate samples when each fruit may be assessed only once. In the former the selected sample is

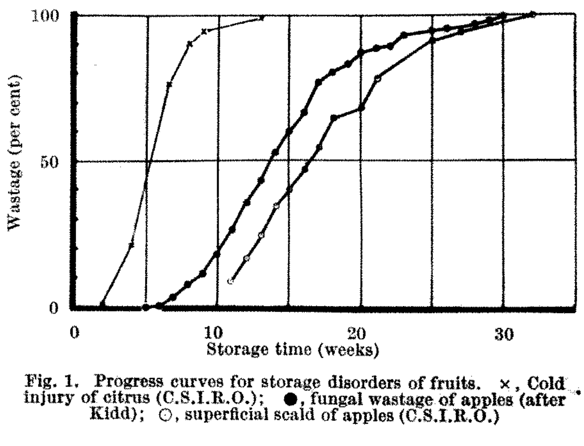

Fig. 1. Progress curves for storage disorders of fruits. ×, Cold injury of citrus (C.S.I.R.O.); ●, fungal wastage of apples (after Kidd); ○, superficial scald of apples (C.S.I.R.O.)

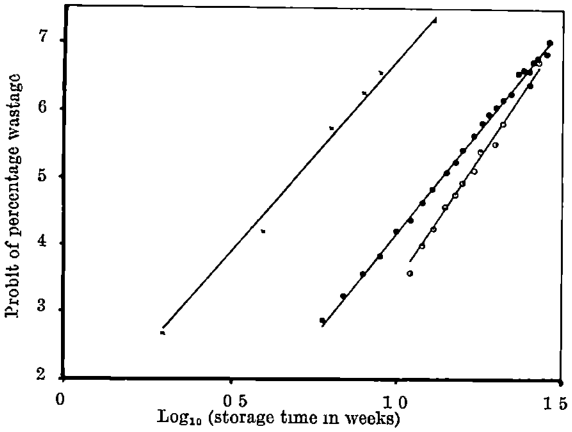

Fig 2 Data for storage disorders in fruits reduced to transforms giving linear relationship Symbols as for Fig 1

stored as a unit and the individual fruits examined periodically for development of disorder, the mean and standard deviation of storage life for the sample may be calculated as for any grouped data The latter situation, common in cool-storage experimentation, requires provision of a number of samples or units, each to be examined at only one of a series of times, this yields quantal data from which the methods of probit analysis provide an estimate of the log (time corresponding to a particular proportion of disordered fruit) together with its standard error

The probit method has been applied to data for superficial scald of Granny Smith apples in cool storage, to be reported in detail elsewhere

E A Roberts

C S I R O,
Division of Mathematical Statistics,
North Ryde

K. J Scott

N S W Department of Agriculture,
North Ryde,
New South Wales

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A Specific and Predictable Biochemical Anomaly in Interspecific Hybrids of *Baptisia viridis* × *B. leucantha*

In a chromatographic analysis of twenty-one putative hybrids of *Baptisia viridis* × *B leucantha* each of the hybrid types contained in the leaves 4 distinctive substances which were absent from the leaves of more than 25 specimens of each of the pure parental species examined The 4 hybrid-specific components were distributed in a novel pattern and reacted characteristically in ammonia vapour when observed in ultra-violet light Accordingly, they were considered to be bona fide hybrid-specific substances[1] Since there has been considerable interest in the appearance of new components in hybrids, and attendant theoretical implications touch on aspects of plant biochemistry and evolutionary mechanisms, it is important to establish conclusively that such substances actually are hybrid-specific and, if possible, their identity

As a result of a more comprehensive chromatographic study of *B viridis*, *B leucantha* and their natural hybrids we are now able to clarify somewhat the circumstances of the appearance of the 4 hybrid-type substances The same 4 hybrid-type substances occur as constant species-specific components of the flowers of *B viridis* The components are absent from the leaves of *B viridis* and entirely absent from *B leucantha* They are present in the flowers of hybrids of *B leucantha* × *B viridis* and in essentially unreduced amounts in the leaves of the hybrid While the substances cannot be considered as *de novo* products in the hybrid, they nevertheless are hybrid-specific in the leaf We wish to emphasize that these 4 components are major spots with unique attributes and they are consistently clearly identifiable in the circumstances described

B viridis also hybridizes with *Baptisia leucophaea* var *laevicaulis* and with *B nuttalliana*, but in these hybrids the 4 components are not detected in the leaves Of the 3 species with which *B viridis* hybridizes, *B leucantha* is most different both morphologically and, on the basis of present knowledge, biochemically Therefore, a greater genetic difference is probably represented in the hybrid, *B viridis* × *B leucantha*, and accordingly a breakdown in certain regulatory mechanisms is more easily explained in this hybrid We infer from the appearance of the 4 components of *B viridis* in the leaves of the hybrid *B viridis* × *B leucantha* that the mechanism which regulates the distribution of these components is disrupted so that the substances accumulate in sites in which they normally do not occur Presumably, the other crosses, which are thought to involve genetically closer species, do not result in as great a modification of leaf biochemistry

The interspecific hybrids of *Baptisia* generally contain the sum of the species-specific components of both parents, and most of the other components of the flower are present in the leaves as well[2] Therefore, it is not likely that a common precursor which is normally diverted in the leaves to form some other type of compound is available for conversion to the 'hybrid-type' components Certain properties of the 'hybrid-type' components indicate that they may be flavonoid in nature Thus, an abnormal build-up of these compounds could be analogous to that of the anthocyanin pigments in which a number of conditions deleterious to the plant as a whole encourage the formation of anthocyanin in atypical sites in the plant

It is possible that the phenomenon reported here could be used in artificial crosses with other species of *Baptisia* and *B viridis* to provide further knowledge of species affinities in the genus by a unique method Interspecific hybrids of *Baptisia* are unusually common Beyond this, the phenomenon cited falls into the category of examples which may at some future time provide an experimental tool for the analysis of genetic regulatory mechanisms operative indirectly on a biochemical pathway

R E Alston
Janiece Simmons

Department of Botany and Plant Research Institute,
University of Texas,
Austin,
Texas

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An Inhibitory Growth Correlation in the Apothecial Stipe of *Sclerotinia sclerotiorum*

ALTHOUGH growth correlations have been widely studied in the higher plants, there have been few observations of this phenomenon in the lower plants, and none at all in the fungi The occurrence of such a correlation in the apothecial stipe of the fungus *Sclerotinia sclerotiorum* (Lib) D By , 1886, is described here

The *S sclerotiorum* used was isolated from a diseased bean pod of *Phaseolus vulgaris* from Spearwood, Western Australia, during September 1958

Under certain conditions of moisture and temperature, one to several stipes, 0 5–0 8 mm in diameter, may develop on the surface of sclerotia No external supply of nutrients is necessary for this to occur If the sclerotia and attached stipes are held in darkness, the stipes continue to grow in length without branching and may attain lengths up to 50 mm under laboratory conditions If their apices are exposed to light each develops a single apothecium

As extensive studies were being made on factors affecting the formation of apothecial stipes by sclerotia, germinating sclerotia were readily available The sclerotia formed by each culture had been air-dried at 25° C for 3 weeks, sterilized in 1 1,000 mercuric chloride, washed in distilled water and placed in a Petri dish containing 1 per cent water agar The dishes were then placed in a dark incubator at 15° C to induce the formation of apothecial stipes This usually occurred within six weeks

Twenty of the dishes were selected at random In each dish one sclerotium with an apothecial stipe more than 10 mm in length was located The apical regions of 1–2 mm were removed with a razor from ten such stipes The remaining ten stipes were left intact as controls The dishes were then partially sealed with 'Sellotape' to prevent desiccation and replaced in the incubator Observations were made weekly The experiment was repeated using 20 apothecial stipes longer than 20 mm , from half of which the apical 10 mm was removed

Decapitated stipes ceased to grow in length Lateral branches, morphologically similar to the parent stipe, developed in 1–2 weeks at lower levels on all except two such stipes (Table 1) The withering of the two stipes on which no branches formed probably resulted from damage to the point of attachment of the stipe to the sclerotium during decapitation Sometimes only one branch developed, but frequently several branches formed Occasionally, several formed at one point on the stipe In one or two instances, the apices of the lateral branches rested in water on the surface of the agar Such apices withered and branches formed on them at lower levels No branches developed on the intact stipes Under suitable light conditions all lateral branches, except the few which had withered, formed apparently normal apothecia at their apices (Fig 1)

Table 1 FORMATION OF LATERAL BRANCHES ON DECAPITATED AND INTACT APOTHECIAL STIPES

Treatment of stipes		No of branches formed per stipe									
		1	2	3	4	5	6	7	8	9	10
Experiment 1 (stipes > 10 mm long)	Decapitated (2 mm removed)	1	3	1	2	4	3	2	2	4	2
	Intact	–	–	–	–	–	–	–	–	–	–
Experiment 2 (stipes > 20 mm long)	Decapitated (10 mm removed)	3	3	–	6	1	2	4	5	–	2
	Intact	–	–	–	–	–	–	–	–	–	–

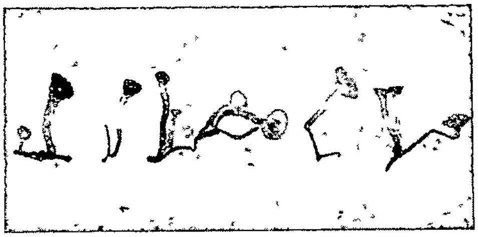

Fig 1 Mature apothecia at the apices of lateral branches on decapitated apothecial stipes The sclerotia have been removed Natural size

The origin of the lateral branches of the apothecial stipes of *S sclerotiorum* is uncertain They may originate from microscopic areas of meristematic tissue normally prevented from developing by the presence of the stipe apex However, no such areas were observed in the few longitudinal sections examined It is doubtful, therefore, if the phenomenon can be likened to that of apical dominance which occurs in higher plants It is more likely that they arose as a result of induced meristematic activity in mature cells following the removal of the stipe apex In this event the phenomenon would be one of regeneration comparable, for example, with the development of adventitious shoots below the cut end of decapitated hypocotyls in certain higher plants[2,7] Robertson[5] suggested that branching of the hyphal apex of *Fusarium oxysporum* following the arresting of the growth of the hyphal apex for more than 60 sec might be explained similarly

Auxins are thought to be involved in correlation phenomena in higher plants[1,6,7] However, though auxins are known to be produced by many fungi, they have not been assigned a growth-regulating role in these organisms[3] This may be because most experimental work has been concerned with the effect of auxins on growth of fungal mycelia rather than of fruit bodies The inhibition of the development of lateral branches by the intact apex of the apothecial stipe of *S sclerotiorum* may be determined to some extent by growth substances originating in the apex Certainly the apothecial stipe of *S sclerotiorum* appears to merit investigation as a source of growth-regulating substances in fungi

RUTH M HENDERSON

CSIRO,
Division of Forest Products,
Yarra Bank Road,
Melbourne

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Germination of Resting Bodies in *Verticillium* Species

ALTHOUGH detailed investigations have been carried out on the morphology, physiology and pathology of *Verticillium* species, there are very few references to the function and germination of the resting bodies formed by these fungi Reinke and Berthold[1] drew diagrams of structures resembling germ-tubes emerging from resting mycelium but they gave no details Basu[2] and Van den Ende[3], working with *Verticillium* spp , failed to induce germination of microsclerotia Wilhelm[4] has stated that resting bodies of *V. albo-atrum* in diseased tomato tissue germinated by becoming covered with conidial heads This, however, does not directly prove, but merely implies, germination

In experiments to investigate the germination of resting bodies in *Verticillium* spp , we used the following species so that all types of resting bodies could be tested *V albo-atrum* forming resting mycelium, *V dahliae* forming microsclerotia, *V nigrescens* forming small chlamydospores, *V nubilum* forming large chlamydospores, and *V tricorpus* forming resting mycelium, microsclerotia and chlamydospores

Since resting bodies are difficult to separate macroscopically from the hyaline supporting hyphæ and conidia, a technique was developed whereby these structures could be dissected out under the microscope

In initial experiments, material containing resting bodies taken from 3/4 week-old cultures on Dox's agar medium was squashed in a drop of sterile distilled water between a glass slide and a coverslip and then spread on freshly poured nutrient agar in Petri dishes The preparations were then placed under the low-power objective of the microscope and the resting bodies separated from the surrounding material with a micro-needle operated from a Saunders-Singer micromanipulator[5], and placed in rows or in a series of squares marked out on the agar The plates were then incubated at 22 5° C in the dark for 12 hr Of all the resting bodies tested under these conditions, only the chlamydospores of *V nigrescens* germinated, giving 85–100 per cent germination The suggestion may be made, therefore, that the resting bodies of *V albo-atrum*, *V dahliae*, *V nubilum* and *V tricorpus* are dormant, according to the definition of Gottlieb[6], a "dormant spore is one which does not germinate under the same nutritive and environmental influences which later allow the production of germ-tubes"

Germination failed to occur also when the above technique was used together with the usual methods of breaking dormancy in fungal spores, namely, the addition of nutrients, vitamins, amino-acids, enzymes, detergents, soil extracts, root exudates and extracts from appropriate host-plants to the agar medium on which the resting bodies were spread, and alternate wetting and drying of resting body material in test-tubes, and passing oxygen over the resting structures on agar plates Heat-shock and treatment with sub-zero temperatures also failed to bring about germination

Germination did occur, however, when the above procedure was modified to allow for prolonged soaking of the resting bodies as follows after squashing the material containing resting bodies, a loopful of the preparation was transferred to 0 5 c c of sterile distilled water in 'Pyrex' glass tubes and incubated at 22 5° C in the dark for 12 hr A 0 1-c c sample was then spread on distilled-water agar in Petri dishes, the resting bodies isolated by micromanipulation and the plates incubated for a further 12 hr at 22 5° C in the dark Non-soaked resting bodies were used as controls

Germination achieved by the foregoing method was approximately 56 per cent for resting mycelium of *V albo-atrum*, while for *V dahliae*, whereas groups of cells from microsclerotia showed 75 per cent, single cells gave only 5 per cent germination Chlamydospores of *V. nubilum* gave 84 per cent germination In *V tricorpus*, it was assumed that the resting mycelium, microsclerotia and chlamydospores were randomized in the preparation and so all three types were included in the counts, which showed approximately 91 per cent germination Non-soaked controls showed no germination.

The low percentage germination of single cells of microsclerotia of *V dahliae*, as contrasted with clusters or groups of cells which may be part or whole microsclerotia, raises the question as to whether there is division of function between the individual cells of these resting structures, that is, whether some cells are destined to germinate whereas others are not, and serve some other function Although no distinct morphological distinction has yet been discerned between the cells, this problem is being further investigated

Prior to germination, hyaline granules appear in the resting bodies of all the species investigated In *V albo-atrum*, the individual cells of resting mycelium frequently become somewhat rounded, while in *V dahliae* the cells of the microsclerotia often become invaginated No swelling occurred, neither was any operculum or germination-pore observed in any of the resting bodies

During germination, one or sometimes two hyaline germ-tubes developed from all types of resting bodies, although not in any constant position

In *V albo-atrum*, these germ-tubes branched and produced conidia within 6 hr , whereas in the other species this occurred very much later

These investigations on the germination of the resting bodies may indicate their role in the survival of *Verticillium* species in the soil, and this is the subject of further studies

Ivor Isaac
Q D MacGarvie

Department of Botany,
University College of Swansea

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Longevity of Conidia of *Peronospora tabacina* Adam.

During the three years ending 1961, *Peronospora tabacina* Adam has spread from north-western Europe east to the U S S R and Turkey and south to Algeria and Tunisia The evidence[1] indicates that this extensive distribution has been brought about by dissemination of the thin-walled, almost hyaline conidia that are produced in very large numbers I have found up to 750,000 conidia per cm ^{2} of infected leaf surface, while Corbaz[2] records twice this number The effectiveness of conidia in initiating infections in previously free areas depends, *inter alia*, on their ability to survive during transfer Survival (viability) on glass slides to which water is afterwards added to obtain germination is favoured by low temperatures and low relative humidities[3], but with this procedure germination is extremely variable More recently, Cruickshank[4] and Shepherd[5] have shown that reliable data on germination of conidia can be obtained on agar.

In the experiments summarized in this preliminary report, conidia were produced on disks cut from leaves in the pre-sporulation stage[6] The disks were transferred to filter paper to remove excess water, then stored, at different relative humidities, in desiccators held at constant temperatures Disks were withdrawn, at intervals, from the desiccators, washed in water to remove conidia and a drop of the conidial suspension so obtained was placed on an agar block for incubation at 15° C for 24 hr Table 1 shows the period in days over which germination, down to as low as < 1 per cent, occurred in conidia stored at the stated temperatures and relative humidities, and, for comparison, the period of survival of approximately 15 per cent of the conidia

Table 1. LONGEVITY (IN DAYS) OF CONIDIA OF *P tabacina* STORED AT CONSTANT TEMPERATURES AND RELATIVE HUMIDITIES

Temperature (° C)	30		25		20		15		10		5	
Relative humidity (per cent)	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
80–100	0	0	2	2	9	7	6	6	12	6	12	8
70–80	0	0	5	3	9	7	21	14	30	22	46	36
50–70	–	–	8	4	14	7	—	—	—	—	—	—
40–50	0	0	10	6	—	—	43	27	—	—	—	—
30–40	–	–	21	7	36	20	75	43	106	70	131	90
20–30	–	–	29	10	69	22	—	—	—	—	—	—
10–20	–	–	42	7	106	40	22	19	—	—	—	—
Nominal nil	3	0	5	1	13	2	15	2	20	2	20	2

A, <1 per cent survival, *B*, approximately 15 per cent survival

The results confirm the earlier conclusions of Angell and Hill[3] They also show that optimum longevity of conidia at all temperatures did not occur at the same relative humidity As temperature increases, optimum longevity occurs at lower relative humidities It is considered that under the dynamic situation imposed by temperature and relative humidity interaction, viability of conidia is conditioned by the balance established between transpiration and respiration If the results are considered in terms of water potential, which is regarded as identical with diffusion pressure deficit, then it can be shown that maximum longevity at all temperatures occurs at approximately the same low water potential value

Thus conidia have a remarkable capacity for survival under the dry conditions characteristic of the regions in which *Nicotiana* spp , the natural host plants, occur In general, it would appear that dry, hot conditions such as may occur in field or glass-house cannot be expected to eliminate conidia as a factor in spread of *P tabacina*

A V HILL

CSIRO
Division of Plant Industry,
Canberra

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B_{12} Coenzyme Content of the Nodules from Legumes, Alder and of *Rhizobium meliloti*

IN our recent communication with the above title[1], a dilution factor was unfortunately omitted in the calculation of results, thus the results given in Table 1 are incorrect

The coenzyme content per gm of fresh tissue (mμmoles) for the various species tested is as follows *Medicago sativa*, age 78 days, 64, *Glycine max*, age 39 days, 39, *Glycine max*, age 73 days, 30, *Pisum sativum*, age 26 days, 47, *Pisum sativum*, age 45 days, 16, *Trifolium pratense*, age 81 days, 40; *Alnus oregona*, age 2 years, 62, *Rhizobium meliloti*, age 36 hours, 77

MARK KLIEWER
H J EVANS

Department of Botany and Plant Pathology,
Oregon State University, Corvallis, Oregon

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Distribution of Certain Strigeid Trematodes in Great Britain

THE genus *Apatemon* has been recorded frequently from members of the Anatidae in Europe and the United States[1,2] In Britain only one published record of this genus seems to be available Baylis[3] recorded *Apatemon gracilis* (Rudolphi, 1819) from *Anas penelope* from Cheshire Two unpublished records exist of the occurrence of this species in Wales Davies[4] described this species from a domestic duck, and Williams[5] recovered 12 specimens of *Apatemon gracilis* (Rudolphi, 1819) from *Anas crecca* and 15 specimens from *Anas penelope* The host–parasite lists of Nicoll[6] are not considered valid in this context as they do not differentiate between European and British records

Over the past eight years, parasitological studies on the invertebrate fauna of freshwaters in Glamorgan (South Wales) have shown the larval stage of this genus to be quite widely distributed in leeches (particularly *Erpobdella*) and in snails, especially *Lymnaea pereger* (Fig 1) Collections of leeches from Breconshire (Llangorse Lake) and Radnorshire have also been found to be infected The rate of infection of *Erpobdella* varies from approximately 12 per cent for Llangorse Lake to roughly 90 per cent for Roath Park Lake, Cardiff At a recent vacation course in parasitology at Windermere, one (*Erpobdella octoculata*) out of four species of leech was found to carry *Apatemon* metacercariæ A total of 194 *Erpobdella octoculata* from three different habitats (Lake Windermere, Drunken Duck Tarn and Clea Barrow Tarn) were examined, and the percentage infection for the total collection was 30 per cent All three habitats were infected, and the highest single infection-rate was at Windermere where approximately 50 per cent of the leeches were infected The numbers of metacercariæ varied from 1–13 and a sample of 26 infected leeches yielded 144 metacercariæ At the end of the course, 200 *Erpobdella* from Windermere were brought back to Cardiff and fed to helminth-free, 16-day-old ducklings The ducklings were killed on the 15th and 18th day after infection (exps 88 and 89) and adult *Apatemon gracilis minor*, Yamaguti 1933, recovered In the same way samples of the metacercariæ from South Wales leeches have also been correlated with *Apatemon gracilis minor*, Yamaguti 1933 One of the adult populations obtained in this way[7] has its metacercaria in *Erpobdella octoculata* and its furcocer-

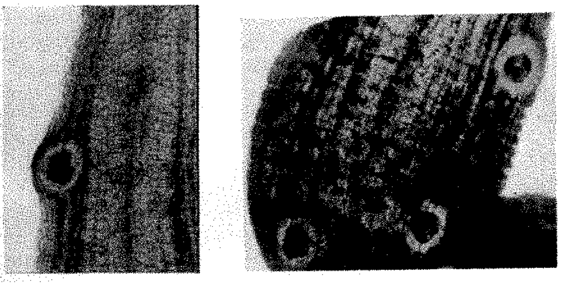

Fig. 1. *a*, A superficial cyst, lateral in position in *Erpobdella octoculata*; *b*, A multiple infection with more deeply situated cysts. Note the hyaline cyst wall. The cysts are easily observed in living leeches by compressing the leech between two slides and examining with a low power lens, using transmitted light (both × 21)

carial stage in *Lymnaea pereger*. Another variety (*Apatemon gracilis pellucidus*, Yamaguti 1933), also obtained experimentally in the duck[8], has its metacercaria in the body-cavity of bullheads (*Cottus gobio*) present in the streams near Cardiff. Thus there seems to be a fairly abundant reservoir of *Apatemon* metacercariæ in fresh-water leeches and snails, although the frequency of the occurrence of this genus does not seem to be reflected in the lists of adult trematodes recorded from birds in Britain. The reason for this may be in the short adult life of *Apatemon*, which under experimental conditions in the duckling seems to survive for only three weeks. This brief life-span would reduce the chances of its recovery from a natural population of birds.

The metacercaria of *Diplostomum phoxini* (Faust, 1918) parasitic in the minnow has been recorded from many parts of Scotland[9,10] and from the Aberystwyth area[11] in Wales. Samples of minnows from Windermere and Grasmere showed an infection rate of 100 per cent and 65 per cent respectively with a diplostomulum-type metacercaria. In both cases the metacercariæ were free in the posterior ventricles of the brain and sometimes occurred in the spinal cord. Material sent to Cardiff and fed to 1-month-old helminth-free herring gulls produced a heavy infection of *D. phoxini* (Faust, 1918) adults (Expt. 92). The life-cycle of this species has been described by Rees[12] and a longifurcous, pharyngeal cercaria resembling that described by Rees has been obtained from *L. pereger* in Windermere. Unfortunately not enough material was obtained to allow a complete morphological description and an experimental proof of its identity.

I thank the Director and his staff at Ferry House, Windermere, for facilities.

Note added in proof. Since this communication was submitted for publication the records of I. C. Williams have been published (*Ann. Mag. Nat. Hist.* (13), **4**, 467 (1961).

DAVID A. ERASMUS

Department of Zoology,
University College, Cardiff.

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ENTOMOLOGY

Structure of the Micropyle in Eggs of *Nasonia vitripennis*

IN insects a micropyle is formed at the anterior pole of the egg and often has a very complicated structure. Sometimes it is a single pore or it may consist of numerous canals grouped together. The formation of the micropyle is due to a number of follicle cells or nurse cells having protoplasmic prolongations towards the egg, around which the chorion is secreted[1]. Snodgrass[2] pointed out that although a micropylar area is present in the egg of *Apis mellifera* no actual pores have been demonstrated. There is no published work on the structure of the micropyle in chalcids.

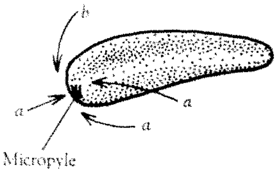

Fig. 1. Egg of *Nasonia vitripennis*

The eggs of *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae) are of a typical hymenopterous shape, like that of a curved sausage but more pointed at one end than the other. They are formed in polytrophic ovarioles, and the pores in the chorion which constitute the micropyle are the result of protoplasmic strands from the trophocytes and are situated at the rounded end of the egg, farther away from the oviduct end of the ovariole.

The ovaries were dissected from 50 specimens of *N. vitripennis* and, having been stained with aceto-orcein, were made into temporary mounts and examined with a light microscope. This, therefore, entailed examination of 100 ovaries each with four ovarioles, and each of these with one or two eggs showing micropyles, the total number of eggs being 480.

Owing to its curvature, each egg has in its meridional plane a convex and concave side. The micropyle is invariably situated in a slightly sub-apical position on the concave side of the meridional plane (Fig. 1). It consists of five radiating grooves in the chorion of the egg which narrow as they converge. Each of these grooves leads into a tube which narrows as it passes through the chorion and opens to the egg beneath by a pore (Fig. 2). The grooves radiate over an angle of 270° of arc from the centre of the micro-

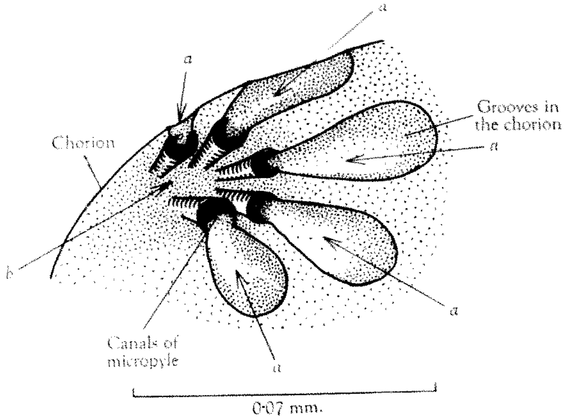

Fig. 2. Micropyle

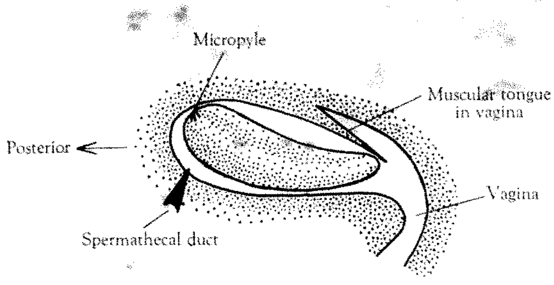

Fig. 3. Egg passing through uterus, with convex meridian orientated ventrally

pyle. The other 90°, which is situated on the convex side of the meridional plane of the egg, has no grooves.

When a population of *N. vitripennis* has ready access to host puparia, so that the parasites are not resorbing their eggs, each generation of offspring has a sex ratio of about three females to one male, the letter derived from unfertilized eggs[3]. Within each ovariole the eggs are orientated so that the oldest eggs (those nearest the oviduct end) have their convex sides pointing away from the axis of the ovary[4]. The spermathecal duct in *N. vitripennis* opens into the postero-ventral surface of the vagina in the mid-line, and it has been suggested that when eggs enter the vagina with their concave face either ventrally or laterally orientated the spermathecal duct can make contact with the micropyle allowing the sperms to enter the egg (Fig. 1*a*), but that when the egg has its convex face ventrally orientated (Fig. 3) the spermathecal duct is remote from the micropyle and fertilization much less likely. The structure of the micropyle would help this suggested mechanism of sex determination because if the spermathecal duct approaches a micropyle within an arc of 45° on each side of the convex meridian (direction *b*, Figs. 1 and 2) there would be no grooves there to guide the terminal portion or the discharge sperm towards the pores of the micropyle. The structure of the micropyle, in addition to providing a point in the chorion for sperm entry, helps to control the proportion of eggs fertilized and hence the proportion of females in the next generation.

I thank Mr. R. F. O. Kemp for help, and Prof. E. W. Knight-Jones in whose department this work was done.

PHILIP E. KING

Department of Zoology,
University College of Swansea.

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Parallel Evolution in European and North American Populations of a Geometrid Moth

THE phenomenon of industrial melanism in the Lepidoptera has provided an exceptional opportunity for the observation of minor evolutionary changes within relatively short periods of time. Many species of moths, have, in the past 100 years or so, developed melanic forms which have spread and largely replaced the predominantly pale-coloured 'normal' forms. Melanic forms first appeared and have reached their greatest relative frequencies in or near centres of heavy industry. Selective predation by birds appears to have contributed toward the decrease in relative frequency of the original pale forms[1]. *Biston betularia* (L.) (Geometridae) is the species which has been most extensively studied in Britain, and since a very similar moth, *B. cognataria* Guenee, occurs in North America, some comparison of the evolution of melanism can be made. It can be assumed that geographical barriers prevent gene flow between North American and European populations of these moths.

Although *B. betularia* and *B. cognataria* are now regarded as distinct species, they may, in fact, be so similar genetically that they should be regarded as conspecific: no difference has been found in the structure of the male genitalia[2], and they will interbreed in captivity[1]. Even so, the pale forms of each are quite distinct: in *B. betularia* the wings are white, speckled with black, and the postmedial and antemedial lines are broken up into spots and blotches, while in *B. cognataria* the wings are much darker, more heavily speckled, and the postmedial and antemedial lines are clearly defined. The melanic forms are extremely similar in both species: the entire wings and body are black and the intricate pattern of the pale form is absent, except in a few specimens in which traces of the postmedial line on the wings can be distinguished.

In Britain, the first melanic *B. betularia* was recorded in Manchester about 1848[3], while in North America the first melanic *B. cognataria* was recorded in Philadelphia in 1906[4]. In many parts of Britain[1] and in some areas of north-eastern North America (for example, near Detroit)[4], melanics now comprise up to 90 per cent of the population. It appears that the spread of melanic forms began about 50 years later in North America than in Britain, but in both areas melanic forms first occurred, and are now at their highest frequency, in or near centres of heavy industry.

It is, of course, possible that the potentiality for producing melanic mutants was present before North American and European populations became isolated and that, until recently, selection has prevented their spread through the population. But the rapid changes in gene frequency which have occurred in North American and European populations of these moths have taken place independently in response to a similar environmental change: increased industrialization. Hence for the first time we have direct evidence of parallel evolutionary changes in isolated populations of moths. Evidently many other species of North American and European moths are being similarly affected, especially in such genera as *Phigalia* and *Ectropis*[4]. What happens in the future will be of considerable interest.

D. F. OWEN *

Department of Zoology,
University of Michigan,
Ann Arbor.

* Present address: Department of Zoology, Makerere University College, Kampala, Uganda.

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Mode of Host Selection by an Aphid

CONCLUSIVE evidence of the properties of plants which determine host selection in aphids has been lacking[1]. The host plants of the cabbage aphid, *Brevicoryne brassicae* (L), are restricted to the Cruciferae and Resedaceae[2] in which mustard oil glucosides are characteristically present[3] and known to stimulate feeding by certain lepidopterous larvæ[4]. To test the

Table 1. BEHAVIOUR OF *B. brassicae* DURING THE FIRST 10 MIN AFTER ARRIVAL ON THE SHADED SIDE OF HOST (*B. rapa*), NON-HOST (*V. faba*) AND SINIGRIN-TREATED NON-HOST LEAVES

	Time spent on shaded side of leaf (sec)	Total time spent probing (sec)
Brassicae rapa L.	600	444
Vicia faba L.	148	68
Sinigrin treated *Vicia faba* L.	580	470

effect of such a substance on *B. brassicae*, the mustard oil glucoside sinigrin was introduced into young leaves of the broad bean, *Vicia faba* L., which is not a host of this aphid. The leaves were set with the cut petiole in a 2 per cent aqueous solution of sinigrin for 2 h before and also during each experiment. Apterous virginoparous adult aphids were given a choice between such a sinigrin-treated leaf and a similar but untreated leaf, standing side by side under a 2 × 1 in. glass vial. 45 aphids were used in a total of five experiments. After 24 h an average of 93 per cent of the aphids were found settled and feeding on the sinigrin-treated leaf, with an average of four new-born larvæ each. Only two larvæ altogether were found on untreated leaves and the few adults seen there were restless. *B. brassicae* were reared on sinigrin-impregnated broad bean leaves continuously to the fourth generation when the culture was still healthy.

The time taken to show discriminating behaviour on a host or non-host substrate varies with the responsiveness and consequently the pretreatment given the aphid. To test the initial behaviour young virginoparous apterous adults were kept in plastic containers for 24 h in darkness and 10 min in light. The main act of host selection then occurred after a brief probe. When a leaf of turnip (*Brassica rapa* L.), which is a host of *B. brassicae*, was held upright and lit from one side only, and an aphid was led on to the shaded side where it received only the light transmitted through the leaf, the aphid made a brief probe. If during its subsequent movements it reached the edge of the leaf where it met the direct light, it turned back into the shade. Probes were repeated on the shaded side, and the aphid had often settled there in the feeding posture within 10 min. On a non-host such as broad bean, presented in the same way, the aphid again made a brief probe and walked to the leaf edge but, instead of turning back, continued over on to the illuminated side and walked off the leaf without further probing. Thus stimuli from the leaf determine the reaction to light. On a broad bean leaf presented in the same way but treated with sinigrin through the petiole, the aphids turned back from the edge and remained probing on the shaded side of the leaf exactly as if it had been a host leaf (Table 1).

These values are averages for 14 insects, each tested on the three leaves in turn.

That the sinigrin itself acts as the distinguishing stimulus was shown by means of disks of pith moistened either with a 0·2 per cent aqueous solution of sinigrin, or with water alone, both being covered with a thin celloidin membrane and lit with green light. As expected from Moericke's work[5], the green light was enough to induce one probe regularly, even on non-host surfaces. 27 aphids were each tested on both types of disk in the course of five experiments. The presence of sinigrin increased the average number of probes per aphid by 1·3 times and the average duration of a probe by 2·4 times. Similar results were obtained when 'Millipore' filters were used instead of pith disks.

In both the preceding experiments aphids discriminated only after probing. Further experiments with celloidin membranes showed that the essential stimuli were received via the stylets as they penetrated the substrate rather than externally by sense organs on antennæ, rostrum or tarsi. When aphids were tested as here on upright turnip and bean leaves which had been ensheathed in a thin layer of celloidin, the aphids discriminated between them clearly at once when the membrane was quite fresh. In a typical experiment all of 13 aphids tested walked off ensheathed non-host (bean) leaves within 3 min whereas all of 13 aphids were still on the unlit side of the ensheathed host (turnip) leaf after 10 min. This was not likely to be a response to material diffusing outward through the celloidin membrane because when similar discrimination tests were repeated at 5, 20 and 60 min after the membranes were applied there was no improvement in the response to the host. Nor did thickening the membrane reduce or delay the response. Furthermore, on broad bean leaves ensheathed and then treated externally with sinigrin the aphids displayed some tendency to turn back from the direct light at the leaf edge but did not settle. On leaves similarly ensheathed but treated with sinigrin through the petiole only, the aphids settled as readily as they did on the naked leaves so treated above. In fact, once the aphids could be induced to probe, they would settle down on a host leaf even when it was smeared with a substance such as crushed fennel leaf or clove oil which had a visibly repellent effect.

It is concluded that sinigrin is a specific stimulus for host selection by *B. brassicae*, and is received via the stylets after they have penetrated the leaf surface. A fuller account of this work and of the sense organs concerned will be published elsewhere.

I thank Dr. J. S. Kennedy for suggestions and discussion, Prof. V. B. Wigglesworth for granting facilities with the Agricultural Research Council Unit of Insect Physiology, and the National Research Council of Canada for a supporting grant.

R. J. D. WENSLER

Department of Zoology,
University of Cambridge.

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MICROBIOLOGY

Chain Formation by *Phaeodactylum*

Phaeodactylum tricornutum (Bohlin) is a small marine diatom, with relatively unexacting growth requirements. Since the isolation of the original culture[1] several others have been started, and subcultures have been sent to many countries. The rate of growth in relation to nutrition and environmental conditions has been widely studied in unialgal and bacteria-free culture, as well as in mass culture. The species is also extensively cultured as food for rearing marine larval forms and filter-feeding invertebrates. Considerable speculation has centred on its taxonomic position[2].

Phaeodactylum is pleomorphic, producing ovoid, fusiform, triradiate, and cruciform cells[3]. A siliceous valve is possessed only by the ovoid form and that on one side only[4,5]. Despite its long history in culture, under a great variety of conditions, no mention has been made to date of chain formation by cells of this species.

During a routine examination of cultures maintained in this laboratory, it was found that *Phaeodactylum* had successfully invaded a declining culture of *Isochrysis galbana* (Parke) and was present as curved chains of up to 52 cells, as well as single cells. These chains consisted of either wholly fusiform or wholly triradiate cells, the component cells being of normal appearance and dimensions. The monotypic nature of the chains might suggest that the cells were dividing longitudinally, as do diatoms, replicating the original. No ovoid cells were observed in any of the chains examined. The exact medium conditions under which the chains arose are unknown, but they are persisting in heat-sterilized sea-water enriched with 0·8 p.p.m. phosphorus (disodium hydrogen phosphate) and 6·0 p.p.m. nitrogen (as ammonium sulphate).

I thank Mr. N. I. Hendey for assistance, and the Central Electricity Generating Board for financial support.

John Coughlan

University of Southampton,
Department of Zoology,
Plankton Laboratory,
Generating Station,
Poole, Dorset.

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Streptomyces Species producing Oxytetracycline

Since the isolation of *Streptomyces rimosus*[1], a number of other species have also been described which produce the antibiotic oxytetracycline. The status of some of these species has been questioned[2,3], and comparative studies on induced mutants of *S. rimosus*[4] suggest that other members of this group might be naturally occurring variants. One recently described species, *S. albofaciens*[5], however, was stated to be distinct from *S. rimosus* as it produced a white aerial mycelium bearing numerous sporophores with monoverticillate spirals. The morphology of the sporophores is considered to be an important diagnostic character in the study of this genus, and if this culture falls into the section *Monoverticillus–Spira* of the scheme proposed by Pridham *et al.*[6] it would be far removed from *S. rimosus* and might even be placed in the separate genus *Streptoverticillium* Baldacci[7].

I have isolated independently two oxytetracycline-producing strains from soil which appear to be very similar to the described species *S. albofaciens*. The vegetative mycelium of the two strains is long and branching, penetrating the agar medium and also forming the tough, compact colonies which on certain media exhibit the wrinkles, folds and cracks characteristic of *S. rimosus*. The colonies are convex to umbonate and yellow to yellow-brown to golden-brown in colour, with a pale yellow-brown to brown soluble pigment diffusing into the medium. The aerial mycelium when present is coloured white to very pale grey. Both isolates show only sparse growth on Czapek's agar, moderate liquefaction of nutrient gelatin, only very slight peptonization of skim milk, good hydrolysis of starch and positive reduction of nitrate. They were also very similar to *S. rimosus* in the utilization of various carbon sources. They did, however, have a distinct aerial mycelium morphology.

The aerial mycelium of one of the isolates (*A*5279) was thin and limited almost entirely to very long hyphæ growing upright but usually forming a loose network with hyphæ from the same colony or adjacent colonies. Spore chains appear to be borne in whorls on these very long hyphæ, the individual chains in each whorl showing very irregular spirals or loose coils often encircling the main axis, so giving the aerial mycelium a beaded or knotted appearance when viewed under low-power magnification (Fig. 1). The actual structure of the individual whorls is difficult to determine owing to the coiling of the spore chains. Examination of extremities of the long hyphæ shows few whorls of two or sometimes three short and immature sporophores, arising from the same point or close together on the main axis, which become coiled during elongation to produce the typical knot-like structures. In most cases there appears to be only a single side branch which either coils several times around the main axis or branches almost immediately into two or three coiled secondary branches which fragment into spores.

The other isolate (*B*7648) shows the same gross morphological appearance as *A*5279. The aerial hyphæ are again very long and bear the sporophores in knots at intervals along their length. In this case, however, the whorl structure is less apparent, and sporophores are mostly single side branches, sometimes showing secondary branching, which again show the loose and irregular coiling.

This pseudo-whorl structure of the aerial mycelium in *Streptomyces* has been described previously for several species[8,9], and its more pronounced appearance in these strains (*A*5279, *B*7648, *S. albofaciens*) is related to the greatly extended main axis of the aerial

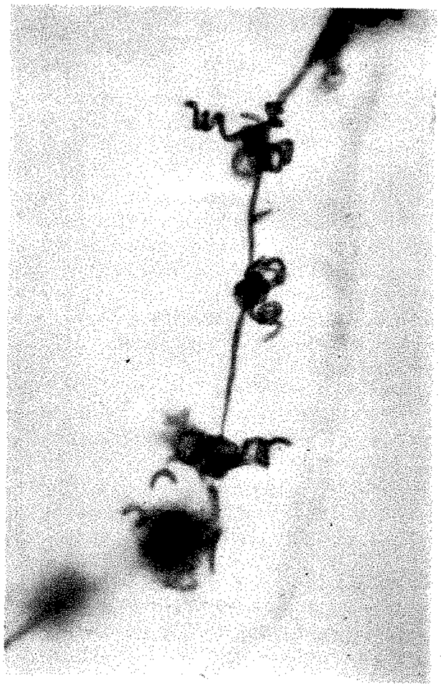

Fig. 1. Aerial mycelium of isolate *A*5279 (× *c*. 530)

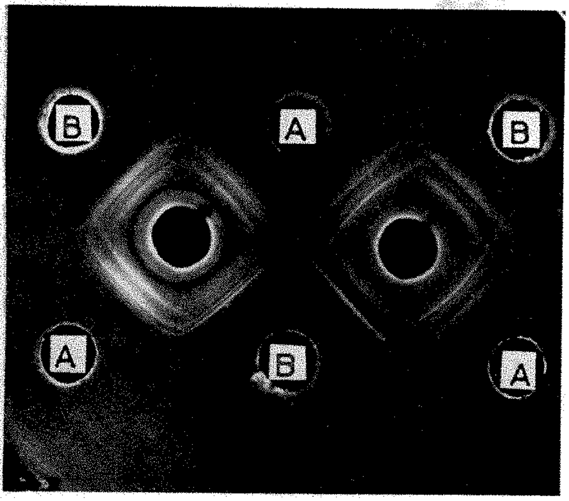

Fig. 2. Photograph of agar diffusion plate. Centre wells contain immune serum to isolate *A*5279, surrounding wells contain antigens (*A*), *A*5279; (*B*), *S. rimosus*

hyphæ. Because of the close similarity of these strains to *S. rimosus* and the common production of oxytetracycline it is suggested that they are variants of *S. rimosus* with coiled to spiral sporophores separated by long internodes and would be more satisfactorily included under the name *S. rimosus* forma *pseudoverticillatus*.

Further evidence of the relationship between *A*5279 and *S. rimosus* has been obtained serologically using the Ouchterlony gel diffusion method (Fig. 2). It appears that *A*5279 and *S. rimosus* have four and possibly five common antigens. *A*5279 has one strong antigen which cannot be detected in *S. rimosus* and conversely all the *S. rimosus* antigens are to be found in *A*5279. This would again suggest a very close relationship and at the same time a strain difference which is illustrated by the different aerial mycelium morphology.

T. Cross *

Antibiotics and Fermentation Division,
Research Department,
Boots Pure Drug Co. Ltd.,
Nottingham.

* Present address: Department of Biological Sciences, Institute of Technology, Bradford, 7.

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GENETICS

Possible Genetic Evidence of Somatic Segregation in *Allium cepa* L.

Reduction in somatic nuclei has been noted in various plants and animals by a number of workers. This communication reports an instance in which both glossy and semi-glossy seed-stems were found on the same onion plant. Huskins[1] has reviewed many examples in the plant field, thus a full review will not be given here, but only a few more pertinent examples will be cited. Segregation and/or reduction in chromosome number was induced in cells of onion root tips which had been treated with 1–4 per cent sodium nucleate. Regular genetic segregation occurred with greater frequency than would be expected if the distribution of the chromosomes were a random process[2]. Onion bulbs stored at 5°–6° C. showed a reductional grouping[3].

During the growing season of 1954, 17 plants with both glossy and semi-glossy seed-stems were found in a population of about 500 of the Yellow Sweet Spanish onion inbred *B* 12132. The inbred *B* 12132 is not homozygous for the semi-glossy character. Jones, Clarke and Stevenson[4] reported that glossy foliage was recessive to non-glossy. Apparently, semi-glossy is dominant to glossy but recessive to non-glossy. Bulbs stored at the University of Idaho, Branch Experiment Station, Parma, Idaho, at temperatures of 5°–7° C. were set in the field in late March.

Two bulbs—one a *B*-line (fertile) bulb and the other an *A*-line (male-sterile) bulb—were selected for further study. The *B*-line bulb produced three seed-stems, one of which was semi-glossy and two were glossy; however, only a few seeds were obtained. No plants survived from seed produced on the umbel of the semi-glossy seed-stem. The foliage of the progeny of the two glossy seed-stems was glossy.

The *A*-line bulb produced two seed-stems, one of which was semi-glossy and the other glossy. The *A*-line bulb was crossed to a glossy plant. No seed was obtained from the glossy seed-stem. All the plants from the seed on the semi-glossy seed-stem, which had been crossed to glossy, were semi-glossy. The F_1 when crossed to glossy gave a segregation ratio of 1 : 1.

The F_1 hybrid of a cross between glossy and semi-glossy foliage is semi-glossy. The F_2 segregates in the ratio of 3 semi-glossy to 1 glossy. In this particular instance, both types appeared on the same plant. Progeny from the glossy seed-stem bred true for glossy. Similarly, progeny from the semi-glossy seed-stem bred true for the semi-glossy character. Only true-breeding individuals were obtained. A change occurred in the inheritance pattern of the glossy seed-stem character of the onion. The change could have been caused by the loss of a segment of chromatin carrying the dominant gene, though the high percentage of plants with both glossy and semi-glossy seed-stems makes this unlikely. The change could have been caused by a point mutation, but two simultaneous mutations would have to occur. Somatic reduction of chromosomes could be responsible for two true-breeding lines in an otherwise heterozygous individual. If the latter possibility is true, then the common bulb onion, *A. cepa*, offers an excellent opportunity for study of somatic reduction in plants.

Elmo W. Davis

Crops Research Division,
Agricultural Research Service,
U.S. Department of Agriculture,
Plant Industry Station,
Beltsville,
Maryland.

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A Human Double Cross-Over

An X-linked blood group system, Xg, was described this year jointly by investigators in the United States and at the Medical Research Council, Blood Group Research Unit, at the Lister Institute[1]. More recently it was shown, at the University of North Carolina[2], that while 'classical' hæmophilia (hæmophilia A) is closely linked to colour blindness, as was already known from the work of Haldane and others, hæmophilia B (ptc deficiency, Christmas disease) is very loosely linked to colour blindness, if linked at all. In English families the loci for Xg and for hæmophilia A have been found to be widely separated[3]. Though work in progress is showing no hint of linkage between *Xg* and colour blindness the two should, in theory, be within measurable distance of each other because colour blindness is close[4-6] to the locus for glucose-6-phosphate dehydrogenase deficiency (g-6-pd) and g-6-pd is within measurable distance[7] of *Xg*.

During an investigation of the Xg-hæmophilia B relationship we have tested a family which can be scored for *Xg*, hæmophilia B (*h.ptc*) and colour blindness (*cb*). This is the first human family in which three genes on one chromosome have been seen to segregate with crossing-over between each, and furthermore, it shows at least one double cross-over.

The pedigree chart (Fig. 1) is a modification and extension of that published elsewhere[2]. The mother, known to be heterozygous for hæmophilia B and protanopia, is also heterozygous for Xg, Xg^aXg, for she is Xg(a+) and has Xg(a−) children. Each of the five sons is unique; they demonstrate five of the eight single chromosome combinations possible from a triply heterozygous mother.

It is intriguing to speculate about the linear order in which the three loci are arrayed. Three orders (and their opposites) are possible: (I) *cb—Xg—h.ptc* ; (II) *cb—h.ptc—Xg* ; (III) *Xg—cb—h.ptc*. Order II, with *h.ptc* in the middle, appears to be excluded for the following reason: the present family[2] shows that *cb* and *h.ptc* cannot be close to each other, for at least two of the five brothers must be recombinants; work in progress is showing *Xg* and *h.ptc* to be widely separated, yet *Xg* and *cb* are likely, for reasons given here, to be within measurable distance of each other.

There is at present little to guide the choice between orders I and III. In Fig. 1 we have used I. The reader can verify that all three orders involve double cross-overs.

Whichever order is correct the three pairs of genes could be aligned in four different fashions in the two X-chromosomes of the mother. However, at least one double cross-over must be accepted, whatever the alignment. The family data[2] indicate that configuration A is more likely than configuration B, but it was not possible to distinguish between options 1 and 2 because the Xg groups of the maternal grandfather and grandmother are not known.

The occurrence of at least one double cross-over in this family combines with other linkage studies to show that these three genes are well separated; they may span as much as 100 map units of the X chromosome.

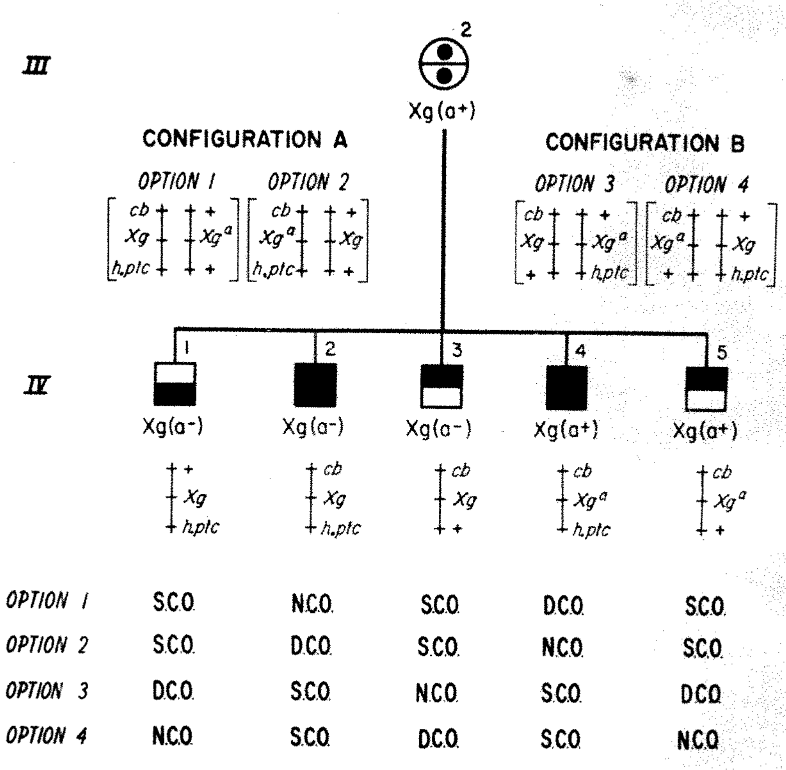

Fig. 1. Segregation in one family of three pairs of X-linked genes: those for colour blindness (protanopia), for hæmophilia B (ptc deficiency, Christmas disease) and for the Xg blood groups. Squares, males; solid upper half, colour blind; solid lower half, hæmophilia B; open, normal. Circle, female; dots represent a carrier of the colour blind and hæmophilia B genes. Genetic notation: +, normal gene; *cb*, colour blindness gene; *h.ptc*, hæmophilia B gene; Xg^a, the gene responsible for the antigen Xg^a and *Xg* represents its allele which causes no antigen yet identified. N.C.O., non-cross-over; S.C.O., single cross-over; D.C.O., double cross-over

John B. Graham
Harold L. Tarleton

Department of Pathology,
University of North Carolina,
Chapel Hill.

R. R. Race
Ruth Sanger

Medical Research Council,
Blood Group Research Unit,
The Lister Institute,
Chelsea Bridge Road,
London, S.W.1.

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VIROLOGY

Simultaneous Activation of Pentose Phosphate Shunt Enzymes in a Virus-infected Local Lesion Host Plant

In the course of work on the biochemical basis of plant virus localization it has been found that around the necrotic lesions of *Nicotiana glutinosa* leaves infected with tobacco mosaic virus (TMV) the dehydrogenases are highly activated as shown by the *in vivo* application of the triphenyl tetrazolium chloride (TTC) technique[1] Because of the possible implication of oxidative metabolism in resistance phenomena, the nature of the enzymes involved was examined Our attention was directed to the pentose phosphate cycle enzymes as alterations in their activities were recently reported to occur in host tissues infected by fungal pathogens[2,3] Isotope investigations also suggested an increased *in vivo* role of the pentose phosphate cycle in these hosts[4,5] Virus diseases have not been investigated from this point of view so far, although the host–virus systems offer distinct advantages, the direct contribution of the pathogen to the enzymatic changes occurring in the host was in this way excluded

In the present work *Nicotiana tabacum* var White Burley plants were inoculated mechanically with the *para* strain of TMV[6] The infection resulted in the development of necrotic local lesions surrounded by a yellow ring of living tissue Infiltration with 0 6 per cent TTC led to the rapid appearance of red colour around the lesions, indicating the area of enhanced dehydrogenase activity From the corresponding tissues of a number of infected leaves not infiltrated with TTC, disks were punched out with a miniature cork borer (2 5 mm *d*) The cell-free extract of 120 disks was used for the spectrophotometric assays An equal number of disks punched from the normal green areas of the same leaves served as controls

A representative experiment is shown in Fig 1 It may be seen that in the tissues surrounding the lesions the activity of glucose-6-phosphate dehydrogenase is increased by about 300 per cent over the control Similar or even higher activation of 6-phosphogluconate dehydrogenase was found in several consecutive experiments

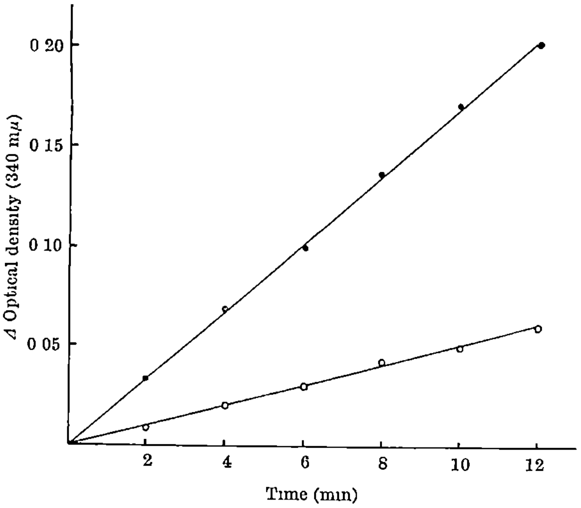

Fig 1 Activity of glucose-6-phosphate dehydrogenase in White Burley tobacco leaves infected with the *para* strain of TMV ●, Extract from tissues surrounding local lesions, ○, extract from normal green areas of the same leaf Composition of the system 2 5 μM glucose-6-phosphate, 0 25 μM triphosphopyridine nucleotide, 1 μM magnesium chloride Enzyme corresponding to 20 mg fresh weight Glycyl-glycine buffer *p*H 7 5 Final volume 1 5 ml Light path, 0 5 cm

From a biochemical point of view, it is quite remarkable that two members of a cycle are affected simultaneously in the same sense despite the fact that the two enzymes are distinct, soluble proteins The nature of increased activity (*de novo* protein synthesis, removal of inhibitors, activation) is under investigation

The role in host–virus interactions of the phenomenon described here is not fully understood, however, it might be postulated that the enhanced production of reduced triphosphopyridine nucleotide by the dehydrogenases involved plays a part in maintaining a high phenol/quinone ratio, thereby hindering the growth of the lesions[7]. Further investigations along these lines might contribute to the elucidation of the biochemical nature of localized acquired resistance of the tissues surrounding the virus-induced lesions[8,9]

F Solymosy
G L Farkas

Research Institute for Plant Protection,
Budapest

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Serological Relationship of Chrysanthemum Virus *B* to Carnation Latent Virus and Potato Viruses *M* and *S*

When leaf extracts from chrysanthemum plants infected with chrysanthemum virus *B* (CVB) were tested against antisera to 22 different elongated plant viruses, those prepared against carnation latent virus (CLV), potato virus *M* (PVM) and potato virus *S* (PVS) gave a positive serological reaction In reciprocal tests an antiserum prepared against CVB itself reacted positively with partially purified preparations of CLV, PVM and PVS

These results indicate that CVB should be included in the group of plant viruses hitherto represented by CLV, PVM and PVS, the serological relationships of which have already been established among previous authors[1–4]

In Table 1 the average homologous titres of the antisera to CLV, CVB, PVM and PVS respectively are summarized together with their titres against the heterologous viruses of the group

Table 1 Virus Preparation Tested

Antiserum to	CLV	CVB	PVM	PVS
CLV	8192	32	128	32
CVB	256	2048	32	64
PVM	16	16	2048	16
PVS	64	128	32	4096

The relatively large differences between homologous and heterologous titres suggest a major antigenic fraction specific for each virus together with minor fractions common to two or more viruses of the group.

On the morphological basis of 'normal length', estimated by electronmicroscopy, Brandes[5] has ranged chrysanthemum virus *B* in a group of viruses which among others includes CLV, PVM and PVS.

D. H. M. VAN SLOGTEREN
N. P. DE VOS

Laboratorium voor Bloembollenonderzoek,
Lisse, The Netherlands.

F. A. HAKKAART

Proefstation voor de Bloemisterij in Nederland,
Aalsmeer, The Netherlands.

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PSYCHOLOGY

Theoretical and Empirical Confusion Functions

CROSSMAN[1] produced an empirical 'confusion function' with a value for a pair of stimuli proportional to the time taken to discriminate between them.

Hammerton[2], from a mathematical model of perception, has derived theoretical confusion values in good agreement with Crossman's for, roughly, $3 > x_2/x_1 > \frac{1}{3}$, where x_1 and x_2 are the two stimulus values. Welford[3] has pointed out that $K \frac{(x_2 + x_1)}{x_2 - x_1}$ differs by very little from Crossman's confusion function $\frac{1}{\log_2 x_2 - \log_2 x_1}$ (K a constant) except when x_1 and x_2 are very different, and then Welford's formula gives good agreement with Hammerton's confusion values. Unfortunately, this agreement is coincidental and results from an unjustified assumption made by Hammerton.

The model assumes "that (*a*) an act of perception is based on a population of values—perhaps, for example, of neurone firing-rates—normally distributed about the correct magnitude, that (*b*) the standard deviation of such a population bears a constant ratio (taken as unity for simplicity of computation) to the value of the mean", that is, if we take $\frac{\text{standard deviation}}{\text{mean}} = k$, then it is assumed that $k = 1$. This is the unjustified assumption.

The theoretical confusion function is $C \propto \frac{1}{1 - A}$ where A is the area of the overlap of the distributions of x_1 and x_2, and

$$A = \frac{1}{\sqrt{2\pi}\, k} \left\{ \frac{1}{x_1} \int_{-\infty}^{p} \exp\left[- \frac{(x - x_1)^2}{2k^2\, x_1^2} \right] dx \right.$$

$$\left. + \frac{1}{x_2} \int_{p}^{q} \exp\left[- \frac{(x - x_2)^2}{2k^2\, x_2^2} \right] dx + \frac{1}{x_1} \int_{q}^{\infty} \exp\left[- \frac{(x - x_1)^2}{2k^2\, x_1^2} \right] dx \right\} \quad (1)$$

and p and q are the roots of

$$\frac{(x - x_1)^2}{2k^2\, x_1^2} - \frac{(x - x_2)^2}{2k^2\, x_1^2} - \ln \frac{x_2}{x_1} = 0 \quad (2)$$

Now it is seen that if the population of values is in fact that of neurone firing-rates, then the substitution $k = 1$ is unrealistic or unjustified, since negative firing-rates are impossible and consequently the distribution of values must be markedly non-normal, so that putting $k = 1$ in equations (1) and (2) would give incorrect values for A.

Further, we should expect that C would bear some relation to errors. In fact, if $p(x_1) = p(x_2) = 0.5$, and if $p(e)$ is the probability of an error, then we must have $p(e) \geq A/2$. In that case, if $k = 1$ and $x_2/x_1 = 2$, $p(e) > 0.3$, which is far too high a value, even for Crossman's experiments, in which errors were appreciably higher than the minimum possible.

Finally, $p(e)$ decreases with increase in observation time (up to about $\frac{1}{2}$ sec.[4]) and so, conceivably, k changes during an observation.

Thus it is difficult to justify the assumption that k has any particular value, and especially difficult to justify the assumption that it has a value as large as 1, and yet p, q, A, and consequently C, are all sensitive to changes in k.

Table 1. VALUES OF THEORETICAL AND EMPIRICAL CONFUSION FUNCTIONS

	x_1/x_2							
Hughes	0	0.5	0.67	0.75	0.80	0.83	0.86	0.88
Crossman	0.00	1.00	1.71	2.41	3.13	3.82	4.51	5.18
Welford	0.33	1.00	1.67	2.33	3.00	3.67	4.33	5.00
Hammerton								
$k = 1$	0.34	1.00	1.64	2.28	2.92	3.57	4.35	4.86
$k = 1/14$	1.00	1.00	1.00	1.05	1.11	1.25	1.39	1.54

Values of the theoretical C are tabulated for $k = 1$ (from Hammerton), and $k = 1/14$, together with the corresponding values of the expressions put forward by Crossman and Welford. The values for $k = 1$ were all multiplied by Hammerton by a constant factor to give $C = 1$ for $x_1/x_2 = 0.5$. Similarly, a constant factor $K = \frac{1}{3}$, is required in Welford's formula to give $C = 1$ for $x_1/x_2 = 0.5$, and since in both formulæ the uncorrected value of $C \rightarrow 1$ as $x_1/x_2 \rightarrow 0$ or ∞, the agreement of corrected values of C for extreme values of x_1/x_2 clearly depends on the value of k chosen in Hammerton's model. For $k = 1/14$ $A < 0.000005$ and $C = 1$ very nearly when $x_1/x_2 = 0.5$, so that no correction factor is needed. It is seen that values of C for $k = 1/14$ are markedly different from those of the other formulæ apart from the arranged agreement at $x_1/x_2 = 0.5$, and yet this value of k, far from being unrealistically small, may still be over-large, since it corresponds to $p(e) = 0.02$, 0.08 and 0.17 for $x_1/x_2 = 0.75$, 0.8 and 0.875. It would, of course, be possible to arrange agreement among the formulæ at some value of x_1/x_2 other than 0.5, but this would produce no general improvement, nor would it remove the apparent paradox, which is in fact the result of adjusting Hammerton's formula: that the smaller the value of k, that is, the greater the separation of x_1 and x_2 in standard deviations, the larger the adjusted value of C for $x_1/x_2 = 0$.

I. M. HUGHES

War Office Clothing and Equipment
Physiological Research Establishment,
Farnborough, Hants.

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FORTHCOMING EVENTS

Wednesday, August 29—Wednesday, September 5

British Association for the Advancement of Science (at Manchester)—124th Annual Meeting

Wednesday, August 29

At 8 p m —Sir John Cockcroft, F R S "Investment in Science" Presidential Address

Thursday, August 30

At 10 a m —Dr B E J Pagel "The Analysis of Starlight" (Kelvin Lecture)

At 10 a m —Prof R D Haworth F R S "The Chemistry of the Tannins" (Presidential Address, Section B)

At 10 a m —Prof O E Lowenstein, F R S "Frontiers of Knowledge in the Study of Sensory Function" (Presidential Address, Section D)

At 10 a m —Prof G D Christopherson, F R S ' The Exploitation of Research" (Presidential Address, Section G)

At 10 a m —Prof W O James, F R S "Plant Respiration and Microstructure of Plant Cells" (Presidential Address, Section K, K*)

At 10 a m —Mr Percy Wilson "The Unity of Knowledge—a New Dynamic for the Schools" (Presidential Address, Section L)

At 10 a m —The Earl De La Warr "Science and Hunger" (Presidential Address, Section M)

At 10 30 a m —Prof P M S Blackett, F R S "Science and the Developing Countries" (York Lecture)

At 11 30 a m —Prof M Ginsberg "Facts and Values" (Presidential Address, Section N)

Friday, August 31

At 10 a m —Sir Basil Schonland, F R S "Lightning and the Long Electric Spark" (Presidential Address, Section A)

At 10 a m —Mr A Digby "Time the Catalyst or Why We Should Study the Material Culture of Primitive Peoples" (Presidential Address, Section H)

At 10 a m —Prof R A Morton, F R S "Lipid Cofactors in Biochemistry" (Presidential Address, Section I)

At 10 a m —Mr M V Edwards "Marginal Aspects of Modern British Forestry" (Chairman's Address, Section K*)

At 11 30 a m —Mr D McMahon "The Identification and Use of Talent" (Presidential Address, Section J)

At 8 p m —Prof J D Bernal, F R S "Space and Life (Evening Discourse)

Sunday, September 2

At 11 a m —Service in Manchester Cathedral Preacher The Lord Bishop of Manchester, the Right Reverend Dr W D L Greer, D D

Monday, September 3

At 10 a m —Prof F W Shotton, F R S "The Physical Background of Britain in the Pleistocene Period" (Presidential Address, Section C)

At 10 a m —Mr L P Kirwan "Nubia—an African Frontier Region" (Presidential Address Section E)

At 10 a m —Mr W B Reddaway "The Economics of Underdeveloped Countries" (Presidential Address, Section F)

At 10 a m —Prof Bruce Pattison "English as an International Language" (Presidential Address, Section X)

At 8 p m —Prof Sir Alister Hardy, F R S "Man and the Beneficent Sea" (Evening Discourse)

Tuesday, September 4

At 10 a m —Dr. M R House "Bursts in Evolution" (Darwin Lecture)

Wednesday, September 5

At 10 a m —Mr R A French ' The Making of the Russian Landscape" (Lister Lecture)

Monday, September 3—Friday, September 7

International Conference on Water Pollution Research (at Church House, Westminster, London, S W 1)

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturer in the Pharmacology Department—The Dean, Guy's Hospital Medical School, London, S E ,1 (August 31)

Leverhulme Student (with a postgraduate diploma in chemical engineering or equivalent qualifications) in Chemical Engineering—The Registrar, King's College (University of London), Strand, London, W C 2 (August 31)

Scientific Assistant in the Pharmacy Department, for the maintenance of physical instruments including spectrophotometers and radiochemistry equipment, and duties will also include certain routine measurement and demonstrations—Chelsea College of Science and Technology, Manresa Road, London, S W 3 (August 31)

Assistant Lecturers (with a good honours degree and preferably with industrial, research or teaching experience) in Physics and Applied Physics—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff (September 1)

Assistant Lecturer in Agricultural Economics—The Registrar, The University, Manchester 13 (September 8)

Assistant Lecturer or Lecturer (with special interests in inorganic chemistry) in Chemistry—The Registrar, The University, Sheffield (September 8)

Assistant Lecturer (with a good honours degree in physiology) in Physiology—The Registrar, The University, Manchester 13 (September 8)

Assistant Lecturer (with a good honours degree in physiology, biochemistry or chemistry with subsidiary physiology) in Chemical Physiology—The Registrar, The University, Manchester 13 (September 8)

Research Assistant (with a good honours degree) in the Department of Agriculture and Horticulture, Research Station, Long Ashton, for work in plant biochemistry, with special reference to photosynthesis—The Secretary, The University, Bristol (September 8)

Lecturers (preferably with a special interest and research experience in one or more of the following plant physiology, mycology, anatomy, or flowering plant systematics) in the Department of Botany, University of Ghana—The Registrar, University of Ghana, P O Box 25, Legon, Accra, or the Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (September 10)

Research Assistants (2) (graduates in geology) in the Department of Geology, to undertake research on Upper Palæozoic conodont faunas—The Registrar, University College of Swansea, Singleton Park, Swansea (September 10)

Lecturer in Physics—The Registrar and Clerk to the Governors, College of Technology, Park Road, Portsmouth (September 14)

Research Student in the Department of Psychology—The Registrar, The University, Hull (September 14)

Reactor Engineer (with a university degree in engineering or an equivalent qualification, several years industrial experience, and preferably some experience in plant operation) in connexion with the projected nuclear reactor to be operated jointly by the University of Liverpool and the University of Manchester—The Bursar, The University, Manchester 13 (September 15)

Chair of Botany in the University of Ghana—The Registrar, University of Ghana, P O Box 25, Legon, Accra, or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (September 17)

Assistant Experimental Officer (graduate) in the Bee Department, for work on field behaviour of bees, including pollination—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 17)

Coal Petrologist (with a university degree or a Technical College Diploma or equivalent with geology as a major subject) in the Division of Coal Research, Commonwealth Scientific and Industrial Research Organization, North Ryde, New South Wales, Australia, to carry out investigations on the microscopy of Australian coals and cokes—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2, quoting Appointment No 480/415 (September 22)

Chair of Zoology—The Secretary, The University, Edinburgh—(September 30)

Lecturer (honours graduate in mathematics or mathematical physics and an interest and experience in the application of mathematics to engineering problems, and preferably experience in programming high-speed digital computers for scientific and engineering problems) in the Department of Mathematics—The Registrar, College of Aeronautics, Cranfield, Bletchley, Bucks (September 30)

Lecturer or Senior Lecturer in Pure Mathematics at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, October 1)

Senior Lecturer or Lecturer (preferably with an interest in chemical spectroscopy) in Theoretical Chemistry in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, October 1)

Research Officer or Research Cadet (with an honours degree in agriculture, science or veterinary science), for research on animal nutrition—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (October 5)

Chair of Pure Mathematics in the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, University of Canterbury (New Zealand and London, October 15)

Chemist or Biochemist (honours graduate), for research on enzymes of liver and other tissues—Dr B Levin, Queen Elizabeth Hospital for Children, Hackney Road, London, E 2

Chief Technician (with experience of general laboratory techniques and equipment, and preferably a knowledge of microanalytical work and animal experimentation) in the Pharmacology Unit—The Secretary, Institute of Animal Physiology, Agricultural Research Council, Babraham, Cambridge

Research Assistant (with a B Sc special chemistry degree of the University of London) in the Chemistry Department, for a research problem in physical-organic chemistry involving a study of the kinetics and mechanism of oxidation processes in solution—The Registrar, Brighton College of Technology, Richmond Terrace, Brighton, Sussex

Research Assistant (with a degree, diploma in technology, or equivalent qualification) in the Department of Applied Physics, for research on subjective brightness—Head of the Applied Physics Department, Northampton College of Advanced Technology, St John Street, London, E C 1

RESEARCH ASSOCIATE (interested in ultra-structural studies related to degenerative changes in human nerve)—Dr J B Finean, Department of Medical Biochemistry and Pharmacology, The Medical School, The University, Birmingham 15

RESEARCH FELLOW (with a Ph D or equivalent and some experience in cryogenics) IN LOW TEMPERATURE PHYSICS, to study nuclear and electronic interactions in ferromagnetic and anti-ferromagnetic metals at very low temperatures—The Secretary and Registrar, The University, Southampton)

SCIENCE GRADUATE (preferably with honours, and experience in the fields of immunochemistry, immune serum production or protein fractionation) with the Edinburgh and South-East Scotland Blood Transfusion Service and Blood Products Unit—The Regional Director, Blood Transfusion Service, Royal Infirmary, Edinburgh 3

TECHNICIAN IN THE INORGANIC CHEMISTRY LABORATORY, to assist in servicing and maintenance of instruments and apparatus—F M Brewer, Inorganic Chemistry Laboratory, South Parks Road, Oxford

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Hopkin and Williams, Ltd Monograph No 45 Alizarin Fluorine Blue Metal Indicator and Reagent for Fluoride By Dr M A Leonard (Organic Chemical Reagents) Pp 8 (Chadwell Health, Essex Hopkin and Williams, Ltd , 1962) [27

The Aluminium Development Association Information Bulletin No 25 Equilibrium Diagrams of Aluminium Alloy Systems Pp 146 (London The Aluminium Development Association, 1961) 21*s* [27

Family, Planning Association 31st Annual Report 1961–1962 Pp 26 (London Family Planning Association, 1962) [47

The British Steel Castings Research Association Ninth Annual Report, 1962 Pp ii+40 (Sheffield The British Steel Castings Research Association, 1962) [47

Microdoc, Vol 1, No 1 Edited by Mark Patterson (Journal of the Council for Microphotography and Document Reproduction) Pp 16 Subscription (non-members) 30*s* per annum (London The Library Association, 1962) (47

Provision and Maintenance of Laboratories in Grammar Schools (1961) (Results of a Questionnaire issued in 1961 by the Science Masters' Association, Association of Women Science Teachers, National Union of Teachers, and Joint Four Secondary Associations) Pp 16 (London John Murray, 1962 Published for the Science Masters' Association) 2*s* net [47

Department of Scientific and Industrial Research National Engineering Laboratory Complex-stress Creep, Relaxation, a Fracture of Metallic Alloys By Dr A E Johnson, J Henderson and B Khan Pp vi+76 (Edinburgh and London H M Stationery Office, 1962) 7*s* 6*d* net [47

Department of Scientific and Industrial Research Laboratory of the Government Chemist Report of the Government Chemist 1961 Pp v+89+3 plates (London H M Stationery Office, 1962) 6*s* 6*d* net [97

The Academic Metallurgist an Inaugural Lecture By Prof J Nutting Pp ii+14+1 plate (Leeds The University Press, 1962) 2*s* 6*d* [97

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Other Countries

Bulletin of the Bingham Oceanographic Collection, Vol 18, Article 1 Ecology of an Estuarine Population of Winter Flounder, *Pseudopleuronectes americanus* (Walbaum), Parts 1–4 By William G Pearcy Pp 78 (New Haven, Conn Bingham Oceanographic Collection, Peabody Museum of Natural History, Yale University, 1962) 3 dollars [47

Atvinnudeild Haskolans—Fiskideild (The University Research Institute—Department of Fisheries) Rit Fiskideildar, III Bindi North Icelandic Waters By Unnsteinn Stefansson Pp. 269 (Reykjavik Atvinnudeild Haskolans—Fiskideild, 1962) [47

Cytogenetics, Vol 1, No 1, 1962 Pp 65 Published six times a year Annual subscription rate 95 Swiss francs, 22 80 U S dollars (Basel and New York S Karger, 1962) [97

Publications de l'Institut National pour l'Etude Agronomique du Congo Série Scientifique, No 93 Étude Phytosociologique des Forets Equatoriales et Tropicales Méthode Mécanographique Par R F E Devred et J D J Dero Pp 69 (Bruxelles Institut National pour l'Etude Agronomique du Congo, 1962) 100 francs [97

Canada Department of Mines and Technical Surveys Geological Survey of Canada Memoir No 323 Stephenville Map-Area, Newfoundland By G C Riley Pp xvi+72+1 plate 1 dollar Paper 61–28 Geology of Tignish Map-Area, Prince County, Prince Edward Island By V K Prest Pp ii+15 50 cents (Ottawa Geological Survey of Canada, 1962) [97

Space Science Reviews, Vol 1, No 1, (June, 1962) Edited by C de Jager Published quarterly Subscription price 150 florins, 42 dollars per volume of 4 issues (Dordrecht D Reidel Publishing Company, 1962 [97

Bulletin of the Moscow Society for Natural Research, New Series, Vol 61 Geological Section, Vol 31, 1st, 2nd, 3rd editions Pp iv+444 (Jerusalem Israel Program for Scientific Translations, 1961 Available from the Office of Technical Services, U S Department of Commerce, Washington, 25, D C , U S A) 4.50 dollars [97

Mauritius Sugar Industry Research Institute Annual Report, 1961 Pp 109 (Reduit Mauritius Sugar Industry Research Institute, 1962) [97

Tanganyika Geological Survey Records of the Geological Survey of Tanganyika, Vol 9, 1959 Pp 94+19 plates Shs 17/50 Memoir No 1 Summary of the Geology of Tanganyika Part 4 Economic Geology By J F Harris Pp 143+map Shs 12/50 (Dar-es-Salaam Government Printer, 1961) [97

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Institut for Atomenergi, Kjeller, Norway Survey of Activities, 1961 Pp 24 (Kjeller Institut for Atomenergi, 1962) [97

University of Natal Wattle Research Institute—(South African Wattle Growers' Union—University of Natal—Government Department of Forestry) Report for 1961–1962 (Fifteenth Year) Pp 36 (Pietermaritzburg University of Natal, 1962) [97

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Museum of Comparative Zoology at Harvard College Breviora No 155, (April 12, 1962) Notes on Hispaniolan Herpetology 6 The Giant Anoles By Ernest E Williams Pp 15 No 156, (April 13, 1962) The Fossiliferous Triassic Deposits of Ischigualasto, Argentina, and Preliminary Description of *Ischigualastia*, a New Genus of Dicynodont By Alfred Sherwood Romer Pp 9 No 157, (May 28, 1962) A Rhachitomous Amphibian, *Spathicephalus*, from the Mississippian of Nova Scotia By Donald Baird Pp 10 No 158, (May 29, 1962) A Fossil Gerrhosaur from the Miocene of Kenya (Reptilia Cordylidae) By Richard Estes Pp 10 (1 plate) No 159, May 31, 1962) Age of a Small Sample of Bluefish (*Pomatomus saltatrix* Linnaeus) By Richard H Backus Pp 4 (Cambridge, Mass Museum of Comparative Zoology at Harvard College, 1962) [97

Bulletin of the Museum of Comparative Zoology at Harvard College Vol 126, No 3 The Carabid Beetles of New Guinea Part 1 Cicindelinae, Carabinae, Harpalinae through Pterostichini By P J Darlington, Jr Pp 321–564+4 plates Vol 127, No 1 The Genera of the Spider Family Theridiidae By Herbert W Levi and Lorna R Levi Pp 1–74–14 plates Vol 127, No 2 American Spiders of Genus *Argyrodes* (Araneae Theridiidae) By Harriet Exline and Herbert W Levi Pp 75–202+15 plates (Cambridge, Mass Museum of Comparative Zoology at Harvard College, 1962) [97

Institut des Corps Gras, Paris Centre Technique Industriel Rapport d'Activité pour l'Année, 1961 Pp 51 (Paris Institut des Corps Gras, 5 Blvd de Latour-Maubourg, 1962) [137

India Council of Scientific and Industrial Research Annual Report of the National Metallurgical Laboratory, 1960–1961 Pp iii+153 (Jamshedpur National Metallurgical Laboratory, 1962) [137

New Zealand Dominion Museum Records in Ethnology Vol 1, No 7 A Stratified Dune Site at Tairua, Coromandel By C D Smart and Roger C Green With a specialist report by J C Yaldwyn Pp 243–266 (Wellington, N Z Dominion Museum, 1962) [137

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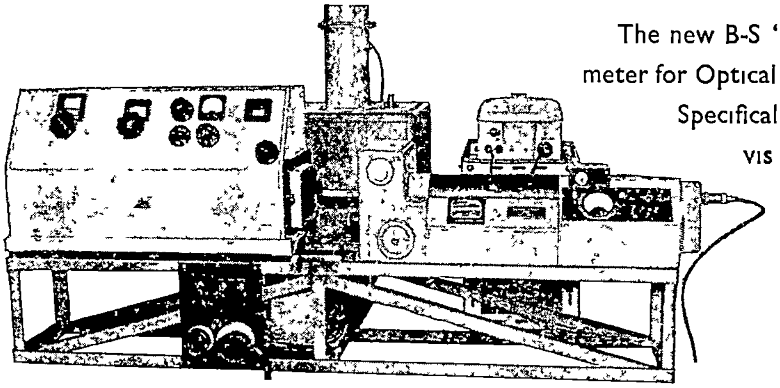

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PLANNING SCIENTIFIC INVESTMENT

IN an admirably balanced presidential address, Sir John Cockcroft used the unique opportunity afforded by the British Association for the Advancement of Science to remind Britain of the vital importance of an adequate and appropriate investment in science (see also p 875 of this issue) His title recalls a recent study by the National Science Foundation, *Investing in Scientific Progress,* which attempted to estimate the investment which the United States should make in science and in engineering education and in fundamental research in its universities (see *Nature,* 194, 320, 1962) It also brings to mind the report of the committee under the chairmanship of Sir Charles Morris which, under the title *Investment for National Survival,* put the expenditure needed for future educational development as likely to approach £1,500 million by 1970 (see *Nature,* 194, 801, 1962) Sir John referred, indeed, to educational development, more especially to the need for more scientists and technologists, and there was no suggestion in his address that supply is anywhere near approaching demand He owed nothing to either of these two studies, however, and his main theme was the need for an adequate and balanced research effort, in which full account was taken of the possibilities for co-operation, both at the national and the international level

Appropriately enough, Sir John began his survey with a glance at the work on radioactivity which was proceeding in Rutherford's laboratory when the Association last met in Manchester It would be difficult to think of any work that seemed less likely to find practical application, yet within five years Rutherford was fairly certain that nitrogen atoms were disintegrated by the impact of his alpha-particles, within a quarter of a century his experiments led to the first controlled release of nuclear energy in Fermi's atomic pile, and within forty years of the Manchester meeting half a century of work by physicists and engineers of many nations had led to the first large-scale nuclear power station in Britain Nuclear power provides, in fact, an outstanding example of the importance of fundamental research—of ensuring that the universities, and, it might be added now, the colleges of technology, have at their disposal adequate resources for research, of choosing men of first-class ability and giving them their head

Sir John was not tempted to dwell unreasonably on nuclear physics as it still remains in an intensely creative phase He noted the immensely powerful tools which have been placed at man's disposal in the synchrocyclotron, the proton-synchroton or Cosmotron and the Berkeley Bevatron, which have permitted the creation of anti-matter and the study of the physics of anti-matter He stayed no more than to point out the challenge presented by the recent discovery of still more ephemeral particles at Berkeley and the possibilities offered of enriching man's understanding of the physical universe as indicating that the study of elementary particles was likely to be in the forefront of physics for several decades and to continue to attract many of the world's best scientists

This, Sir John continued, was sufficient reason for maintaining the present heavy investment in this branch of science, but while this work with very-high-energy particles proceeded, another group of nuclear physicists, working with less-energetic particles of very precisely defined energies, was exploring the detailed structure of the nucleus and its vibrations and oscillations, as well as the nuclear processes which lead to the evolution of the elements in the stars In consequence, it is now known how this evolution starts, and Sir John also referred to the way in which radioastronomers and optical astronomers had extended estimates of the age of our Galaxy and were striking farther and farther out into the boundaries of the universe Moreover, optical astronomers were moving out into space to avoid the interference to their measurements by the absorption of ultra-violet and X-rays in the atmosphere, so that in consequence astronomy had combined with space science to rival nuclear physics in the size of its budget

Sir John said nothing to indicate that he thought a budget of £5 million a year unreasonable for Britain in this field alone, though he clearly thought that economic necessity as well as scientific interest involved collaboration, first with the United States and in future with Western Europe Later in his address he expressed the emphatic opinion that international collaboration in fundamental research was of growing importance—the only way in which sufficient resources could be made available in nuclear physics and space research, for example What he described as space travel was another matter, and he expressed the opinion that the ventures on which the United States and Russia were each spending something of the order of 2,000–3,000 million dollars a year were being undertaken mainly for prestige purposes Frankly, he thought the priorities had gone wrong, and while it was necessary to increase investments in science as rapidly as human and financial resources could permit, it was not for such purposes as these

Sir John said no more on the magnitude of Britain's investment in science than that if Britain wished to maintain her position in the highly technological world of the future it would be necessary to double the 0 2 per cent of the gross national product devoted to basic research during the next decade, and also to devote a much greater proportion of the effort in applied research to furthering the development of new products and new industries The value of his address lies rather in the way it displays the conspectus of the research effort which is demanded and what is involved if Britain's available resources are to be deployed to the best purpose For example, he pointed out that in Britain and in the world generally the physics of the solid state attracts as many research workers as nuclear physics and that their achievements have probably been of more immediate practical use Moreover, like the nuclear physicists, they have been greatly helped by the more powerful and discriminating instruments which advancing technology has made available Again, this is one of the basic reasons for the mounting costs of research equipment which is compelling collaboration up to the international level The development of the transistor industry provides a spectacular example of the interaction of science and technology, and has had far-reaching effects on other fields such as electronic equipment and the computer, and, through these, on the development of sciences such as molecular biology, high-energy nuclear physics, the study of stellar evolution and plasma physics, quite apart from space research

All this was scarcely predictable little more than a decade ago, and the way in which the different fields of science and technology are to-day interdependent could scarcely be better illustrated No more effective answer could be given to those who contend that Britain can afford to leave entirely alone any specific field of basic research where the effort is justifiable on scientific grounds and not on those of prestige The study of semi-conductors, which has led to the development of transistors, only began as an academic subject some thirty years ago, and it has also led to rectifiers of increasing importance in power generation, to devices for producing refrigeration, to solar batteries and many other products

Sir John pointed out that the sciences dealing with the Earth and its environment were in an equally lively state, and he referred incidentally to the belief of oceanographers that more extensive studies of the nutrient content of the deep ocean and methods of increasing the turnover could lead to greatly increased production of fish protein which, at present, provides only about 1 per cent of the Earth's food Here we infringe on fisheries research, which is probably one of the most important ways in which Britain could continue the overseas research effort to which Sir John referred in the latter passages of his address, particularly if the needs of the increasing population for greater food supplies are to be met This may be the more important in view of the disinclination of the emergent territories to devote their own resources to such research

The development of the biological sciences likewise has been greatly helped by the new techniques and instruments which technological developments in other fields have placed at their disposal Furthermore, a remarkable example of inter-disciplinary collaboration has led to a discovery which Prof Medawar has said puts into man's power, in principle in the very remote future, the possibility of exercising a primary and direct control over genetic make-up even to secure a direct and fundamental cure of inborn disorder Biochemists also seem to be making progress in the control of fertility, and the application of technology to increase agricultural production in under-developed countries may be supplemented by turning fishing from a hunting to a farming operation and by new approaches to providing protein for human food.

Sir John concluded his review of some of the more outstanding recent developments in these and other fields, including that of energy, by asking the question whether the organization of applied research could be improved and whether Government assistance could be more effective He thought that Britain might have something to learn from some American organizations, but that there was a critical size for an industrial research establishment if creative work was to flourish Obviously, to-day, there is a limiting size below which an establishment will be unable to provide the highly expensive equipment which is essential in many fields of research, but otherwise it is arguable whether organization rather than size is not the critical factor When this question was discussed in a symposium on the direction of research establishments at the National Physical Laboratory in September 1956, there was no suggestion that size was of any importance The provision of conditions favourable to creative work is what matters, and this can be done in the large or the small establishment Indeed, the large establishment may be the better able to provide stimulating inter-disciplinary contacts

Everything in fact depends on the outlook and understanding of management and, as Sir John pointed out, we must get a much higher proportion of scientists and engineers into managerial positions So far from there being any likelihood of an excess of scientists and technologists in the next decade, he thought that the major obstacle to university expansion would be a shortage of highly qualified mathematicians, scientists and technologists He remarked that the best way to reduce the present loss of scientists to the United States was to improve the research facilities at home, and that Britain needs a steadily continuing increase in research grants to the universities to match the growth of the research schools and the increasing complexity of research tools This must include adequate funds for the social sciences also, which have hitherto been allowed to depend too much on funds from foundations in the United States

In thus displaying the opportunities for investment in science which confront us to-day, Sir John was content to indicate the magnitude of those opportunities, the way in which the different fields are interrelated and the extent to which co-operation is now indispensable, even to nations with resources and traditions such as Britain still possesses He made no attempt to suggest how Britain's effort should be deployed nor did he enter on any discussion of the adequacy of the existing organization for research and development These all involve political issues, as does the question of Britain's future policy in overseas research, for which he saw a clear and urgent need, and in fact suggested that if the political difficulties in achieving disarmament could be overcome, enormous resources would be released for diversion to the urgent needs of the under-developed countries

Fundamentally, Sir John's address was a challenge to his audience, and to the many thousands whom his words will have reached by radio or in print, to see that the question of adequate and effective investment in science is made a real political issue and one that is honestly faced by all political parties Understandably and correctly, the Government in power realizes that there are definite limits to the extent of Britain's investment in science set by finance, material resources and manpower, but neither major political party has yet been honest enough to put to the country what would really be involved by an adequate investment in science and technology Neither has had the courage to admit that such an investment may be incompatible with unlimited expenditure in non-essentials, even on things that to-day in Britain, if nowhere else, are taken into account in calculating the cost-of-living index

Political courage and honesty are prerequisites in determining the level of investment in science, and without them the financial resources are unlikely to be forthcoming Whether or not Britain's existing organization for determining the allocation of resources, which is at present under scrutiny, is the most appropriate and effective, something more is needed Ultimately the allocation of resources, like the wise selection of research projects, the determination of priorities and the choice of policies, is a matter of sound judgment What matters most in these questions of science and Government is that the structure for presenting scientific advice should both ensure that that advice is presented effectively at policy-level to those capable of appreciating its significance, and that in the formulation of that advice there is full opportunity of authoritative discussion and criticism Above all, it should ensure that the advice presented does not reflect the prejudices, obsessions or limitations of a single mind It is as a contribution to the task of educating public opinion as to what is really implied by adequate investment in science that Sir John's presidential address to the British Association was most welcome Scientists and technologists will best show their appreciation by responding to his challenge and making what contribution they can, not only in solving the technical problems of controlled and complete disarmament but also in continuing the task of educating public opinion and securing the appropriate action required to increase the investment in science in the widest sense

DEATH OF NAPOLEON

Who Killed Napoleon?

By Dr Sten Forshufvud Translated from the Swedish by Alan Houghton Brodrick Pp 256+8 plates (London Hutchinson and Co, Publishers, Ltd, 1962) 25*s* net

THIS book is unlikely to gain general popularity, if only for the lengthy descriptions of the more unpleasant features of Napoleon's illnesses in St Helena Its one merit lies in the convenient presentation of the lives and characters of those persons in attendance on the Emperor during his last exile The merit that the author would ascribe to it lies in his contention that Napoleon was systematically poisoned over many years by the administration of arsenic and, finally, in the classic style of the French arsenic poisoners of the seventeenth century, that he was given antimony so as to cause evacuation of the previous doses of arsenic The *coup de grâce* is stated to be a dose of 10 grains of calomel ordered by four doctors, three of whom were British (the fourth, Antommarchi, disapproving) This calomel was changed into hydrocyanic acid and corrosive sublimate through interaction with a fruit drink containing bitter almonds!

The author has a doctor's degree but practises as a dental surgeon, from his presentation of clinical material it is difficult to believe he has any proper knowledge of medicine and one presumes his degree is in dental surgery In his wearisome discussions on the effect of the 'climate' of St Helena on Napoleon's health, he speaks entirely as a layman and seems to know nothing of the bacterial infections which exist in such places

Although the British Government dealt harshly with anyone who claimed that Napoleon's health was adversely affected by his sojourn in St Helena, it is very probable, and certainly fully acceptable, that he did indeed suffer during his five years these recurrent attacks of an infection that was associated with features of hepatitis This, indeed, is supported by the small portions of intestine that were presented by Barry O'Meara to Sir Astley Cooper and were found among Cooper's specimens after his death, and were labelled as Napoleon's It is probable that O'Meara secured them from Antommarchi, who visited him in London These fragments were from the lower part of the small gut and showed swollen and inflamed Peyer's patches such as would be found in a chronic bacterial infection that he could have acquired on the island The specimens were unfortunately lost when the Royal College of Surgeons was bombed in 1941

Whether or not the author is right in claiming that arsenic was administered to Napoleon there can be no doubt that post-mortem revealed a large cancer infiltrating the greater part of the stomach and with a perforation in one place sealed by an adherence to the left lobe of the liver Napoleon himself, during his last days, was convinced that he was suffering from a cancer of the stomach, a disease that caused his father's death, and he made a specific request that this lesion should be sought for so that his son could be warned about the danger of himself developing such a cancer

The presentation of the evidence for chronic arsenical poisoning is unsatisfactory, irritating and tortuous For most of the book the impression is gained that it was the "wicked British" who were responsible, but finally it is implied, although not specifically stated, that the poisoner was the Comte de Montholon (whose wife, incidentally, was Napoleon's mistress in St. Helena). The Count, in true thriller fashion, persuades Napoleon to change his will in his favour and when this is done recommences his final administration of poison!

It is implied, but not definitely stated, that he administered poison at the instigation of Talleyrand, and the author even claims that the administration must have been begun at Elba, and that certain of the indispositions Napoleon exhibited at the Battle of Waterloo "fit in very well with the general picture of arsenical poisoning"! It is also claimed that even earlier in his reign Napoleon was being drugged, at the instigation of the Great Powers fighting against him.

Napoleon is obviously the author's hero, but even this should not allow him to make errors in the assessment of history; he states that after his escape from Elba the whole of France welcomed him with joy. The truth is that the great mass of the French people were heartily sick of his antics, which had brought so many years of war and sorrow, and had resulted in almost every family grieving a father or sons. The only real support Napoleon met with on his last adventure was from the scores of thousands of his veterans who had been demobilized after peace was signed and, having known nothing but war because of his campaigns, were largely unsuited to return to civilian life and were, at that moment, glad of a chance to return to the life they knew. They were soon disillusioned. The rest of the nation had already been disillusioned. RUSSELL BROCK

LINEAR SPACES

Linear Algebra and Geometry

By Prof. Nicolaas H. Kuiper. Translated from the Dutch by A. van der Sluis. Pp. viii + 284. (Amsterdam: North-Holland Publishing Company, 1962.) 48s.

An Introduction to the Theory of Linear Spaces

By Prof. Georgi E. Shilov. Translated from the Russian by Richard A. Silverman. Pp. ix + 310. (London: Prentice-Hall International, 1961.) 50s.

Proceedings of the International Symposium on Linear Spaces

Held at the Hebrew University of Jerusalem, July 5–12, 1960. (A publication of the Israel Academy of Sciences and Humanities.) Pp. xi + 452. (Jerusalem: Jerusalem Academic Press; London and New York: Pergamon Press, 1961.) 100s.; 14 dollars.

LINEAR algebra is occupying an increasingly prominent place in the undergraduate mathematics curriculum. In the first place it is the basic mathematical tool in the study of the analytic geometry of n dimensions and in affine and projective geometry. Secondly, the notions of vector space and linear transformation are central to the study of systems of linear equations (and linear differential equations). Thirdly, these notions form a necessary and fairly elementary introduction to those concepts proper to group theory, to topology and to functional analysis, with which the student will have to familiarize himself later in his course. Fourthly, there is a growing realization of the importance and variety of the applications of linear algebra outside the domain of pure mathematics, for example, in statistics, linear programming, operational research. Fifthly, functional analysis and topological algebra constitute a very active area of present-day mathematical research and it is essential for any student aspiring to work in this field to familiarize himself thoroughly with the theory of finite-dimensional spaces. (Actually, the algebraic topologist also needs much of this basic theory; I myself, as an interested party, regretted the absence of a treatment of tensor products and exterior powers from the two text-books under review.)

The book by Kuiper is an excellent undergraduate text-book in which (as one would expect of the author) the geometric motivation is strongly, but not obtrusively, emphasized. Thus while the abstract concepts of vector space and mapping are given due prominence and the treatment is very largely co-ordinate-free, there are chapters devoted to Euclidean motions, to projective geometry and to non-Euclidean planes. Two further features of the book deserve mention. First, there is an interesting chapter on the applications in statistics, and, secondly, each chapter (except for the first two, which are brief) carries a number of stimulating and appropriate exercises. By way of mild criticism, however, it must be admitted that the book has clearly not been translated into English by an Englishman!

The book by Shilov is much less overtly motivated by geometric considerations. Whereas Kuiper is a geometer, Shilov is a functional analyst, and it is a measure of the ubiquitousness of linear algebra in mathematics that the actual contents of the two works overlap so substantially. In fact, it is Shilov's avowed object to introduce the reader to functional analysis. Not only is this intention made evident to some extent by the choice of material and style of presentation of the earlier chapters, but also the book closes with a long, and exceedingly valuable, chapter on infinite-dimensional Euclidean spaces, which constitutes one of the most accessible introductions to the basic concepts of functional analysis. The notions of completion, of orthogonal expansions, of bounded and completely continuous operators are discussed and there is a treatment of the eigenvector problem for a Fredholm operator and of the immediately relevant parts of the theory of integral equations. The book is well provided with examples, exercises and bibliography and its appearance bodes well for the series of translations of Russian texts of which it is the first. It was not to my taste that the book opened with a substantial chapter discussing determinants with Victorian formality (in the book by Kuiper, determinants appear on p. 91 as functions of endomorphisms of finite-dimensional vector spaces), and throughout the early chapters the treatment is perhaps excessively 'co-ordinatized'. But tastes vary, and the later chapters should suit most palates.

The third work under review contains the 32 papers read at the International Symposium on Linear Spaces held in Jerusalem during July 1960. The topics discussed range widely over the field of functional analysis and bear eloquent testimony to the vitality of this mathematical topic to-day. The Israel Academy of Sciences and Humanities is to be congratulated on the speedy publication of this record of an important conference.

P. J. HILTON

QUALITY AND QUANTITY IN SPACE AGE LITERATURE

Rocket Engines

By S L Bragg Pp 158+6 plates (London George Newnes and Co , Ltd , 1962) 35*s* net

Ballistic Missile and Space Vehicle Systems

Edited by Prof Howard S Seifert and Kenneth Brown (Engineering and Physical Science Extension Series, University of California, Los Angeles) Pp xii+526 (New York and London John Wiley and Sons, Inc , 1961) 96*s*

OF the many things with which we have been inflicted since the U S S R made the first moves into the 'Space Age' in October 1957, one is a proliferation of space literature and most of it is mediocre Perhaps editors and authors, inspired by the vastness of space, are making desperate efforts to fill the void with words Fortunately, we occasionally find an author who imposes strict limitations on himself and gives himself a precise objective It would seem that Bragg is such an author

With only a few innocent digressions from his objective, Bragg presents the important processes within a rocket engine in a logical and concise manner The overall impression is that one is following the thoughts of a man, sufficiently alert to discard trivialities, who is ascertaining what is of basic importance The result is a pleasant and readable book which, with a few words of caution, can be firmly recommended to students and anyone interested in rockets Some of the lapses from strict accuracy will be discerned immediately by the knowledgeable reader and later by those who are inspired to delve more deeply into rocket technology, but I myself would like to comment on statements made in sections 4 7 and 5 5 In the former section the relationship between propellent density and specific impulse is a sufficiently accurate approximation for the example quoted, but this example is scarcely typical of guided missiles The spectrum of missile designs is so wide that a single typical example cannot be quoted and the relationship between propellent density and specific impulse is more complicated than the author implies The statement, on p 91 in the latter section, to the effect that the drop in pressure generated in a combustion chamber by heat addition is not really a loss is both puzzling and incorrect When heat is added to a gas flowing in a duct, it suffers a fundamental loss in total pressure which cannot be recovered by subsequent cooling The extent of this fundamental loss is dependent on the degree of acceleration permitted, which in turn is dependent on the geometry of the duct, as Fig 5 4 indicates

In marked contrast to Bragg, Seifert and Brown have endeavoured to cover the whole gamut of disciplines and technologies necessary to design a launching vehicle and inject a satellite into orbit If they had succeeded in this task a really remarkable book would have been created, but it would have been of stupendous size As it is the book is too large when measured by the authority which it wields The first chapter, "Systems Engineering", carries tautology to extreme limits Its fourteen pages may be summarized as "Authority is vested in the systems engineer to ensure concentration, coordination and collaboration" If the two editors had assumed this authority something much better would surely have emerged What in fact has emerged is a series of articles each of a superficial character, and there is little evidence that the authors have perused their own efforts and still less that they have collaborated between themselves I am unable to visualize the reader who will derive much satisfaction from *Ballistic Missile and Space Vehicle Systems* It is certainly not the specialist in propulsion nor structures nor guidance and control Who is left but some enigma ! He may call himself a "Systems Engineer", but he is more likely to be a "Jack of all Trades"

Our language is flexible enough to meet the requirements of modern technology yet we abuse it in an appalling manner Bragg departs from his grandfather's precepts and meekly accepts "propellant' , but worse still, Seifert and Brown have accepted such abominations as "outage" and "fabricability" In both books the word "parameter" is frequently used, but never with its correct meaning Few of us who claim to have any scientific training, are endowed with much literary sense If we humbly acknowledge this we are likely to be more careful and less likely to make mistakes J E P DUNNING

REACTOR PHYSICS PRINCIPLES SIMPLY EXPLAINED

Textbook of Reactor Physics

An Introduction By J F Hill Pp 227 (London George Allen and Unwin, Ltd , 1961) 36*s* net

THIS book is a useful addition to the already numerous texts on reactor physics, and will provide easy reading to the many non-specialists who, although working in atomic energy, are not involved in detailed reactor physics calculations It also provides a stepping-stone between existing elementary and advanced works for those wishing to specialize in the subject In the writing of this book the author has drawn on the valuable experience he gained in teaching reactor physics to engineers and scientists while principal of the Harwell Reactor School He has therefore given clear physical pictures, without using involved mathematics, of the complex processes taking place in a nuclear reactor and anticipated the difficulties which come to the mind of the engineers and scientists who have little or no physics background

The book starts with an excellent summary on basic atomic and nuclear physics followed by a discussion on chain reactions and physics problems of reactor design A section on binding energy in this chapter, relating to the mass number-binding energy curve, is slightly misleading These topics are followed by a chapter on neutron behaviour in moderators, including an appendix clearly explaining the laboratory and centre of mass systems, neutron scattering and energy losses in collisions

Chapter 5 describes reactor parameters and discusses the critical size of a homogeneous reactor, starting with the 4 factor formula and including many useful graphs showing how these main parameters are affected, for example, by the ratio of the number of moderator to uranium atoms This chapter, of necessity, includes sections on the diffusion and slowing down lengths of neutrons, and it would be most useful if in future editions the author could add small appendixes on these topics

Chapter 6 shows the effect on the reactor parameters of changing from a homogeneous to a heterogeneous system It also gives numerical examples and provides information sufficient to carry out further simple, but useful, and instructive calculations

Chapters 7 and 8 are extremely valuable as they give a very clear description of the processes taking place in a reactor during start up, normal operation, shut down and power changes during normal operation The effects of fission product poisons, temperature changes and changes in the content of the fuel are discussed and illustrated by a number of figures The last chapter is on reactor kinetics and starts by considering the rate of change in the total number of neutrons assuming they are all prompt This is then followed by a very good description of the effects of delayed neutrons, assuming, to simplify the mathematics, that there is only one group

There are a few small transcription errors, which fortunately are fairly obvious, otherwise the printing and production are good and the price is reasonable The book can be thoroughly recommended to both students and professionals interested in the subject

K J DURRANDS

TOWARDS TAILOR-MADE POLYMER MOLECULES

Block and Graft Copolymers

By Dr E J Ceresa Pp xvi + 196 + 4 plates (London Butterworth and Co (Publishers), Ltd , 1962) 42*s*

NUMEROUS methods have been used to prepare block and graft copolymers The author has reviewed these in separate chapters (2–7 inclusive) according to the processes used, that is, transfer and addition, chemical, radical and irradiation, mechanicochemical, condensation and ionic These syntheses follow an introductory chapter outlining a clear and satisfactory nomenclature for describing the numerous products which can be classified as block and graft copolymers. Chapters 9 and 10, on the characterization and properties of such copolymers, reveal the chief difficulty in this field, that of obtaining a block or graft copolymer free from homopolymer As the author points out, only about 20 out of more than 2,000 block and graft copolymers synthesized or claimed have been fully characterized

Information on this topic is widely scattered Dr Ceresa has collected it together in one volume and has classified it very thoroughly The copolymer index at the end of the book is a most useful reference table, including roughly 900 of the block and graft copolymers so far synthesized, it is well cross-referenced to papers dealing with every copolymer listed The index is, of course, a guide to claims, rather than to completely characterized products

This book is a review of the literature and the author makes no other claim for it I feel that it could have been shortened quite appreciably without losing its admirable thoroughness Thus, the chapters on the preparation of copolymers contain numerous abstracts of experimental procedures, with references to the original papers As many of these are examples of the same class of synthesis, it is difficult to see what purpose is served by repetition Similarly, Chapter 8 (24 pages), dealing with industrial systheses, contains a list of published patent applications with an indication of the method used and the products claimed in each case. Most of these products are listed in the copolymer index and an extension of the references given in the index would have absorbed Chapter 8

To expect a critical survey of this field is to be optimistic in view of the limited data available Further progress should lead to interesting results relating the structure of macromolecules and their physical properties and will extend, still further, the contributions of polymer chemistry to our knowledge of free radicals and their reactions

In writing this very thorough review of the literature, Dr Ceresa has performed a service to those already familiar with the principles of polymer chemistry and who are interested in block and graft copolymers. The book is well produced and is remarkably free from printing errors—only four were noticed

S SMITH

PALÆOBOTANY OF *PTERUCHUS*

Bulletin of the British Museum (Natural History)

Geology Vol 6, No 2 On *Pteruchus*, a Microsporophyll of the Corystospermaceae By John A Townrow Pp 287–320 + plates 24–26 (London British Museum (Natural History), 1962) 20*s*

MICROSPOROPHYLLS called *Pteruchus* described thirty years ago by the late Dr Hamshaw Thomas and attributed to *Dicroidium*, are by far the commonest leaf in the Triassic of the southern hemisphere He placed the whole group of organs, leaf, microsporophyll and fruits, in the Corystospermaceae and included them in the Pteridosperms The present publication is the first thorough-going revision of these microsporophylls published since then

We may begin by noting with pleasure that Townrow describes no new species, but instead reduces some twenty ill-characterized ones to three He strongly supports Thomas's attribution of *Pteruchus* to *Dicroidium* and its close relatives, and tentatively attributes his three species to particular leaves His main contribution is, however, morphological and consists in restricting too wide possibilities Thomas regarded *Pteruchus* as a microsporophyll, that is a fertile leaf, and at the same time as an inflorescence, a kind of catkin bearing little bracts and fertile appendages This idea he put forward deliberately as involving no conflict but instead a triumphant illustration of his "New Morphology" This "New Morphology" is an extremely plastic treatment of the categories of 'leaf' and 'stem' which are considered not as fundamentally different but as alternative specializations of the *Rhynia*-like shoot system He went so far as to suggest that the poplar male catkin might have originated in such a form as *Pteruchus* and that *Pteruchus* itself was also to be compared closely with the leaf-like microsporophylls of Carboniferous Pteridosperms It is only fair to add that he did not claim fully satisfactory knowledge of *Pteruchus* and he allowed the possibility that it might be purely a microsporophyll or purely an inflorescence

Townrow eliminates most of this, *Pteruchus* he finds is entirely leaf-like in its origin on the stem and in the fine details of its branching Its symmetry is purely dorsiventral Any little photosynthetic outgrowths it bears are just small pinnules, borne in exactly the manner of pinnules and not like bracts It has no inflorescence-like feature at all This makes it fit comfortably into the Pteridosperm idea as that existed before the "New Morphology" However, within the Pteridosperms it stands by itself, and Townrow concludes that each of the 'Mesozoic Pteridosperms' seems remote from all the others We may hope soon to see a work from Townrow revising the seed-bearing organs of these strange plants

T M HARRIS

THE SOUTH AFRICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

DIAMOND JUBILEE CONGRESS

By A. C. BROWN

Department of Zoology, University of Cape Town

THE diamond jubilee of the South African Association for the Advancement of Science (usually referred to as S_2A_3) was celebrated in the University of Cape Town during the first week of July 1962. As befitted such an important event in the history of South African science, the planning of the Congress was considerably more ambitious than usual, and a number of scientists from Great Britain, France, America and Portuguese East Africa were invited to read papers and particularly to participate in a series of symposia. The organization of the congress was undertaken by a local committee under the chairmanship of Prof. Ronald Singer, a vice-president of the Association and associate professor of anatomy in the University of Cape Town. The congress was marred only by the recent death of Dr. J. S. Henkel, who was the last surviving foundation member of the Association and was to have been a guest of honour at the diamond jubilee celebrations.

It was most appropriate that the attention of members be directed to the history and achievements of their Association over the sixty years of its existence; this was done in several ways during the course of the congress, and by several speakers, including Dr. J. P. Duminy, principal of the University of Cape Town, in his presidential address on the "Challenges of Science to-day". Dr. Duminy pointed out that the rapid advances in science in South Africa and elsewhere have raised moral and ethical problems which are by no means to be lightly dismissed and that the scientist's obligations are still, and must always be, to the community of which he forms such an essential part. An article also appeared in the handbook issued to delegates, dealing with the birth of the Association—the outcome of a meeting of South African engineers in 1901—and with some of the men and women whose personalities have been an inseparable part of the Association over the years. The Association was patterned on the British Association for the Advancement of Science, which was represented at the Congress by Prof. R. W. James.

Concurrently with the senior congress, a junior congress was organized for advanced pupils of local schools. Many members consider the encouragement of scientific interests among high-school children the most important work of the Association, and the efforts of those who arranged the junior congress were indeed rewarded, for the numbers attending (320 from 27 schools) outweighed those attending the senior congress. Several junior members read papers which they themselves had prepared, and the general standard of these was very high. A very full programme was arranged for both junior and senior sections; from a scientific point of view, however, the highlights were certainly provided by the symposia.

A completely new departure for the Association was a symposium on palæontology. This was also the most extensive of the symposia and continued for most of the duration of the congress. The majority of the papers read dealt with vertebrate palæontology and the accent was on the reptiles and mammals of the South African Karoo. Never before has such a number of eminent palæontologists been present in any one place in South Africa at the same time, and members of the Association were not slow to take advantage of such a unique opportunity, the audience throughout being large and enthusiastic.

A paper which gave much pleasure and aroused considerable interest was that of Dr. A. W. Crompton, director of the South African Museum, who spoke on the origin of the mammalian lower jaw, drawing much of his evidence from *Diarthrognathus broomi*, an advanced therapsid Triassic mammal in which both the reptilian and mammalian jaw articulations were present. Dr. A. S. Brink, of the Bernard Price Institute for Palæontology, posed the question "How mammal-like are the mammal-like reptiles?" while Dr. J. Kitching, of the same Institute, discussed the fossil localities and mammal-like reptiles of part of Northern Rhodesia, concluding that "closely comparable conditions controlled deposition throughout central Africa in Karoo times and since these successions are broadly similar to those of the Republic of South Africa, such conditions were probably widespread over much of Gondwanaland". A guest from Paris, Mlle D. Sigogneau, read a paper on the classification of the Gorgonopsia, which she is studying in South Africa. Dr. E. H. Colbert, representing the American Museum of Natural History and an expert on Triassic reptiles, delivered two most interesting papers, one on the relationships of the Triassic reptilian fauna of southern Brazil—the other on the evolution of Triassic dinosaurs, ending with the suggestion that the so-called carnosaurs of the Triassic may possibly be a separate line of carnivorous pachypodosaurs, paralleled in later ages by the giant middle and late carnosaurs of the Mesozoic. The nineteen papers read at this symposium led to a considerable amount of lively and penetrating discussion, for which ample time had been allotted.

The symposium on marine biology was originally to have had as its theme "Food Relationships in Marine Environments", and though this plan had to be abandoned, several speakers delivered papers on this topic. The progress of marine research in South Africa—pioneered by John D. Gilchrist in the early years of the century—has indeed been rapid and has now reached the stage when the data and material collected over sixty years can be analysed with the view of greater understanding of the inter-relationships between different sections of the flora and fauna. Until recently, South African marine biology has been mainly concerned with the ecology of intertidal rock, estuaries, and shallow bays; this has proved an excellent apprenticeship for a programme which now pushed down to the edge of the continental shelf While benthonic studies are the concern mainly of Prof J H Day and his associates at the University of Cape Town, the marine plankton is being studied both at the South African Museum and by the Division of Fisheries A recently formed Department of Oceanography is also in operation, mainly interested in physical and chemical oceanography and providing valuable data which are being correlated with the distribution of the fauna and flora The results emerging from these studies—both with regard to distribution and to food relationships—are not only of considerable academic interest but also of urgent economic importance so far as the fishing industry is concerned Also under way is an investigation of the fauna of intertidal sandy beaches, an ecosystem which has been almost entirely ignored by South African zoologists until recently This oversight is probably due to the obvious paucity of sandy beaches as compared with intertidal rock (there are less than one hundred permanent inhabitants of intertidal sands described from the whole of South Africa), nevertheless this very paucity of species (though not of numbers) allows each one to be studied in great detail and the picture which begins to emerge may possibly be more complete than has been obtained from more popular intertidal environments Some attention was given to all the above aspects during the symposium, those taking part including Prof J H Day, Dr A de Decker, Mr J R Grindley and Mr F J R Taylor

A symposium on "Current Problems in the Behavioural Sciences in South Africa" was convened by Dr S Biesheuvel, president-elect of the Association and director of the National Institute of Personnel Research He opened the proceedings with a paper on the growth of abilities and character, which gave rise to much discussion and speculation "The Industrialization of an Indigenous People" was dealt with by Mrs Y Glass, while Prof K Danziger outlined "Some Social Psychological Aspects of Economic Growth" Another notable contribution was made by Prof C Wyndham, of the University of the Witwatersrand, who discussed the role of physiological factors in human performance This paper was of special interest to many delegates in that it supplemented to some extent an address given earlier in the week by Prof A W Sloan (Department of Physiology, University of Cape Town) in his capacity as president of the section dealing with human biological sciences Prof Sloan's topic was "Human Fitness" In an admirably clear and concise way he reviewed the methods of measuring human fitness and the standards of fitness encountered in college and university students in England the United States of America and South Africa It came as a surprise to many members of the large audience to learn that British standards of fitness are higher than those found in South Africa and very much higher than those of American students

In the middle of the congress week the delegates were transported to Stellenbosch University about thirty miles distant from Cape Town, to hear a symposium on "Fruit Research" The organizing committee had also arranged various entertainments and excursions, and the delegates were the guests of the Mayor of Stellenbosch at a luncheon given in their honour Those directing fruit research in South Africa are well aware of the need for a larger production of suitable foods for a rapidly increasing world population, indeed, this problem is particularly acute in countries such as South Africa where the majority of the population is underprivileged The development of high-protein foods to combat malnutrition among the lower income groups is thus of the greatest possible importance and is by no means being neglected by our food research institutes The organization of food research in South Africa was outlined by Dr C J B Smit, who also made a plea for closer co-ordination of food research activities in order to make the best use of existing facilities and personnel Although the Fruit Research Institute at Stellenbosch is largely concerned with applied and economic aspects of the subject, this does not imply that pure research has been ignored On the contrary, these scientists realize fully the necessity for research in all aspects of their subject This is illustrated clearly by Dr P G Marais's paper on the use of radioactive isotopes and ionizing radiations in agricultural research, the studies range from the essentially academic to those on the seasonal uptake of fertilizers and the irradiation of stored potatoes to prevent sprouting The symposium, covering these and other topics, did much to bring to the notice of fellow scientists, and the public as a whole, the valuable work being done at South African food research establishments generally, and by the Fruit Research Institute in particular

Two other symposia were held during the congress One organized by H M Astronomer at the Cape, Prof R H Stoy, dealt with astronomy in South Africa and covered not only recent developments in the observation of the southern sky but also the practical business of tracking man-made satellites both by optical and radio methods Of interest was the description of the Radio Space Research Station at Hartebeesthoek, near Johannesburg, which is operated by the South African Council for Scientific and Industrial Research and is designed primarily for the tracking of satellites and interplanetary research vehicles However, it is also expected that the Station will be able to make valuable contributions towards radioastronomy as such, when not required for its primary purpose Dr R Citron and Dr G Courtes, visiting scientists from the United States and France respectively, also read papers at this symposium

A short but intensely interesting symposium was held on "Early Man and his Environment" Prof J D Clark of Berkeley, California, discussed Pleistocene environments and culture change in Africa, while Dr R F Ewer, of Rhodes University, spoke on the contribution made by investigations of the associated mammalian faunas Among other aspects, she discussed the possible direct effect of mammals on human evolution, concluding that they provided an environment in which hunting skill could be perfected by easy stages and in which the development of such skill would be of great survival value Prof van Zinderen Bakker (of the University of the Orange Free State) and Dr G Bond (of the University College of Rhodesia and Nyasaland) both stressed the importance of different aspects of palæobotanical and geological studies in assessing the climate in which early man evolved The symposium was under the chairmanship of Mr R R Inskeep, senior lecturer in archæology in the University of Cape Town

In addition to the symposia there were the usual sectional meetings at which members of the Association read short original papers The standard of these papers was more variable than that of the symposia, but taken as a whole, they provided an interesting cross-section of science in South Africa to-day, the subjects ranging from the ecology of mangrove swamps to the decipherment of the Minoan scripts and from Stone-Age sites to the description of a versatile fraction collector Papers of particular interest included a report by Prof P V Tobias on the brain capacity of *Zinjanthropus*, a fossil ape-man from Tanganyika, and a paper by Dr Margaret Kalk on the occurrence of vanadium in tunicates, together with some suggestions concerning the intracellular mechanism which concentrates the metal and the possible part it plays in tunicate metabolism The difficulties of making a taxonomic study sound fascinating to non-taxonomists were admirably overcome by Prof H B Rycroft, director of the National Botanic Gardens at Kirstenbosch, in a paper on the history of the taxonomy of the Cape species of *Protea* during three and a half centuries, this was delivered as the presidential address to Section B (Botany) The presidential address to Section F (Humanistic and Social Sciences) was delivered by Dr M D W Jeffreys, who dealt with the cult of twins in Negro Africa Prof E Boné, official delegate from the University of Louvain, read a thought-provoking paper on human speciation as viewed in the light of palæontological species

Mention should certainly be made of the popular evening lecture, given this year by Prof D W Ewer, head of the Department of Zoology and dean of the Faculty of Science at Rhodes University, Grahamstown Prof Ewer's lecture was on "Mendelism and Macromolecules", but, as it was the intention to attract the general public, who are traditionally encouraged to attend this annual event, it was officially entitled "Brown Eyes, Blue Eyes, Short or Tall ?" We were much impressed by Prof Ewer's ability to explain the most complicated chemical phenomena with the utmost clarity Making brilliant use of models, he described the composition and replication of the chromosome and the nature and expression of the gene with such simplicity and logic that only the few of us who have lectured on this subject ourselves realized what penetrating and meticulous preparation lay behind his easy and almost informal delivery This was a fine and rare example of the art of lecturing and one which we shall remember for many years to come

A number of excursions organized for those attending the congress had a botanical bias and included a trip over the mountains into the Little Karoo, a route carefully planned to cross a maximum number of distinct types of floral habitat It is highly probable that following this route it is possible to see as many species and genera as over a similar distance anywhere in the world Prof H B Rycroft and Mr A V Hall acted as guides Though all the excursions were well supported, the most popular was a visit to the property of Dr S H Skaife, an authority on the life of social insects Parties were shown around Dr Skaife's laboratory, with its living colonies of ants and termites, and given first-hand explanations of the experiments which were being conducted The many people who generously welcomed parties to their laboratories, factories or private homes helped in no small measure to make this the outstanding congress it undoubtedly was It is generally felt that a standard has been set for future congresses in South Africa, and it is hoped that many of the innovations introduced at this diamond jubilee congress will become a regular part of South Africa's science time-table

BLAISE PASCAL (1623–62)

By Dr J ROSE

Vice-Principal, Coventry Technical College

"The heart has its reasons of which the reason knows nothing" (*Pensee*, 477)

IN the seventeenth century France was the most unified State on the Continent and numerically by far the strongest power in Europe Thanks to her large population and her wealth, France succeeded Spain as the dominant power on the Continent In some respects, however, seventeenth-century France was close to the Middle Ages, since it was essentially a semi-feudal State, based on a predominantly agricultural society The country was still in a transitional state from feudalism to Modern Age Socially, France was even more semi-feudal than seventeenth-century England, for the nobility and clergy were still privileged orders, divided in the legal and social sense from the artisans, peasants and traders, members of the inferior classes The peasant was at the mercy of his landlord in many respects, for example, taxation, marital status, residence, etc It was the peasant who bore the greatest share of the burdens of the country On the other hand, the middle class or *bourgeoisie* began to increase in importance at the expense of the nobility Owing to the general chaos in tax collection, some of the members of the *bourgeoisie* built up for themselves great fortunes, while acting as civil servants and advisers to the Government, and acquired by various procedures nobility status Indeed, the old social hierarchy based on birth was beginning to crumble, since the aristocracy suffered great impoverishment owing to their extravagant living Hence, under Richelieu and Louis XIV, only strict obedience to royal rule could allow them to survive and live in grand style, so that the scions of the great nobility were gradually transformed into flatterers and lackeys at the Court of "Le Roi Soleil" Even the revolts, known as the first and second Fronde, could not avert the evolution of France towards absolutism, which reached its height in the period 1661–85

As regards the position of the clergy, the Catholic Church presented a great contrast of piety and indifference, of deep dissensions and religious fervour The age of Saint Vincent de Paul, Fénelin, Bossuet and Pascal was also the period in which high offices of the Church were held by great noblemen and the sons of the King's ministers, the lives of most of them were thoroughly worldly, as shown by the memoirs of Saint-Simon In fact, the Church was a mirror of society, since in it the wealthy bishop and *abbé* looked down with contempt on the poorly paid and socially inferior parish clergy

In the first half of the seventeenth century, however, winds of change began to blow across France

The aristocracy and the rich were adorning the capital with imposing mansions, new districts were being built, and wide streets and spacious squares were laid out The religious awakening found its expression in new convents and monasteries, among them Port-Royal, presided over by the indomitable Mère Angelique, Saint Vincent de Paul began his work of charity and conversion Fashionable life glittered in the *salons*, while the open squares were being used as promenades by the gentry, *les honnêtes gens*, a species of cultivated gentlemen 'Honesty' in this context was identified with the art of 'pleasing' in the deepest sense of the word, that is, making oneself loved The *honnêtes gens* avoided everything pedantic and low, and appeared not to be interested in their profession or business. These notions and the glitter of the *salons* were, however, unknown to the mass of the people wrapped in poverty and ignorance

Despite the wretchedness of the masses, seventeenth-century France rings with great names of men of letters, for example, Corneille, Racine, Molière, La Fontaine and Boileau, and mathematicians, such as Fermat, Pascal and Desargues Their genius raised them above the spirit and conditions of the age, so that they were not the mirror of society The year of rampant ignorance saw the foundation of the Paris Academy of Sciences in 1666, four years after the Royal Society received its charter in England The Academy arose from an association of a group which used to meet at the cell of Father Mersenne, the originator of the Mersenne Numbers, who was responsible for spreading Galileo's teachings The original members included Descartes, Pascal, Fermat and Gassendi, whose commentaries on Epicurus revived the atomistic speculations of the early Greek materialists Mersenne acted as a sort of post-office, transmitting mathematical and scientific letters of his friends from one to another In fact, he founded a society of genuine scientists in 1635, the first centre of purely scientific life in Europe, and the members were Pascal's father and the mathematicians Roberval and Desargues He kept up an assiduous correspondence with Descartes in Holland and Fermat in Toulouse

The middle of the seventeenth century was the great age of mathematics in France In that country alone there were as many great mathematicians as Europe had produced during the preceding millenium The integral calculus grew out of the work of Cavalieri (1589–1647) and his successors, among whom one of the greatest was Pascal Indeed, Leibniz was influenced more by Pascal than by Newton in his mathematical thinking, so much so that we owe to Leibniz the record of parts of Pascal's work which would otherwise have been lost The theory of probability, projective geometry, the first calculating machines, and some remarkable predictions concerning quantum physics, saw the light of day in this age of greatness when the shackles of the Middle Ages began to disintegrate in the glow of the revival of critical thought The habits of mind were mathematical, and its methods were considered necessary for an exact philosophy Modern philosophy then began with Descartes, who built up a system by framing its axioms and postulates in such a way as to make philosophy acceptable to mathematicians Despite the great advances in science and mathematics there was, however, a manifest lack of its application to industrial processes, since industry was mainly conducted on traditional and small-scale lines

The life of learning was nearly completely cut off from the fashionable world and the people It was confined to groups or 'academies', in which various topics were discussed, ranging from physics to bibliography Moreover, these academies became the meeting places of the so-called free-thinkers These libertines, no idle scoffers, included such diverse attitudes as religious unbelief, philosophical detachment, or submissiveness to religious and political authority It was the sort of a *milieu* in which the mind was educated, and which influenced Pascal's attitude Even during Pascal's childhood, mathematicians and philosophers were his companions owing to his father frequenting such academies Indeed, Pascal's father reacted against the bonds of Aristotle's physics and thus spared his son the usual 'scholastic' training of the Middle Ages This is shown quite clearly in a letter addressed to Fermat in 1636 in which the value of experimenting and physical demonstration of proof was considered to be of primary importance in formulating a hypothesis

Pascal the Mathematician

Even in the age of Descartes, Fermat and Desargues, Pascal's mathematical brilliancy was unsurpassed At the tender age of twelve Pascal, stimulated by his father's refusal to allow him to study mathematics, devoted his spare time to geometry As a result of his deriving several leading properties of the triangle, his father repented and agreed to let him study Euclid and the conics of Apollonius Four years later Pascal published an original essay on conic sections based on a medieval geometrical theorem known as "l'hexagramme mystique", which states that if a hexagon is inscribed in a conic, the three points of intersection of pairs of opposite sides always lie on a straight line This idea was later taken up by Naclaurin, who introduced general methods for solving problems which cannot be attacked by the Cartesian procedures, in fact, he laid the foundations for the progress in pure science in the nineteenth century by Chasles, Clifford and Salmon, while the theory bore its finest fruit in the many-dimensional geometry of Segré

At the age of eighteen, in the year of Newton's birth (1642), Pascal made the first calculating machine in order to help his father in his tax-collecting activities The machine performed the carrying of tens automatically by means of wheels and ratchets In 1652 he sent one of his machines to Queen Christine of Sweden, the great patron of writers and scholars of the age A much more versatile machine made by Leibniz in 1671 was capable of adding, subtracting, multiplying and dividing, Pascal's denary notation having been replaced by a binary scale This invention, and that of a prototype of a wrist-watch, brought Pascal fame, so much so that this achievement was praised in prose, verse and sonnet, and a hundred years later Diderot described it in his *Encyclopedia* On the other hand, his health suffered greatly as a result of his labours The first symptoms of Pascal's life-long illness actually appeared at the age of one, probably due to a malformation of his skull There was a gradual worsening in his health, and the constant studies, as well as the endless controversies with his contemporaries, took their toll

Pascal's gift as a mathematician was overwhelming While lying awake and suffering in the last years before his untimely death at the age of thirty-nine, he established the chief properties of the cycloid by a brilliant geometrical argument This particular problem had baffled Galileo and Descartes, while Pascal, by using "the method of indivisibles", recently invented by Cavalieri, put the mathematics of this curve in its proper perspective within eight days In his letters to Sluse, Carcavi and Huygens dealing with the cycloid, Pascal laid the foundations of integral calculus

As a geometer Pascal ranks as one of the great of the millenium This is especially evident in his contribution to projective geometry in which the object is to discover those properties of figures which are invariant under projection The technique is one of the most elegant parts of mathematics, and its fascination extends to the historical origins in the work of Renaissance artists In this field Pascal was urged by the architect and engineer Desargues to investigate the properties common to sections of projections The theorems derived by Pascal and Desargues showed that there are significant properties common to sections of any projection of a given figure, and thus interpreted to some extent certain features of painters' work Owing to the antagonism exhibited towards projective geometry by the mathematicians of the period, with the honourable exception of Descartes, Desargues abandoned this field of geometry, and his publication was lost Fortunately, a book published in 1648 by Desargues's pupil, Bosse, who reproduced his master's results, was rediscovered in 1804, while Pascal's work published in 1640 remained unknown until 1800 The work of these two geometers thus lapsed into obscurity until the nineteenth century, when Chasles picked up by accident in a bookshop a copy of Desargues's work written by his pupil, P de la Hire It is also of interest to note that analytical geometry created by Descartes and Fermat, and the calculus, invented chiefly by Leibniz and Newton, proved extremely useful in the physical sciences, while scientists and mathematicians displayed no interest in Desargues and Pascal with their novel and baffling terminology Indeed, the great success of Descartes's analytical geometry with its "insistent clatter of the co-ordinate mill" almost completely diverted attention from pure geometry, so that it languished for two centuries, especially since Newton, Euler and Lagrange continued in the Cartesian tradition In our times, projective geometry has had an important bearing on mathematical and physical sciences Thus the emphasis on invariance of scientific laws, as recognized by the theory of relativity (the laws of the Universe varying from one observer to another) is no stranger to the mathematical physicist or geometer, since the concept of invariance plays a prominent part in the work of projective geometry

So far as algebra is concerned in the modern sense of the word, Pascal might be considered as one of its founders Following discussions with Fermat about games of chance and probability, Pascal adopted the arithmetic triangle, a device used earlier by Napier, to the solution of problems involving combinations of given things In fact, he utilized the diagonals of the triangular of numbers for working up the binomial expansion of $(a + b)^n$, where n is a whole number, thus introducing a geometrical pattern into configurations of numbers and creating higher algebra Newton investigated this pattern further and thus discovered the binomial theorem in its general form, while Bernoulli, Euler and Cayley, very much later, built on the foundations laid down by Pascal in 1653

The year 1654 witnessed the birth of the theory of probability, when Fermat formulated its fundamental principles in a short correspondence with Pascal This discussion had actually arisen as a result of a request by two gamblers disputing the distribution of winnings in an unfinished game, thus, to use a metaphor, the probability theory has been born under a gaming table to become the dictator of modern science Pascal went even farther in his famous wager with the Devil Since the expectancy of winning a prize in a lottery is the value of the prize multiplied by the probability of winning it, though the average sinner's probability of winning eternal bliss may be very small, the prize is so great that it will pay him to take a chance to reform his ways and live a good life Furthermore, Pascal asked whether probability itself is probable, thus anticipating some of the postulates of quantum physics

Despite Pascal's preoccupation with theological activities at that time, he scarcely interrupted his scientific pursuits He had kept in touch with Roberval and others, and in 1657 he entered into correspondence with the mathematician Sluse in order to solve certain problems of the geometry of curves

Contributions to Physics

As a result of discussions with P Petit, the commissioner of ports and fortifications, Pascal became interested in Torricelli's experiments on vacuum He devised a series of experiments involving tubes of various sizes and shapes, in combination with diverse liquids, for example, mercury, oils, wine and water Pascal found that the space in the Torricellian tube above the liquid was indeed a vacuum, thus eliminating the Aristotelian axiom that Nature abhors a vacuum His experiments became widely known in scientific circles in France and abroad It was the fear of conceding originality to others that prompted Pascal to publish *Nouvelles Expériences touchant le Vide* in a great hurry in 1647 His postulate of the existence of a vacuum was a revolutionary step, since even the great Descartes assumed the existence of "subtle matter" in the Torricellian space, in accordance with the medieval principle that Nature abhors a vacuum In addition, he devised a series of experiments in the period 1647–48 in order to demonstrate the truth of Torricelli's assertion that the height of the column of mercury in Torricelli's tube is balanced by the weight of air The *Traité de Vide*, published in 1654, summarizes this work His writing is also notable for its clearness and brevity, in contrast to the verbose Latin dissertations of his scientific contemporaries

Pascal then applied himself to the study of liquids in general and formulated the law that the pressure exerted anywhere on a confined liquid or gas is transmitted without loss in all directions throughout that fluid These researches led to the manufacture of the first syringe and the understanding of the relation between barometric pressure and height above the Earth's surface Indeed, the first calculation of altitude was made by Pascal in 1648 by studying the heights of columns of mercury in Torricellian tubes at various places

Pascal as Thinker and Theologian

As already described, religious life in seventeenth-century France was undergoing a profound change Outward respect for religious observance was combined with a lack of inner piety, while the scholastic aspect of theology was neglected in favour of delving into Biblical texts and non-metaphysical contemplations. In order to avoid any possible conflicts between reason and faith it was assumed that anything which is the concern of faith cannot be the concern of reason. This age is also marked by a religious revival, by the emergence of the doctrines of Saint-Cyran and Jansenius, and by the Jesuit–Jansenist controversy. The spirit of this upheaval penetrated into the very citadels of the agnostics, so that many great families abandoned the life of dutiful religious practice in favour of inner piety, austerity, penitence and virtue. Thus, the brawling and fiery brothers Deschamps, who later exerted a great influence on the Pascals, became 'converted' and devoted their lives to works of charity. Blaise Pascal experienced his first 'conversion' in 1640 by becoming an ardent Jansenist, but this did not detract him from scientific pursuits, though the Jansenist tradition did insist on abandoning the desire for knowledge, since the latter was equated with intellectual arrogance. His zeal in matters religious was, however, quite strong, as shown by his spirited attacks on some eccentric theses propounded by the Capucin friar Jacques Forton, who tried to eliminate the mysterious element in Christianity by pushing to the extreme limits the agreement between reason and faith. At the same time, scientific integrity prompted Pascal to indulge in a bitter argument with the Jesuit Father Noel, who defended the physics of Aristotle and refuted Pascal's interpretation of Torricelli's experiments.

Pascal's life was divided between a deep attachment to science and a perfect submission to his faith. His enemies ranged from the Jesuits to Voltaire, but his inner piety and religious sentiments were unimpeachable. This is clearly shown in his letters written in the period 1647–51. In one of these he described marriage as "most dangerous and the lowest of the conditions of life permitted to a Christian", though he apparently contemplated matrimony for some time, at least in order to improve his finances. Although he cultivated the society of 'free-thinkers' and he felt a deep passion for science, popularizing his discoveries in lectures at various gatherings, he felt increasing uncertainty and confusion, in the words of his sister Jacqueline, "he has experienced a great contempt for the world and a great distaste for people who compose it". All this led to the 'second conversion' on the night of November 23, 1654, which culminated in a mystical ecstasy, contrite humility and fervent exaltation, as shown by the text of the "Mémorial" which he carried inscribed on parchment around his neck. He renounced marriage, but did not withdraw from the world. In fact, this event was followed by a brilliant series of letters, known as *Les Provinciales*, in which he pleads the defence of the Jansenist theology against the Jesuits and the doctors of the Sorbonne. Though Pascal did not belong to Port-Royal, the citadel of Jansenism, in a formal sense, he was one of its effective allies.

The 'Provincial Letters'

One of the results of the Reformation in France was the emergence of a strong Counter-Reformation movement. The protagonists of the latter movement were, however, divided among themselves, and a number of groups could be distinguished. The members of the Society of Jesus were the first to enter this intellectual battlefield. They sought to reconcile traditional Church doctrine with intransigent modernism. On the other hand, the Jansenists (led by Bishop Cornelius Jansenius, 1585–1638, and Saint-Cyran, 1581–1643) abandoned modernism and preferred the dogmas of the ancient Church. They believed that in Saint Augustine there was an antidote to lax morality and collapsed standards. A profound pessimism appears to invade the Jansenist doctrine, since they assumed that from the moment of Adam's fall man has lost his will to do good, so that he is in need of omnipotent divine grace to lead a good life, these teachings are contrary to Jesuit principles according to which man has absolute liberty to choose good or evil, divine grace being a mere prompting, and not a compulsion. While the Jesuits were preoccupied with 'minimal' levels of living, sufficient to attain salvation, the Jansenists demanded total submission to God and maximum perfection. Jansenism thus represented an intense reaction against religious abuses and the Jesuitic teachings because of the latter's opportunism and spirit of compromise. Some of these teachings were published by Jansenius in *Augustinus* in 1640, the book was later condemned by Pope Alexander VII in 1657.

Though the convent of Port-Royal became the citadel of the Jansenists, it did not always follow the doctrine with exactitude. Great controversies arose in the 1640's, so much so that the Pope himself became involved, and the Sorbonne was asked to sit in judgment on certain aspects of the Jansenist doctrine. The *Provincial Letters*, which were placed on the *Index* in 1657, form the defence of Jansenism during the protracted proceedings at the Sorbonne. It is a collective work by Pascal and other theologians, an anonymous and clandestine book, addressed to all men of good will. In order to interest the widest possible public Pascal sought to enliven it with wit and endow it with a clarity of style, exposition and composition. Parts of the letters are also concerned with fierce battles with Jesuits. Pascal seeks to define the relation between God and man, and considers the problem of human responsibility, he opposes the two conceptions of human personality, as defined by the Jesuits, by delving deeper into man's intimate selfhood corrupted by original sin. At the same time, he attacks their morality founded on casuistry, using irony, comedy, indignation and vehement eloquence to smash his opponents. For his pains the enemies of Port-Royal called him a heretic, slanderer and Calvinist. As a result of this spirited reaction and the persecution of the Jansenists, Pascal had to adopt in his last letters a more conciliatory attitude, to some extent, the ideas propounded in his last letters departed from the fundamentals of Jansenism.

In the *Provincial Letters* Pascal reveals to some extent his true nature. He shows himself to be the religious apologist seeking deep conversion of sinners, he uses scientific methods and appeals to experience wherever possible. Mystical qualities are sometimes infused with polemical vehemence, bias and a lack of charity so much so that Chateaubriand called him "a slanderer who had genius". In his letters there is a deep anxiety of the soul, an aspiration after the infinite a spiritual ferment, an attack on lax moral theology prevailing in the seventeenth century—all this clothed with magnificent prose. The points raised in the letters are elaborated in greater detail in *Ecrits sur la Grâce*.

The impact of Pascal's writing on the cultural history of France was tremendous, though at the

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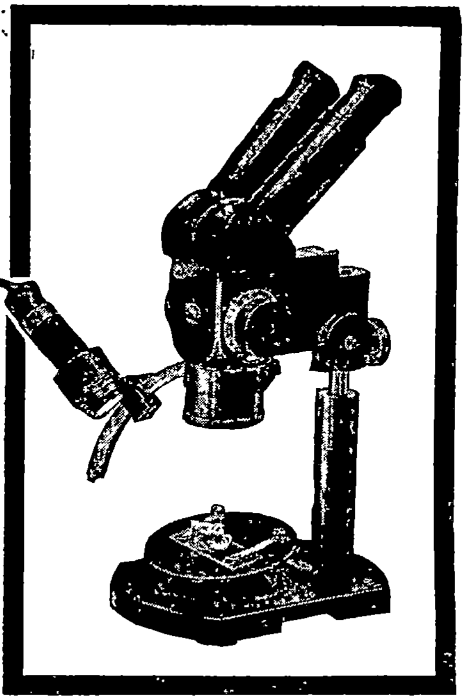

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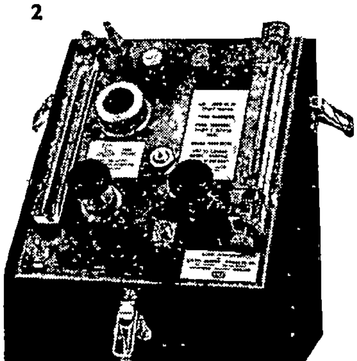

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time his defence of Jansenism was of no avail Though his health declined rapidly, he pursued his scientific inclinations He invented a new method of teaching reading for use in the Port-Royal schools in 1655, and established in Paris a regular omnibus service—the first of its kind His illness, however, grew worse, and he died on August 19, 1662, without disavowing his past life or beliefs (J Mesnard, *Pascal* 130, Harvill Press, London, 1962) Among the many manuscripts left, some of them unfinished, was a collection of notes, which were given the title *Pensées* by the first editor, these notes appear to be a product of reflection, suffering and deep insight The actual arrangement of these fragments is not known with certainty, despite many editorial attempts (*loc cit*, 134) In this 'tract for the time' Pascal seeks to awaken in the 'free-thinker' a religious sense, an inner ardour and æsthetic appreciation of the Christian faith by a skilful mixture of rational demonstration, emotional appeal and sense of drama He attacks the unbeliever by asserting the necessity of reason submitting itself to the mysteries of faith, as defined by the theologians of Port-Royal This Apology is based on The Bible and also on the writings of his contemporaries, the Jansenists, Descartes and various Christian apologists The work is original and coloured by a powerful imagination, it is written in the style of a scientist and mystic, and it helped to keep alive in France a certain Christian sensibility among unbelievers

Pascal as Man and Artist

Pascal meant different things to different people To the eighteenth-century Condorcet he appeared to be an agnostic to the Romantics he was the type of an anguished man, Claudel and Aldous Huxley looked at him as a sick man and a life-hater, respectively, some modern thinkers view him as a Christian humanist, on non-believers of all ages Pascal exerts a fascination owing to his scientific achievements and ruthless analysis of the world in terms acceptable to the free-thinker, to certain existentialists he is the inspired founder To the French he is the father of modern prose and a superb artist

This diversity of opinion may perhaps be ascribed to the very perplexity of Pascal's personality He was a bundle of contradictions, charitable in daily life, but merciless in controversy, truthful, but a cunning advocate, deeply religious, but a defiant opponent of certain aspects of established beliefs, a humble Christian, though a dauntless sceptic, man of science and high society, though an ascetic and of simple tastes—a veritable cauldron of opposing tendencies He combined the pursuit of science and attention to the fashionable world with austerity and anguish A neurotic racked by pain of a life-long illness, a deep believer and rational theologian Violent and merciless towards his adversaries, somewhat arrogant, conscious of his genius and impetuous as a religious apologist, but with a passion for truth and goodness He could experience great ecstasy, but preserve a lucidity of reason Even in the biography written by his sister, Pascal gives the impression of being hot-tempered, irascible, obstinate and discourteous He was a child of a troubled age, of an epoch of religious and social upheavals, an age of Copernicus and Galileo, and of cruel religious wars

As a thinker Pascal was original in his emphasis on the primacy of the experimental method, contrary to his contemporary Descartes, who asserted that experiments merely confirm mathematical deductions. He discarded deductive reasoning in science and theology, in both, reason plays a limited part Experience reveals two classes of facts, religious and scientific, independent of each other In philosophy the fundamental role of experience is equated with the primacy of existence, though man by considering his own existence realizes the necessity of the divine filling a vacuum in his mind, without God, man is wretched and powerless However, the heroic element in man allows him to rise from the slough of despair and acquire greatness by turning to God, who clarifies human thought, so that man perceives the truth True religion, according to Pascal, can only consist of love of God, hence ceremonial rites lose their importance, as religion becomes based on a personal link between God and man on the path to perfection Though Pascal was condemned posthumously as a Jansenist and heretic, his reputation as a theologian grew, for he diagnosed the anguish of the soul and prescribed the remedy

Pascal was a superb man of letters He eschews everything that smacks of 'false beauties', specialization and 'unnaturalness' and he paints passions in their natural colour and distinct order His style is a blend of a poet and scientist As a poet he creates images by using original metaphors, a sense of rhythm and superb lyricism, his thought, however, shapes itself in a scientific mould His life is a desperate attempt to try to comprehend as he put it "I know nothing about all things" There is a profound unity in his life and work He expresses the aspirations of the ages, the spirit of restless generations in search for an ideal and concrete reality Pascal sought truth in life, religion and science, so that his vocation was to run from one high place to another to light the fires (Guitton, J, *Pascal's Pensées*, 11, Librarie Générale Française, Paris, 1962)

SOME RECENT MEASUREMENTS OF CÆSIUM-137 AND ZIRCONIUM-95 IN HUMAN BEINGS

By J. RUNDO and D. NEWTON

Atomic Energy Research Establishment, Harwell, Berks

MEASUREMENTS of the mean level of fall-out cæsium-137 in a small group of control subjects have been made at this Establishment since early 1956, using body radioactivity measurement with scintillation γ-ray spectrometry[1] The results of such measurements to September 1960 have been reported[2] Cæsium-137 has been the only γ-ray emitting fission product detectable except during and very shortly after periods of nuclear test explosions, when iodine-131 was present in thyroid glands However, we have had occasion to suspect the intermittent presence of an emitter of 0 75-MeV γ-rays

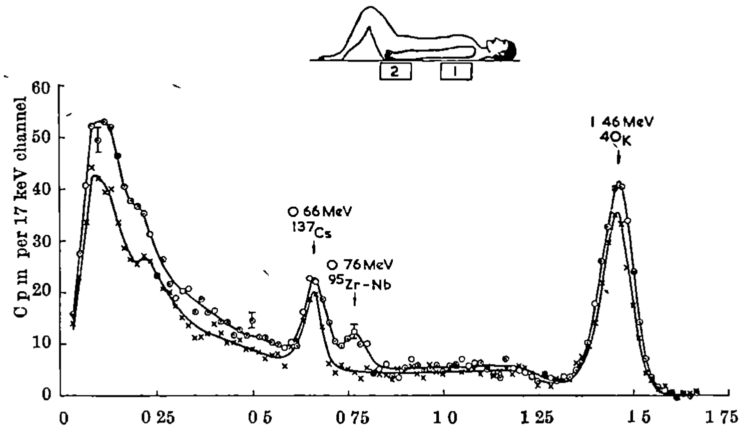

Fig 1 Gamma-ray spectra from subject *M* measured on April 24, 1962, with a crystal of sodium iodide (Tl) 23 cm in diameter by 15 cm thick
O, Crystal in position 1, ×, crystal in position 2

(presumably zirconium-95/niobium-95) during periods of intense fall-out (for example, the spring of 1959), when clothing contained easily detectable amounts of these nuclides The modest sensitivity and energy resolution of our equipment[1] precluded accurate energy measurement of the suspected contaminant *in vivo* and also of localization in the body In this article we are presenting the results of our measurements of fall-out cæsium-137 since September 1960, we also wish to report the recent detection of radiation from human lungs which we attribute to inhaled zirconium-95/niobium-95. During April and May 1962 we used a crystal of sodium iodide (Tl) 23 cm in diameter by 15 cm thick for measuring the cæsium-137/potassium ratios of our control subjects As we suspected the presence of fission products in lungs, two 20-min measurements of body radioactivity were made at each observation, the first was with the centre of the crystal placed under the mid-point of the sternum of the supine subject and about 2 cm below the back, while the second was with the crystal under the subject's sacrum as indicated in the inset of Fig 1, which shows the two γ-ray spectra obtained from subject *M* In addition to the prominent photo-peaks at 0 66 MeV and 1 46 MeV due to cæsium-137 and potassium-40 respectively, the first spectrum shows a well-defined photo-peak at 0 76 MeV which we attribute to zirconium-95/niobium-95 in the lungs To eliminate the possibility of this activity being in clothing or on the skin, all subjects showered immediately before the measurements and changed into clean clothes which had been stored in plastic bags The counter background was measured either immediately before or after the subject with a distilled water phantom in position on the stretcher, 'dressed' in the clothes worn by the subject All persons examined have shown evidence of this radiation from the lungs, including a 6½-year-old girl

Caesium-137 In order to determine the cæsium-137/potassium ratios *in vivo* the following measurements were made Spectra from known amounts of cæsium-137 and potassium in solution in a phantom were measured separately and these were used to estimate the cæsium-137/potassium ratio from the results obtained at the second measurement on each subject, that is, those which showed no indication of a peak at 0 76 MeV Because of the geometrical arrangement of subject and detector the absolute values of potassium and cæsium-137 were not considered reliable, but in most cases the values of their ratio were consistent with the values obtained for the same subjects measured by our usual method[1] in January 1962, and they were thought to be correct to within the statistical error of not more than ± 2μμc cæsium-137/g potassium The results of all measurements of the mean cæsium-137/potassium ratios in the control group since September 1960 are set out in Table 1, these values are plotted in Fig 2, where the earlier measurements (from February 1956) are included together with those of the subjects with the highest and lowest values of the ratio The values for January and April 1962 call for comment as zirconium-95 was present on both dates The scale of cæsium-137 content at the right of Fig 2 is approximate and applies only to the average values, and not to the measurements on subjects *C* and *R* This is because the potassium contents of these subjects were not the same as the average for all the control subjects, thus the cæsium-137 content of subject *C* in July 1959 was 16 3 mμc (99 μμc /g potassium) while the scale of Fig 2 indicates only 14 5 mμc The mean value for April 1962 was determined from the measurements with the single large crystal as already described All the γ-ray spectra from the subjects measured in January 1962 were averaged and a small photo-peak due to zirconium-95/niobium-95 at 0 76 MeV was then apparent (it was not as prominent as shown in Fig 1 because of the much less favourable geometry) The mean potassium content was calculated and its contribution in the region of the cæsium-137 and zirconium-95 photo-peaks was determined and subtracted The mean cæsium-137 content was then determined by trial-and-error, and its spectrum was subtracted, leaving a surplus counting-rate due to the average zirconium-95 content (see below)

By October 1961 the mean cæsium-137/potassium ratio had decreased to a lower value than at any time since our measurements began The rate of decrease from March 1960 indicated a halving time of about 16 months, which is very similar to the value found by other workers[3] This time is much longer than the

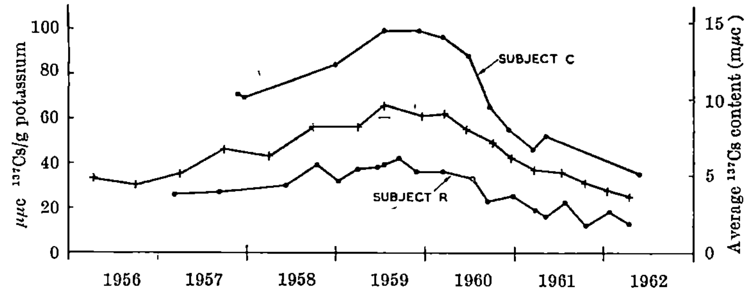

Fig 2 Mean cæsium-137/potassium ratios in a group of control subjects from 1956 to April 1962, and the ratios for subjects *C* and *R* The scale of cæsium-137 content at the right is approximate and applies only to the mean value of the group

Table 1 CÆSIUM-137/POTASSIUM RATIOS AND AVERAGE CÆSIUM-137 CONTENTS IN A CONTROL GROUP OF SUBJECTS RESIDENT IN BERKSHIRE AND OXFORDSHIRE

Date	No of subjects	^{137}Cs/g K ($\mu\mu$c)	Average ^{137}Cs content (mμc)
September 1960	13 (14)	48 (49)	7 0
December 1960	14 (15)	41 (42)	6 4
March 1961	14 (15)	36 (37)	5 5
July 1961	12	36	5 2
October 1961	14	31	4 5
January 1962	10	28*	3 95
April 1962	15	25*	3 5

Values in parentheses show the effect of including subject *C* (Fig 2) whose Cs-137/K ratio was the highest of all the control subjects

* See text

biological half-life of cæsium in man (our data, which will be described in detail elsewhere, indicate an average value of about 110 days), showing that the daily intake is not directly proportional to the rate of fall-out, which decreased faster than did the cæsium-137/potassium ratios *in vivo*

Zirconium-95 In the average spectrum from the controls measured in January 1962, the counting-rate at 0 76 MeV, after subtraction of contributions due to potassium-40 and cæsium-137, was attributed to zirconium-95/niobium-95 The best estimate which we can make of the average content is about 300 μμc (total γ-ray activity at 0 76 MeV)

To estimate the amounts of zirconium-95 in the lungs of the subjects measured in April 1962, we used special chest phantoms similar to that described by Miller[4] These were constructed with sheets of 'Mix D' phantom wax[5], with air-spaces to simulate the low density of the lungs, a standard source of zirconium-95 was placed in turn in each of the air spaces and all the spectra were added together in order to simulate the effect of a distributed source. This was repeated for each of several phantom thicknesses (15–24 cm) to cover the range of chest thicknesses of the subjects The spectrum obtained using the 20-cm phantom, together with the spectra from the normal cæsium-137 and potassium phantoms, were used to calculate the cæsium-137/potassium ratio and a value for the lung content of zirconium-95 by a computer method of least squares analysis[6] Because the shapes of the spectra at low energies from the zirconium-95 showed considerable variations with the various phantom thicknesses, and because we suspected the presence in the spectra from the subjects' lungs of contributions from ruthenium-103 and cerium-141 (0 5 MeV and 0 145 MeV quanta respectively), the least squares fitting was confined to the energy range 0 57–1·675 MeV, but the computer was programmed to calculate the spectrum over the entire energy range measured (0 025–1 675 MeV) The cæsium-137/potassium ratios were in excellent agreement with those calculated from the results of the second measurements

An alternative method was also used to determine the amounts of zirconium-95 in some of the subjects, the spectrum obtained with the crystal near to the subject's sacrum was normalized in the energy range 0 83–1 675 MeV to that obtained with the crystal close to the lungs, and the spectral difference (energy range 0 63–0 83 MeV) was attributed to zirconium-95, the amount of which was calculated The statistical errors were considerably greater than in the computer method, but the results from the two methods were in good agreement except where relatively large normalizing factors were needed

To allow for the differences between the chest thicknesses of the subjects and the thickness of the chest phantom chosen as standard, the estimated zirconium-95 contents were multiplied by appropriate correction factors deduced from the measurements with the various phantoms These corrections were all small (< 20 per cent) except in the case of the child The results for 15 subjects are set out in Table 2, where the values for zirconium-95 are the total γ-ray activities at 0 76 MeV Only the statistical error is indicated, there may be a systematic error due to the calibration method, but we have not been able to assess this In calculating the values for the concentrations of zirconium-95 in the lung it has been arbitrarily assumed that the mass of the lung is 1 43 per cent of the body-weight The radiation dose-rate from an average concentration of 0 35 μμc /g of zirconium-95 with niobium-95 in equilibrium is 0 11 μrad/h, if the zirconium-95 is distributed uniformly throughout the lung, but this would not be valid if the zirconium-95 were on a few relatively large particles As we know nothing of the distribution within the lung, the values of 0 05–0 16 μrad/h for the radiation dose-rates to the lungs of the individual subjects are lower limits For comparison, the dose-rates in soft tissues (presumably including lung) due to the naturally radioactive potassium-40 and to the average level of fall-out cæsium-137 are 2 2 μrads/h and 0 49 μrad/h respectively Thus, although the average dose-rate from the zirconium-95 is twice that from the cæsium-137, it is still small compared with the dose-rate from natural background

Table 2 CÆSIUM-137/POTASSIUM RATIOS IN THE WHOLE BODY AND ZIRCONIUM-95 CONTENT OF THE LUNG FOR FIFTEEN SUBJECTS

Subject	Date measured	^{137}Cs per g K ($\mu\mu$c)	^{95}Zr content* ($\mu\mu$c) (*S E* = ± 40 $\mu\mu$c.)	^{95}Zr per g lung† ($\mu\mu$c)	Radiation dose-rates in μrad/h Soft tissues (^{137}Cs)	Lung (^{95}Zr)
N	12-4-62	25	450	0 53	0 050	0 16
T	13-4-62	34	500	0 48	0 068	0 15
R	13-4-62	12 5	360	0 32	0 025	0 10
Miss *M*	16-4-62	20	450	0 48	0 040	0 15
S	16-4-62	27	490	0 42	0 054	0 13
K	17-4-62	15 5	210	0 24	0 031	0 07
Mrs *N*	21-4-62	23 5	200	0 25	0 047	0 08
Miss *R* ‡	24-4-62	19	90	0 27	0 038	0 08
M	24-4-62	21	490	0 46	0 042	0 14
B	25-4-62	26	300	0 33	0 052	0 10
W	25-4-62	26	160	0 15	0 052	0 05
Ri.	3-5-62	29	620	0 53	0 058	0 16
G	26-4-62	25 5	240	0 20	0 051	0 06
Kn	26-4-62	34	240	0 25	0 068	0 08
Sh	11-5-62	31	350	0 33	0 062	0 10
Means		24 6	343	0 35	0 049	0 107

* Total γ-ray activity at 0 76 MeV

† Lung weight assumed to be 1 43 per cent of gross body-weight

‡ Child aged 6½ years

Because the major part of the counting-rate at low energy (< 0 57 MeV) was due to potassium-40 and cæsium-137 it was not possible to detect with certainty the presence in the spectra of photo-peaks at 0 5 MeV and 0 145 MeV, due to ruthenium-103 and cerium-141 respectively, although there were indications of these peaks in the spectra from most subjects Attempts to improve the statistics by averaging spectra, or by averaging the differences between observed and computer fitted spectra, were not successful in demonstrating statistically significant photo-peaks at these energies However, the presence of ruthenium-103 and cerium-141 in the lungs must be assumed because of their presence in the air[7] and in human excreta[8], these nuclides will increase the dose-rates to the lungs It is interesting to note that the average lung content of zirconium-95 of three subjects was 28 times their daily fæcal excretion of this nuclide[8], implying a biological half-life in the lung of about 19 days

Finally, we may note that the amounts of zirconium-95 found by us are similar to the amounts found by Schonfeld *et al.*[9] in the lungs from three subjects examined *post mortem* in Vienna in the Spring of 1959.

Note added in proof. The cæsium-137/potassium ratios *in vivo* in July 1962 were significantly higher than in April. Our provisional results indicate a mean value for six subjects of 35 μμc cæsium-137/g potassium; the mean value for the same six subjects was 29 μμc cæsium-137/g potassium in April. The average lung content of zirconium-95 for these subjects declined from 357 μμc in April to 217 μμc in July (provisional result). From their analyses of human lungs obtained *post mortem*, Schonfeld and his colleagues (paper presented at the third International Congress for Hygiene and Preventive Medicine, Vienna, May 27–30, 1962) calculated zirconium-95+niobium-95 contents of up to about 200 μμc in February 1962. This is similar to our average value for January 1962.

[1] Rundo, J., *Brit. J. Radiol.*, Supp., 7, 125 (1957). *Proc. Second U.N. Conf. Peaceful Uses of Atomic Energy*, **23**, 101 (1958).

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[3] Miller, C. E., and Corcoran, J. B., *Argonne Nat. Lab. Rep.*, *ANL-6398*, 41 (1961). Anderson, E. C., *Los Alamos Sci. Lab. Rep. LAMS-2627*, 223 (1961). Onstead, C. O., Oberhausen, E., and Keary, F. V., *U.S. Atomic Energy Comm. Rep. NP-10390* (1961).

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[7] Fisher, E. M. R. (personal communication).

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[9] Schönfeld, T., Liebscher, K., Karl, F., and Friedmann, C., *Nature*, **185**, 192 (1960).

FUTURE OF OPTICAL ASTRONOMY*

By PROF. D. E. BLACKWELL

Savilian Professor of Astronomy, University of Oxford

I BELIEVE that optical astronomy in Great Britain has now reached, for virtually the first time in its history, a critical stage, and its actual survival is not something that can just be taken for granted without further thought. Physics is studied in universities and in Government and industrial laboratories; but with two exceptions, optical astronomy is studied almost entirely in university laboratories only, and its future largely depends on the attitude of the universities. Too often, the attitude the world over is that an observatory is a place to which one can take one's children on a fine Saturday night. By tradition, astronomers are the most hospitable of all scientists. But the danger of such hospitality is that the visitor sees an almost non-existent aspect of the professional work of an observatory, demonstrated with a telescope perhaps more than a century old, and comes to believe that the astronomer lives in a dream world in which he gazes at the Moon and planets and is constantly watching for the appearance of a new star. Or, if he does not wholly believe this, he thinks of an astronomer as a Victorian scientist using Victorian methods and instruments in a Victorian institution. I do not need to emphasize how damaging such an attitude is to the proper development of astronomy in the universities.

In the early nineteenth century astronomy was receiving whole-hearted support from Government, patron and university, and by the time that physics became established as a proper subject of study in the universities, astronomy was already a firmly established subject, and from then on the two flourished and developed side by side quite independently. This is the beginning of the present unfortunate distinction between physics and astronomy, or between physics and astrophysics, which is often exemplified by the presence in a university of an observatory quite distinct from a physics laboratory. Although each had much to learn from the other they were always, and still are, treated as two entirely separate subjects. In reality, of course, modern astronomy is simply a branch of physics just as much as is low-temperature physics, solid-state physics and nuclear physics. It uses the experimental techniques and apparatus of physics—photoelectric cells, electronics, spectrographs, image intensifiers—and it uses, and contributes to, the basic ideas and data of physics. There is, in fact, no essential difference between a modern university observatory and any other university science department.

I should like to give an illustration of what I have been saying. Astrophysicists were the first to investigate in detail the theory of radiative transfer, and the formation of spectrum absorption lines, and realized before anyone else the importance of collision broadening. All this is now a commonplace part of physics; but it was all started by astrophysicists. I feel that we cannot emphasize this point of view too strongly, and, to remind us that modern astronomy is a part of physics, I hope that we may see the name of the Oxford Observatory become 'Department of Astrophysics'.

Returning now to the main theme, I would say that until the late 1930's one could have expected to predict the future course of astronomy, say, for one decade at least, with some confidence. But he would have been a bold and imaginative astronomer who, in 1940, could have predicted successfully the state of affairs which now exists in 1962, only twenty-two years later. Now a spectrograph attached to a rocket can be used to investigate the solar spectrum in the short wave-length region which is absorbed by the Earth's atmosphere, and we have the exciting possibility of placing our telescope on a satellite well above the Earth's atmosphere. The solar system is being investigated with interplanetary probes, and its scale obtained by radar methods. Photographs have been taken of the other side of the Moon. Men have been put into orbit with their apparatus and brought safely back, although—perhaps heretically—I question the value of this tremendous achievement for astronomy. We have the fact of the astonishing development of radio-astronomy into a flourishing subject of its own. We have all the wonderful techniques of electronics which have been developed for the nuclear physicists and for rocket guidance systems, and we have fast computers which can solve problems a million times as complex as before the War. Is it any wonder that I apologize for my presumption in considering astronomical progress in the next twenty years. There has been as much progress in the past twenty years as there was in the preceding two thousand years.

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* Substance of Prof. Blackwell's inaugural address, delivered on June 12. The full text will be published by the Clarendon Press, Oxford.

Faced with all these exciting achievements and possibilities, what kind of research should a university astronomy department do? I want to consider this problem now, but first I should like to suggest a basic principle. This is, that when a university department undertakes research, it must lead and not be content to fill in the trivial gaps in our knowledge. This is the crucial sentence in this article. But the university astronomer, wherever he is in Great Britain, is heavily penalized by the climate. My theme now is—how can we do significant research under such a disadvantage, and on a relatively small budget. I am going now to consider the various possibilities open to us, leaving until later the question of whether we are in a position to take advantage of these possibilities—a question which will bring us back to the relation between universities and their astronomy departments.

First, space research. For the academically minded there are three attractions of constructive space research, quite apart from any applications. The first attraction is that it enables us to investigate directly the environment of a space-vehicle. By space, here I mean interplanetary space, which hitherto has had to be investigated by somewhat indirect means from the surface of the Earth.

The second attraction of space research is that we are clear of the Earth's atmosphere, which wholly absorbs the radiation from celestial objects at wave-lengths shorter than 3000 Å, and partially in the infra-red at wave-lengths between 20μ and 1 mm. There is a rich field of investigation here which is undoubtedly worth while, although at enormous cost.

The third attraction is concerned with telescope performance. The advocates of space research say that instead of trying to do better by building Earth-bound telescopes larger than 200-in. diameter we should improve the performance of smaller telescopes by putting them into space vehicles.

Do not think that because there are these immense possibilities for astronomy in space research, observing from the ground is out of date and should be abandoned. Far from it—there is still plenty of opportunity for observing from the ground and plenty of scope for imaginative thinking. But I think that it would be foolish to neglect the possibilities of astronomical space research, and I am anxious to see Oxford participating in it in the years to come.

When we come to consider optical astronomy we are, in the light of our maxim, in an apparently difficult position. By optical astronomy I mean astronomical research made in those regions of the spectrum which are transmitted by the Earth's atmosphere, that is, in the range 3000 Å to the near infra-red, these observations being made from ground based observatories. In what follows I shall speak only of astrophysics, and not of astrometry, which is concerned with the measurement of the positions of the celestial bodies.

Britain is heavily handicapped by the climate. What should be done? I am convinced that if optical astronomy is to survive in the universities of Britain—and I emphasize that I am speaking now only of the universities—and to be an effective contribution to modern astronomy, it must, to a large extent, although not entirely, be carried on abroad. I direct attention to this qualification "not entirely", it is important. I do not intend that observing in Oxford should be curtailed. Clearly, it would be absurd to make an investigation in Oxford with the knowledge that the work can be done much better abroad. But there are some astrophysical problems which may be solved equally well in Oxford as abroad, particularly those which most usefully employ a photographic technique rather than a photoelectric technique. Because it is a two-dimensional detector, the photographic plate can gather information much more quickly than the photoelectric cell, which can only measure the flux of radiation through a specified area although the information that the plate gathers may not be so precise as that obtained by the photocell. The photographic technique is therefore a very suitable one for this rather poor climate, for it can gather a great deal of information during a few minutes of clear sky—information which may take some months to analyse.

On the other hand, I believe that the use of highly developed photoelectric techniques in a good climate abroad will lead to far-reaching advances, and I wish to refer to four ways in which I hope that this kind of work will be accomplished by Oxford astronomers.

The first way is by making an expedition to the best possible place for the solution of a particular problem. As an example, I refer to the 1961 Oxford/Cambridge expedition to the mountain site of Chacaltaya at an altitude of 17,100 ft. in the Bolivian Andes. The second way is to make an expedition to observe a particular astronomical event. An obvious example is an eclipse expedition, in which we may be interested in the solar corona. A third way involves making a long-term expedition to solve a particular problem. The physicist often thinks of the Sun as an immensely bright source which is surely radiating energy at a rate sufficient to satisfy the needs of any astrophysicist. On the face of it, this is true. The Oxford solar installation receives from the Sun 10^{21} photons per sec. But there are several aspects of solar physics for which even a flux of this amount is uncomfortably small, and these are the problems that we are beginning to think about at Oxford. In Oxford the investigations that we are thinking of might take centuries, but I am hoping that it may be possible to carry the work through at a station in the Mediterranean where the first part of it should be completed in a period of only five years.

The fourth way is to take our equipment to a large telescope abroad and I hope that in the future, in addition to working at Pretoria, we shall have the opportunity of accepting American hospitality at the National Observatory of Kitt Peak.

I have tried to show that astronomy is a fascinating, flourishing subject which is well worth enthusiastic support. But I think that it will be generally recognized that astronomy is a Cinderella among university subjects, and now I want to take the liberty of making a plea for more active support for it, and more appreciation of, and enthusiasm for, it in the universities. The astronomical world is as hard and as competitive as any other. I will emphasize again, that if a university undertakes research it should lead. Yet how can we progress, let along lead, in the face of the competition from overseas—a competition which originates in a genuine enthusiasm for the subject and an active desire to encourage its development by all possible means. In contrast to this attitude the astronomy departments in Britain are often regarded as expendable, and they are under constant pressure to make way for other larger and more powerful departments and, indeed, are thought unreasonable if they offer resistance These are hard words, but the astronomer's situation in the universities is no less hard Often he must work with no laboratory assistant or computing assistant, perhaps very little secretarial assistance, and only a small amount of workshop assistance Prof P M S Blackett has recently suggested that a research group of six scientists should ideally be supported by six technicians, together with one technician for each research student I suggest that an active astronomy group needs at least this number I have been at pains to say that modern astronomy is not a matter of gazing through a telescope, neither is it a routine matter of photographing stars or putting a photographic plate in a spectrograph, exposing on a stellar spectrum for an hour and then going home Apparatus, often quite complex, must be designed for each problem as it comes up, and for this kind of work a group of six scientists cannot easily manage with less than six technicians In practice, the number of technicians available to the astronomer is only a small fraction of this

It will be said that support must come from sources such as the Department of Scientific and Industrial Research and the Royal Society While this is true—and both these bodies have given generous support to optical astronomy—such support cannot easily provide the basic essentials which the astronomer requires as much as any other scientist, such as floor area and permanent laboratory assistants

A difficulty is that support depends to a large extent on the staff/student ratio, a ratio which is almost meaningless for a postgraduate astronomy department In fact, the Oxford Observatory does teach undergraduates, although it is a largely postgraduate department, and last year the Observatory staff gave more than seventy lectures and a large amount of demonstration time to undergraduates I have no doubt that our problems could be eased by making astronomy into a final honours subject But I am convinced that an astronomer should, like any other physicist, first receive a thorough basic training in physics and mathematics, and take up astronomy seriously only after he has completed his undergraduate course

There was a time when the study of astronomy was thought of as something very remote from everyday life This may have been true at one time, but it is certainly not true now I have not tried to justify astronomical research, but I have tried to show that astrophysics and its more wealthy brother space science, are fascinating, flourishing subjects with a great future I trust that we may have the opportunity to keep Oxford in the forefront of the astronomical world

PRESIDENT FOR 1963 OF THE BRITISH ASSOCIATION

SIR ERIC ASHBY

SIR ERIC ASHBY, Master of Clare College, Cambridge, has been elected president for 1963 of the British Association for the Advancement of Science He will deliver his presidential address at the next annual meeting in Aberdeen The British Association has made an excellent choice, for Sir Eric, who was born in 1904, is a man of many parts, whose services and wise counsel have been sought not only by scientists but also by educationists and politicians He was educated at the City of London School and eventually graduated with first-class honours in the University of London, where (at the Imperial College of Science and Technology) he read botany and was awarded the Forbes Medal in 1926

Sir Eric was then appointed a demonstrator and, after three years doing plant ecological field work in the Carnegie Desert Laboratory at Tucson, he returned to his College as lecturer In 1935 he was appointed reader in botany in the University of Bristol, then in 1938 transferred to the University of Sydney as professor In 1947 he returned to Britain to occupy the important Harrison chair in the University of Manchester There he remained for only three years, for by then Sir Eric's gifts of administration and his work for education had become so widely recognized that he was called to the vice-chancellorship of The Queen's University of Belfast, where he did much for the advancement and extension of that University After nine years, it must have proved a great loss to Belfast, but gain for Cambridge, when he (in 1959) was appointed Master of Clare College, Cambridge In the field of education, Sir Eric has done much, especially towards the integration of technology into higher education Moreover, he has contributed a great deal to extra-mural adult education, and rendered signal service to the establishment and advancement of the new universities in the emergent countries

The Second World War intercepted Sir Eric's academic work in Sydney, but that was to the benefit of Australia's war effort, for he became chairman of the Australian National Research Council, and conducted a survey for the Australian Prime Minister with the object of linking science with the war effort He was the first director of the Commonwealth Scientific Liaison Bureau and spent 1945–46 in the U S S R as counsellor and, for a time, as Chargé d'Affaires in the Australian Legation in Moscow As a result of this experience, he wrote a fascinating book, *Scientist in Russia* Among his other publications is *Technology and the Academics* which has been well received in the educational world As could be expected, Sir Eric has been the recipient of many honours (he was knighted in 1956), and is much sought after as counsellor and lecturer—he gave one of the first Granada–British Association Lectures in Guildhall, London, in 1959

With so many calls on his time, and the demands that his present academic position make on him, it is clear that Sir Eric is unable to carry out practical scientific research, though he is very active in the promotion and advancement of science This is a loss to botany His former botanical research may be said to fall into three categories (1) quantitative analysis of growth (working mainly on *Lemna*), (2) applications of statistical methods to ecology, (3) quantitative investigations of morphogenesis of leaves

NEWS and VIEWS

Henry Bryant Bigelow Gold Medal for Oceanography: Dr. J. C. Swallow

The Henry Bryant Bigelow Gold Medal was established last year in honour of Prof. Bigelow, one of the founders of the Woods Hole Oceanographic Institution (U.S.A.). This year, the Medal, which is accompanied by a cash prize of 2,500 dollars, has been awarded to Dr. J. C. Swallow of the (U.K.) National Institute of Oceanography.

The Swallow Float has become the principal tool for the study of deep-water circulations. The results of these investigations have revolutionized our conceptions of the character of the deep-water motions. Instead of the sluggish, widespread drift anticipated from continuity considerations, the deep layers seem to be moving briskly and not in accordance with any known process. Here, then, is a scientific fact that challenges the imagination. No one is more aware of this than Dr. Swallow. Since 1955, when the first trials of the float were made, there has not been a year in which he has not been at sea in pursuit of further understanding of the character of the deep-layer motions. In 1955 and 1956 he worked in the eastern basin of the North Atlantic aboard *Discovery II*. In the following year he brought *Discovery II* to join *Atlantis* in a co-operative study of a deep countercurrent off South Carolina. In 1957 he worked again in the eastern basin from the Norwegian research ship *Helland-Hansen*. Next, in 1958 he worked off Gibraltar. By 1960 he had concluded a year-long investigation of the deep motions to the south of the Gulf Stream. In 1961 he took part in the International Expedition to investigate the Faroe-Shetland overflow from the Norwegian Sea. During the past winter he joined the *Erika Dan* to measure the deep currents off Greenland and Labrador.

John Crossley Swallow was born at Newmill, Yorkshire, England, on October 11, 1923. He began his education at Holme Valley School. In 1940 he was awarded a scholarship at St. John's College, Cambridge. He read mathematics and physics for two years, after which he joined the Admiralty Signal Establishment, working mainly at the East Indies Station until 1947. He then returned to Cambridge, graduated with first-class honours in physics, and then joined the Department of Geodesy and Geophysics to work on techniques for seismic prospecting at sea. He applied these techniques extensively during a round-the-world voyage of H.M.S. *Challenger* during the period 1950–52 and continued this work in the North Atlantic during 1953. He was awarded the Cambridge Ph.D. degree in 1954 for a thesis on "Seismic Investigations at Sea". He joined the National Institute of Oceanography in 1954 where, one year later, he used his first neutrally buoyant float for tracking deep-water movements.

Scottish Reactor Centre: Dr. H. W. Wilson

Dr. Henry W. Wilson has been appointed director of the Scottish Research Reactor Centre. Dr. Wilson, who is at present a senior principal scientist and a group leader in the Nuclear Physics Division of the Atomic Weapons Research Establishment at Aldermaston, where he has served in the Atomic Energy Authority since 1955, is a graduate of the University of Glasgow and a former member of the staff of the Department of Natural Philosophy, in which he served as assistant, Research Fellow and lecturer prior to 1955. His experience includes a period of several years with Imperial Chemical Industries, and during 1951–52 he was a post-doctoral Fellow in the Radiation Laboratory in the University of California, Berkeley. Dr. Wilson is a leading authority in the field of radiations from radioactive substances, and the research work of his mass spectrometry group has gained international recognition. The Reactor Centre, which is financed by a grant from the Department of Scientific and Industrial Research (see *Nature*, **194**, 338; 1962), is being set up in the grounds of the National Engineering Laboratory at East Kilbride. The Centre is the responsibility of a consortium of the four Scottish universities, the Royal College of Science and Technology, and the Queen's University of Belfast, and will be conducted under the general supervision of a committee of the principals of the participating institutions.

Geology at Hull

Dr. D. T. Donovan

Dr. D. T. Donovan, at present reader in palæontology in the University of Bristol, has been appointed as from next October to the newly founded chair of geology in the University of Hull. Shortly after graduating in 1942, Dr. Donovan was with the British forces in Palestine until he returned to the University of Bristol in 1946 to continue his interrupted researches. He joined the staff of the Department of Geology as an assistant lecturer a year later, was promoted to lecturer in 1950, and in 1962 was appointed reader in palæontology. He was awarded the degrees of Ph.D. in 1951 and D.Sc. in 1960.

Nurtured on the classical sections and exposures of the Jurassic rocks of the West Country, where he gained much from his association with Arkell and, to a lesser extent, with Spath, Dr. Donovan will doubtless direct his attention to the equally famous Mesozoic rocks of Yorkshire and adjacent counties to the south. Here, much has been accomplished and many of the problems of Jurassic stratigraphy and palæontology solved, but there still remains a rich field for research on the sedimentary environments and processes which controlled deposition. His extensive knowledge of Jurassic rocks and fossils abroad, from East Greenland to Italy, will also help to fit British Jurassic geology into a wider palæogeographical setting. Latterly, Dr. Donovan has turned his attention to marine geology and has developed the application of oblique asdic to geological mapping, as witness his recent contribution with Mr. A. H. Stride to the geology of the sea-bed off Dorset. Experience gained in the Bristol and English Channels will prove a useful introduction to the determination of the off-shore geology of Yorkshire, in particular the extension of the Jurassic rocks, and it is hoped that the obvious maritime interests of Hull and the Humber will give him the help he will need of ship's time.

Soil Science in Western Australia

Prof J Quirk

Dr James Quirk has been appointed to the newly created chair of soil science and head of the Department of Soil Science and Plant Nutrition within the Institute of Agriculture of the University of Western Australia This is the first full chair in soil science and department of this name within any Australian university Dr Quirk is aged thirty-seven and expects to take up duties early in 1963 He graduated from the University of Sydney in 1947 with honours in agricultural chemistry and obtained his Ph D in soil physics from the University of London in 1952 while working with the late Dr R K Schofield, head of the Soil Physics Department of the Rothamsted Agricultural Experimental Station For the following four years he worked with the Commonwealth Scientific and Industrial Research Organization Division of Soils in Adelaide and was appointed to his present position as reader in soil science at the Waite Agricultural Research Institute of the University of Adelaide in 1956 Dr Quirk has travelled and lectured extensively in Europe and the United States and has an impressive research record in soil physics He has specialized particularly in soil structure problems, the clay–water systems of soils and the relation of water stress to nutrient uptake by crop plants

Magnanimity

Sir Charles P Snow's rectorial address on "Magnanimity" to the University of St Andrews on April 13 has been issued as a pamphlet together with the laureation address of Prof A J McDonald in presenting Sir Charles, Prof P M S Blackett and Mikhail A Sholoklov for honorary degrees on that occasion (Pp 23 St Andrews, Fife Students' Representative Council, The University, 1962 2*s*) Sir Charles thought that Britain was in danger of losing this major virtue, and that while her society was in some respects more tolerant than the American, it was far and away less magnanimous Integrity had taken on a new meaning and no longer bore the quality of oneness encountered in characters like Einstein, Rutherford or Hardy, moral was coming to mean censorious, and there was a real danger of people forgetting what it is like to be generous to one another. This was happening at a time when the major social task—that of meeting the needs of the under-developed countries—demanded every scrap of courage and magnanimity that could be found The roots of magnanimity were complicated they included love, compassion, charity or brotherhood—whatever one called the 'binding glue' of society—that sense of reality which was also part of humour and a kind of vanity Nor did Sir Charles think we would show social or political virtue if we failed to make the most of ourselves as individuals in our human relations

The National Council for Technological Awards

The report of the National Council for Technological Awards for the period April 1961–March 1962 records 6,201 students as now following courses recognized by the Council and 1,092 diplomas have already been awarded, 620 being conferred during the period under review (Pp 51 London The National Council for Technological Awards, 1962) Of this latter total, 207 were in electrical and 158 in mechanical engineering, 70 in applied physics, 49 in production engineering and 47 in applied chemistry and chemical technology A list of 111 courses at 28 colleges which are now recognized by the Council is included in the report, and appendixes include extracts from written evidence submitted to the Robbins Committee on Higher Education During the period, 17 applications for registration as candidates for membership were received by the College of Technologists, making a total of 51 since the College, in November 1959, issued its memorandum outlining the method of application, and of these 40 have been accepted and a further 4 are under consideration

Political and Economic Planning

The annual report 1961–62 of Political and Economic Planning covers the year ended March 31, 1962, in which the sales of reports were still at the second highest figure in PEP's history, although below the level of 1960–61 The six projects in the present research programme may be grouped under two broad themes (1) change and adaptation to change in economic and social policy, including Britain and the European Common Market, attitudes in British industry, Trade Unions in a changing society, and education and training programmes for developing countries, (2) social policy and family life, including community mental health services and prisoners' families The first project was started in September 1958 and will continue for a further three years from November 1961; a report on Atlantic Tariff and Trade is in the press and an examination of the consequences for industry of setting up the Common Market has commenced Three principal inquiries are being made into community mental health services, covering the collection of data on turnover and interchange of mental patients, on interview survey of psychiatric patients living in the community and of members of their families, and a survey of social work in mental health The three-year project on prisoners and their families is in two main parts an interview sample on a national basis, and a more intensive study of up to a hundred prisoners' families in the London area The study of education and training programmes for developing areas now envisaged is in four parts This comprises compilation of an inventory and analysis of training facilities in the United Kingdom for overseas students wishing to qualify as technicians, technologists, scientists or professional workers, an examination of the types of social facilities available to students, a review of problems which may deter members of the staff of United Kingdom teaching institutions from taking up overseas posts, and a study on the spot in a selected developing area of the provisions for higher technical and scientific training in relation to the level of development of the area and to existing attitudes to change

International Antarctic Analysis Centre

The southern hemisphere is an empty waste, meteorologically speaking The proportion of land mass is comparatively small while the water areas are infrequently traversed by ships The lack of data greatly restricts research Yet the geography of this hemisphere closely approximates the ideal general circulation models which are studied in the laboratory, on the charting bench and by the theoreticians The International Antarctic Analysis Centre was established in Melbourne by the Commonwealth Bureau of Meteorology in 1959 following on a recommendation of the Special Committee for Antarctic Research at its Moscow meeting in 1958. It has now been operating for three years and is staffed by five professional meteorologists from Australia, Argentina, France and the United States, constituting a truly international team The programme of work undertakes to analyse circumpolar 700, 500 and 300 mb constant pressure charts over the area south of 60° S at 00 and 12 G M T daily At 00 G M T the analyses are extended northwards to about 30° S The charts are later re-analysed after the entry of new synoptic data The final product is microfilmed. A library of data is thus being built for future research workers engaged in studies of the meteorology of the southern hemisphere and of the global atmospheric circulation The hope has been expressed that the International Antarctic Analysis Centre may itself soon partake in such research

The Pengelly Cave Research Centre

The Pengelly Cave Research Centre was founded on March 31, 1962, at a joint meeting of the Society for the Promotion of Nature Reserves, the Devon Naturalists' Trust and the Devon Spelæological Society (*News for Naturalists,* 4, No 1, July 1962) The headquarters of this new Centre, which has been named after William Pengelly, the pioneer nineteenth-century excavator of Kent's Cavern, are at Higher Kiln Quarry, Buckfastleigh, purchased in October, 1961, by the Society for the Promotion of Nature Reserves, and is to be developed for the furtherance of cave research in Britain The site is already well known to cavers and naturalists as containing the entrances to Reed's and Joint Mitnor Caves The acquisition of Higher Kiln Quarry by a scientific organization provides an opportunity, for the first time, of developing a much-needed cave research and demonstration centre in Britain The site is exceptionally well suited for this purpose, for not only is it easily accessible, but it also contains a variety of features, usually available only over a distance of several miles

At the present time Britain lags behind countries such as France (which already has a well-equipped underground laboratory at Moulis, in the Pyrenees), Belgium, Yugoslavia, the United States and Cuba in the facilities for cavern research which it has to offer to spelæologists There is no means whereby the naturalist or student who is not a spelæologist can obtain first-hand experience of the natural history of caves. Moreover, the great increase in the popularity of caving as a sport in Great Britain presents a serious threat to the preservation of much of scientific importance that the British caves contain unless steps are taken to educate cavers One of the objects of the Centre will be the development of conservation-mindedness

National Parks of Canada

Two of Canada's National Parks are discussed in booklets issued by the Geological Survey of Canada (Canada Department of Mines and Technical Surveys Geological Survey of Canada *Miscellaneous Report No 4 Yoho National Park—the Mountains, the Rocks, the Scenery* Pp vi+107 1 dollar. No 5. *Cape Breton Highlands National Park—where the Mountains Meet the Sea* By David M Baird. Pp vi+65 75 cents Ottawa Director, Geological Survey of Canada, 1962) Both have been prepared by David B Baird, who has taken some outstanding photographs to add to the attraction of the booklets The text has been prepared with such commendable skill that either booklet would serve as an introduction to petrology as well as a helpful guide to some of Canada's outstanding scenery

Biology and Human Affairs

A novel approach to food production and storage in plants is described in an outstanding editorial comment in *Biology and Human Affairs* (27, No 3, Summer 1962) Among the other articles is one on crop plants by N W Simmonds, an account of his work on the ecology of ants by J W Siddorn, the place of mathematics in biology by M J R Heeley, and various aspects of human learning and understanding by Dr E C Poulton The journal is published by the British Social Biology Council, 69 Eccleston Square, London, S W 1

Space Science Reviews

The first issue of a new international quarterly journal *Space Science Reviews* recently appeared (1, No 1, June, 1962. Edited by C de Jager Pp 154 Published quarterly (Dordrecht D Reidel Publishing Company, 1962) Subscription price 150 florins, 42 dollars per volume of four issues) The editor-in-chief, C de Jager, of the University of Utrecht, explains in the introduction that the journal will not include original research papers, but will consist of invited review articles on particular aspects of space science and technology. Each issue will run to about 180 pages, containing between 5 and 8 contributions Space research, as it expands, is covering an ever-broadening area of science, and the journal should meet the widely acknowledged need for authoritative and up-to-date reviews The first issue has 6 excellent contributions First, H Wexler discusses the results obtained from the *Tiros* weather-satellites Then follow four articles on strong solar disturbances and their effects H Carmichael describes the high-energy solar-particle events of 1959–61, E N Parker and T Gold (in separate articles) discuss the mechanism of geomagnetic storms; D C. Rose and S. Ziauddin consider the polar cap absorption effect Finally, T M Donahue reviews the possible sources of the Lyman-α radiation observed in the night sky

Radioactivity in Drinking Water

Investigations by the Government Chemist show there was no appreciable increase or decrease in amounts of radiostrontium and radiocæsium in United Kingdom drinking water during 1961 (Ministry of Housing and Local Government; Scottish Development Department, Ministry of Health and Local Government, Northern Ireland *Radiostrontium and Radiocaesium in Drinking Water in the United Kingdom—1961 Results* Pp 19 London. H M S O, 1962 1*s* 6*d* net) The investigations do not cover samples taken in 1962, but the evidence so far available indicates that the Russian nuclear tests in the autumn of 1961 are unlikely to have caused higher levels of strontium-90 in drinking water this year than during 1959 In 1961 drinking water contributed less than 5 per cent of the total amount of strontium-90 in the average diet, the amount of cæsium-137 in drinking water was about half the quantity of strontium-90 Details of these and other radioactive substances in diet are given in periodic reports by the Agricultural Research Council's Radiobiological Laboratory, the most recent of which was published in May, 1962 This report on drinking water is the fourth on this subject in the Government's monitoring programme, and states that well water remains virtually free from radioactivity Impounded waters generally have higher levels than river water

U S Committee on Extension to the Standard Atmosphere

THE co-chairmen of the above U S national effort, described in *Nature* of July 14, p 133, are Mr Maurice Dubin, National Aeronautics and Space Administration, Mr Norman Sissenwine, Geophysics Research Directorate, Air Force Cambridge Research Laboratories, and Dr Harry Wexler, U S Weather Bureau

University and College News

Birmingham

THE College of Advanced Technology, Birmingham, was transferred to an independent Board of Governors as from April 1, 1962, and the latest report of the principal, Dr P F R Venables, covers the period September 1960–March 1962 (Pp 40 Birmingham College of Advanced Technology, 1962) During this period 220 students were admitted to the Associateship at the Presentation Ceremony in 1961. and 264 Associates were admitted in March 1962 Grants for research were received in the form of the Wilmot Breeden and the Lucas Research Fellowships and a Ministry of Aviation Research Fellowship. A Research Fellow has been appointed in the Department of Industrial Administration for a research project to investigate end-on arrangements in the development of sandwich courses, which was established with a grant of £6,700 from the Nuffield Foundation One senior Research Fellow and two research assistants were appointed to a research unit established with a grant of £28,580 from the Department of Scientific and Industrial Research to investigate social factors affecting relations and individual performance in factories Of 5,359 students in the session 1960–61, 1,264 were full time, 2,072 in part-time day courses and 2,023 in evening courses only, for the past session 1961–62 the corresponding figures were 1,386, 1,605 and 1,991 Enrolments in sandwich courses totalled 838, 282 being in mechanical and production engineering and 250 in electrical engineering In 1961–62 the total increased to 956

Cambridge

THE following election to fellowships (Title *A*) at Churchill College have been announced Dr J D C McConnell, of Fitzwilliam House (University lecturer in mineralogy, College lecturer in mineralogy), F H McClintock, of Trinity College (assistant director of research in criminology, College lecturer and director of studies in law), A A Sinclair (College lecturer and director of studies in history)

Oxford

PROF. R PEIERLS, professor of mathematical physics in the University of Birmingham, has been appointed to the Wykeham professorship in physics with effect from October 1 Dr V Zussman has been elected to the readership in mineralogy and will take up his appointment in the academic year 1962–63 The following appointments to lectureships have been announced Dr D A Edwards, A W Ingleton, J D Murray (mathematics), F O MacCallum (virology), D J Roaf (theoretical physics), Dr W E Parry (physics)

Announcements

AIR COMMODORE B Samson, has been appointed director, Aircraft Mechanical Engineering Equipment Research and Development, with effect from August 13 Previously he was senior technical staff officer with Near East Airforce

A SYMPOSIUM on "Fungi and their Metabolites" will be held by the Plant Phenolics Group and the British Mycological Society at the School of Pharmacy, Brunswick Square, London, W C 1 during October 1–2 Further information can be obtained from Dr J G Manners, Department of Botany, the University, Southampton

THE Institute of Biology is organizing a symposium on "Colour and Life" to be held at the Harkness Hall, Birkbeck College, during September 20–21 The symposium is open to members and non-members of the Institute, for the latter, however, there will be a fee of 21*s* Further information can be obtained from the General Secretary, Institute of Biology, 41 Queen's Gate, London, S W 7

A SYMPOSIUM, organized by the Nutrition Society, will be held on "Assessment of Nutritional Status in Man" at the Lecture Theatre, Chemistry Building, Meston Walk, Old Aberdeen, on September 29 Further information can be obtained from the honorary secretary of the Scottish Group of the Nutrition Society, Dr D G Armstrong, the Hannah Dairy Research Institute, Ayr, Scotland

A CONFERENCE of European comparative endocrinologists will be held in London at the Rooms of the Zoological Society during September 20–22 Further information can be obtained from the convenors, Prof H Heller, Department of Pharmacology and Practical Pharmacy, the University, Bristol 8, and Prof E J W Barrington, Department of Zoology, the University, Nottingham.

THE Third International Machine Tool Design and Research Conference will be held at the Department of Mechanical Engineering, University of Birmingham, during September 24–28 Further information can be obtained from the Organizing Secretary of the Third International Machine Tool Design and Research Conference Department of Mechanical Engineering, the University, Edgbaston, Birmingham 15

THE Faraday Society is organizing a general discussion on "High Resolution Nuclear Magnetic Resonance" at the University of Oxford during September 17–19 The discussion will be divided into (1) nuclear magnetic resonance in diamagnetic materials, (2) nuclear magnetic resonance in paramagnetic materials, (3) applications to the determination of molecular structure; (4) applications to the study of kinetic processes Further information can be obtained from the Faraday Society, 6 Gray's Inn Square, London, W C 1

THOMAS GRAHAM AND THE DEFINITION OF COLLOIDS

By Prof S G MOKRUSHIN

Urals Polytechnical Institute, Sverdlovsk, U S S R

At present we have two definitions of colloids The 'founder of colloid chemistry', Thomas Graham[1], one hundred years ago, defined the colloids as substances of complicated chemical nature with very high molecular weight and a specific structure of molecules Owing to this fact the molecules of colloids possess the properties different from those of crystalline bodies or crystalloids But Graham emphasized that the process of solution of colloids, that is, the formation of solutions and the phenomena which accompany them, differ from those of crystalloids only in degree The solution of colloids is very slow and the change of temperature is barely perceptible Graham defined the colloidal state as a dynamical state of matter which may change into a crystalloidal statical condition The colloidal state of matter possesses *energia*, and Graham suggested that the phenomena associated with life in those bodies which consisted almost wholly of colloids is due to that *energia*

The world of colloids is particularly the world of organic high-molecular substances, however, Graham showed that inorganic substances composed of molecules of high molecular weight and complicated structure can be also obtained in colloidal state The solutions of colloids are real solutions and they obey the laws of physical chemistry and thermodynamics According to Graham the colloidal state of substances is determined by the high molecular weight

In his papers Graham does not mention the investigations of Faraday[2] on the finest suspensions of metallic gold, nor the works of Selmi[3] on pseudo-solutions The finest suspensions of gold and pseudo-solutions of Selmi are not real solutions but micro-heterogeneous systems Graham, in agreement with his conception of colloids, never assumed that the finest suspensions of gold, silver chloride, sulphur and other similar systems are colloidal solutions

But about half a century after the establishment of colloid chemistry by Graham, a new definition of colloids was proposed by Freundlich, Wo Ostwald and Weimarn[4] which did not conform with that given by Th Graham These scientists proposed to call any substance which is in the dispersed state 'colloidal' The molecular weight of the dispersed substances had no relation to the colloidal state by that definition The finest suspensions or emulsions of dispersed substances in a medium were colloidal solutions according to them

Graham would not have considered the solutions of Freundlich, Ostwald and Weimarn as real solutions but as microheterogeneous (dispersed) systems This second definition of colloids was accepted by most scientists and the finest suspension of gold was considered as a typical colloid[5]

Meanwhile, it is necessary to recall that Graham chose gelatin as the prototype of colloid[6]

The difference between the points of views of Graham and the later three authors as to the question of what the typical colloid is, is evident

Those who supported the dispersoid conception as an explanation of the behaviour of Graham's colloids (high-molecular substances) in solutions, ascribed much greater hydration to them than could be proved by experiment[7] Since Staudinger[8] introduced the conception of 'macromolecule', some scientists[9], fortunately only very few, proposed not to consider the colloids of Graham as colloids, but to refer to them only as high-polymers because these substances made real solutions but by no means microheterogeneous (dispersed) systems

However, it will be noted again that Graham always considered the colloidal solutions as real solutions and never as the pseudo-solutions or microheterogeneous systems But both these definitions of colloids may be reconciled if we assume, with Perrin[10], the definition of 'gigantic physical molecule', which in its physical and thermodynamic behaviour has no distinction from the behaviour of chemical molecules Therefore we may regard the finest suspensions of those particles which show Brownian movement and obey the laws of kinetic theory of heat as real solutions Einstein[11], Perrin[10], Svedberg[12] and Smoluchowski[13] proved this conception to be true by theoretical and experimental investigations

Thus we may conclude that modern colloid chemistry is the physical chemistry of gigantic physical and chemical molecules

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[1] Graham, Th , *Phil Trans Roy Soc* , **151**, 183, 184, 206, 207, 220 221 (1861)

[2] Faraday, M , *Phil Trans Roy Soc* , 145 (1857)

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UNIVERSITY ENTRANCE REQUIREMENTS IN ENGLAND AND WALES

In March 1960 the Committee of Vice-Chancellors and Principals set up a sub-committee, including a substantial proportion of university teachers, to report on university entrance requirements, and the preliminary report of this sub-committee in March 1961 satisfied the Vice-Chancellors and Principals that the time was opportune for a careful reconsideration of entrance requirements by the universities When the draft report was circulated as a confidential document to the universities for comment, there was an almost uniformly favourable response to the proposal for some alignment in form and approximation in substance of present entrance requirements The Committee of Vice-Chancellors and Principals has accordingly asked universities forthwith to restate their present requirements in a common form, and at the same time, where possible, to bring them closer into line when they differed in substance

This restatement is now being carried out, with a view of publication in a combined volume before the end of this year A second main proposal of the draft report*, a new pattern of entrance requirements, was put forward as an educational reform, and after careful examination, Cambridge, London and Reading expressed opposition in principle. Oxford has not yet given any opinion, but the remaining universities approved or welcomed the idea of the proposal, and comments offered were directed to modifications of detail The Committee of Vice-Chancellors and Principals judged, therefore, that there was sufficient university support to justify the wider discussion of the proposal, and its publication was authorized in this report after redrafting to meet most of the points raised in the detailed comments

The report opens with a brief historical review of the development of university entrance requirements in England and Wales in their present form over the past sixty years or so, and this is followed by an analysis of the present difficulties arising from variations and ambiguities in the method of statement of entrance requirements by universities, variations in the entrance requirements themselves, whether in subjects named or in the numbers of subjects in which passes are required, or from the weakness of the general pattern of entrance requirements

The first proposal, already being implemented, is that all universities in England and Wales should publish their examination requirements for entry on first-degree courses in a common form, using standard terms. In this common form the terms 'matriculation requirement' and 'minimum entrance requirement' are replaced by the term 'general requirement', to denote the group of required passes at both levels in the General Certificate of Education, examination equivalent to what is now demanded for Matriculation Similarly, the term 'course requirement' is proposed in place of 'faculty requirement' and 'department requirement' to denote the group of required passes at both levels in the General Certificate of Education examination equivalent to what is now asked for entry into specified faculties or departments, and this Course requirement should be stated in terms of numbers and names of subjects in which 'normally' a candidate should have passed, to hope to be academically acceptable to the department or university concerned As already indicated, the proposal contemplates that each university will print and publish its entrance requirements for first-degree courses in single joint annual volume

The second proposal is more far-reaching and radical, and the Committee believes that adoption of this proposal would not only automatically remove some of the formidable difficulties for candidates which now arise from the interlocking of the minimum entrance requirements and the faculty and department requirements, in terms of General Certificate of Education subjects at both levels, but by placing more weight than hitherto on general studies than special studies in the sixth form, could permit an educational improvement of considerable benefit both to universities and to schools The present balance between general and special studies is the result of piecemeal adjustments made over a long period as conditions in the schools and universities have slowly changed and as the structure of public examinations has been altered Tacitly abandoning their former requirement of a broad intellectual training alone as a condition of entry, the universities have substituted for it a primary demand for competence in subjects of special study, and evidence of general education has become a secondary requirement, the test for which is made at the beginning of the sixth-form course The essential new feature of the proposed new pattern is that it recognizes the importance of general studies in the sixth form by making them examinable, just as are the subjects of special study

The essence of the proposed pattern is to replace the Ordinary Level papers at the end of the fifth-form year by new papers in the second sixth-form year as the university test of a candidate's general education, although it is assumed that most boys and girls will continue to take and to pass the same range of Ordinary Level subjects as they now commonly do before entering the sixth form Under it the entrance requirements would be in two parts, a general and a course requirement, each of which must be fulfilled The latter would be confined to passes in the General Certificate of Education examination, and including at least two Advanced Level passes. The General requirement would consist of required passes in the following new papers, taken not earlier than January in the candidate's second year in the sixth form a paper in the use of English; a general paper, and a paper in the use of foreign language The first would be designed to encourage the serious study of the use of English in the sixth form, the second should be set on the assumption that at an earlier stage candidates will have passed at Ordinary Level in either mathematics or a science subject and also in two or three of the usual arts subjects

The proposals leave room for variation between universities both in the general requirement and in course requirements, and if the new pattern is adopted a transitional period will be necessary during which universities will allow candidates to fulfil requirements either of the existing pattern or the new The timing suggested for the examination for this new general requirement, however, would reinforce the efforts already being made by the Secondary School Examinations Council and other bodies to reduce the excessive weight of Advanced Level syllabuses Moreover, through the firm claim established on a reasonable proportion of sixth-form time for general studies it should afford a wholesome check to excessive specialization and influence those who prescribe syllabuses and the examiners who deploy them to do so in a realistic manner

The note of sanity and realism in these proposals should be widely welcomed in the world of science While they will do nothing to reduce the competition for university places, they should offer a fair prospect of checking premature specialization than earlier measures that have been tried in individual universities, and, particularly by fostering the study of English in the sixth form, assist to remove one weakness in the scientist and technologist that has been a wide source of complaint and criticism The fact that most universities are already known to agree with the proposals is promising, but it is to be hoped that those who have expressed opposition in principle will not fail to come into line Simultaneously, with the re-examination of syllabus in the science subjects themselves, it offers an opportunity of improvement that is unlikely to recur but which if seized with vision and determination could transform the whole training of the science graduate Any university should hesitate before raising obstacles or imposing requirements which could cause the opportunity to be missed.

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* Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom Report of a Sub-Committee on University Entrance Requirements in England and Wales Pp 23 (London Association of Universities of the British Commonwealth, 1962) 2s. 6d

THE MELLON INSTITUTE

THE annual report of the Mellon Institute for the year ended February 28, 1962*, records expenditure of 5 9 million dollars on independent and sponsored research At the end of the year the Institute's staff was 561, and 362 professional and technical members were reporting on some 100 investigations A list of publications of members during 1961 other than patents or periodicals or reports that do not appear in the open literature is included Eighteen appointments at the level of Fellow were made during the year, half of whom joined Independent and half Sponsored Research The Research Services of the Institute in its Instruments Section placed emphasis on systems which completely programme experimental procedures and increase the amount and accuracy of the information recorded An instrument was constructed for the automatic recording of additives during redox measurements, a low-temperature (40° K to ambient) controller designed and built for the magnetic susceptibility programme and a circuit design of the instrument and servo system completed for a 'hot hardness tester' In the Physicochemical Section the study of adsorbed organic molecules on catalysis by infra-red spectroscopy was extended, and a study initiated to find a correlation, if any, between the presence of trace elements and the occurrence of cataracts of the eye The Microbiology and Microscopy Section completed a study of microbiological sludge inhibitors for jet fuels

Under independent research, the report records continuing work on the detailed characteristics and distribution of plasma and red-cell hydrolytic enzymes, with special emphasis on the enzymes of the circulating blood which attack the hypotensive polypeptides, bradykinin and kallidin Investigations are being continued on the interrelation between metabolites of *p*-aminobenzoic acid and aniline hydroxylation by resting cells of acid-fast bacteria, and completion of an electron microscopical study of ribonucleic acid from tobacco mosaic virus is reported. In organic chemistry, besides investigations of the acylamidines, the rotational isomerism in mono- and di-alkylpropenes of the types $R\,CH_2\,CH=CH_2$ and $R_2CH.CH=CH_2$ has been investigated by nuclear magnetic resonance spectroscopy, and a five-step synthesis developed of 1 . 2 3-triphenyl-4 5-benzpentalene Efforts are being directed to develop general routes for the synthesis of branched polymers of the star type and of the comb type, and the stereochemical structures of polymers prepared by free-radical polymerization of methyl methacrylate have been computed from high-resolution nuclear magnetic resonance spectra measured at room temperature in chloroform solutions Preliminary experiments have been made on the effects of heterogeneity in chain length, in chain branching, and of diluent on the relation between molecular weight and flow of high polymers in bulk or in concentrated solution The basic studies in co-ordination chemistry led to the discovery of several unusual complex compounds, including a univalent iridium compound which reacts at normal conditions with a number of molecules commonly regarded as relatively inert In physical chemistry, low-frequency infra-red spectroscopy and the vibrational spectra of inorganic substances have been particularly fruitful fields, and in radiation chemistry the electron-spin-resonance studies of radicals have been extended to a variety of hydrocarbons Photochemical investigations largely focused on such systems as phosgene–olefine mixtures, 1 · 2-dichloroethylene and ethyl and vinyl iodides Work continued on a mathematical theory of fluid behaviour within which many fluid–flow problems can be solved, and attempts are being made to find exact solutions for the steady-state Schrödinger equation for systems of two or more electrons in the potential field existing in an arbitrary molecular framework

In metal physics, low-temperature calorimetry studies are in progress to obtain more reliable data for the copper–zinc and related binary systems The study of stacking-fault probabilities in noble metal alloys has been extended to the silver–cadmium system, where a linear relation between the faulting probability and composition has been observed over compositions containing 0–30 atomic percentage of cadmium

A group of investigations concerned with public welfare range from air purification, the processes involved in the deterioration of artists' materials, toxicity of new organic chemicals, industrial hygiene, mine acid control, steel protection and water resources Notes are also included on progress in projects concerned with the development of solid catalysts from bone products for organic reactions, ceramic chemicals, aspects of forming and treating structural clay products and performance of plant scale equipment, starch-based chemicals as textile warp sizes, the performance of industrial fabrics from felt, ferrous alloys, a hydrogenation method for determining low concentrations of organic sulphur in natural gas, the chemistry of petroleum genesis; the fundamental nature of the activity of catalysts; studies of metal surfaces in hydrogenation, silicones; the synthesis of phosphonitriles; and the degree of susceptibility of selected high-strength case steels for rocket motors and other alloys to stress-corrosion cracking

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* Mellon Institute Annual Report, 1961 Pp 36 (Pittsburgh, Pa Mellon Institute, 1962)

HYDROLOGY OF RIVER FLOOD-PLAINS

By R C WARD

Department of Geography, University of Hull

TO the agriculturist, the flood-plains of rivers have, for a long time, been some of the most interesting hydrological areas of a drainage basin In past centuries these low-lying valley bottom lands were used as flood meadows which were inundated each year In this way the topsoil was enriched by the deposition of silt from the flood waters and a reserve of soil moisture was built up to assist grass growth in subsequent periods of deficiency in rainfall To-day, the old sluices have been replaced by the aluminium pipes and sprinklers of overhead irrigation which can often be carried out most economically in valley meadows owing to the proximity of a readily available source of water in the nearby stream or river As a result of the particular hydrological conditions present in a flood-plain, however, this often abundant source of water can be potentially dangerous The aim of this article is to examine some of these hydrological conditions and to assess their relevance to irrigation practices

The flood-plain in relation to the drainage basin Fig 1 shows the relationship of a flood-plain to the hydrology of a hypothetical drainage basin It is at once evident that there is a direct contrast between the zones of ground-water present in the flood-plain and those present in the valley flanks and terraces In the latter, four zones are represented, that is, the soil zone, the intermediate zone, the capillary fringe, and the zone of saturation below the water-table

The soil zone, limited as its name implies by the depth of soil above the bed rock, contains water which has infiltrated through the surface and which is held by adhesion to the surfaces of soil particles and in interstices between grains and rock fragments Water moves out of the soil zone, either by means of evaporation and transpiration into the atmosphere or when the soil is holding all the water it can against the force of gravity (that is, when it has reached field capacity), by means of downward percolation towards the water-table Immediately above the saturated zone is the capillary fringe, where water is held against the force of gravity by means of capillary action, and between this and the soil zone is the intermediate belt, where water is held by adhesion to particle surfaces Finally, below the water-table is the zone of saturation, where all the cracks and interstices are filled by ground-water

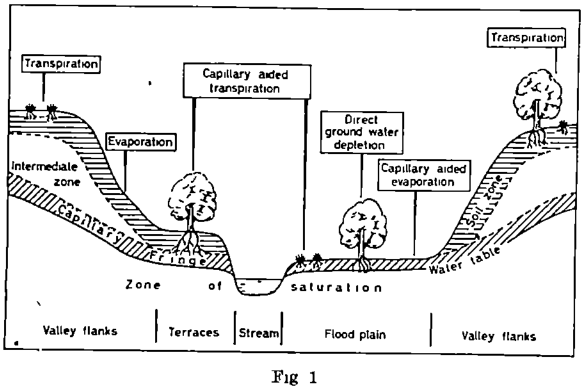

Fig 1

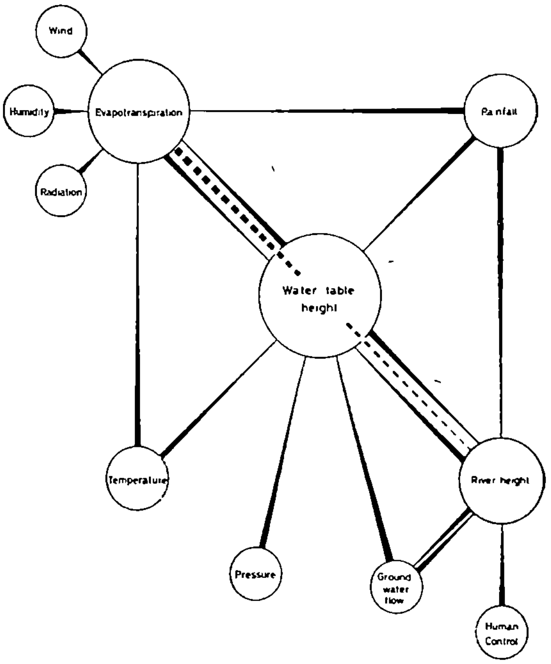

Fig 2

A different situation, however, exists in the flood-plain areas of the drainage basin, where the capillary fringe may extend either into the soil zone or even to the ground-surface itself, depending on the distance between the water-table and the ground-surface and on the height of the capillary fringe Since the water-table is often less than 5 ft below the ground-surface, except in the late summer, it is apparent that, for the greater part of the year, the vegetation of the flood-plain is able to draw on much larger supplies of soil moisture than is the vegetation on the valley flanks and terraces Thus, relatively speaking, losses of water by evapotranspiration will tend to be far higher from the flood-plain than from other areas of the drainage basin Penman[1] emphasized this duality of catchment areas, and pointed out the contrast between the flood-plain, where the high water-table enables the ground-water to contribute to evapotranspiration losses and the higher land, where the soil moisture deficit increases through the summer and ends only when rainfall eliminates it " . from the top downwards"

Although, however, this duality can be recognized in theory, it is virtually impossible to assess quantitatively the individual contribution of either the flood-plain or the higher land to the water-balance of the catchment area as a whole This is due to the difficulty of estimating not only the losses of water from the higher areas, where soil moisture is is not often maintained at a level sufficient to enable evapotranspiration to take place at the potential rate but also the considerable quantities of water which move from the valley flanks to the flood-plain and down the valley through the flood-plain deposits.

Water relationships of the flood-plain. Despite the difficulties of determining exactly the hydrological relationship of the valley bottoms to the remainder of the drainage basin, it is, nevertheless, possible to outline in greater detail the water relationships of the flood-plain areas.

Fig. 2 shows diagrammatically the relationships and interactions between the various factors concerned in the hydrology of a flood-plain. The height of the water-table has been placed in the centre of the 'web' because, alone, it is capable of reflecting the balance of forces exerted by all the other factors. Fluctuations in the height of the water-table are mostly caused by rainfall, evapotranspiration and variations in river-level.

Rainfall, in fact, always exerts some influence on the height of the water-table in any part of a drainage basin since ground-water itself is ultimately derived from precipitation. Its direct effects are of most relevance to the present discussion, however, and can be most striking. For example, if the moisture content of a heavy soil is already high, a rainfall of 1 in. can easily result in a rise of the water-table by some 6–7 in. where, as in a flood-plain, the water-table is already close to the ground-surface. The main influence of evapotranspiration is normally on the moisture content of the soil but again, owing to the height of the water-table in a flood-plain, water lost by evapotranspiration is often extracted directly from the ground-water, resulting in a fall in the level of the water-table. The influence of a river on the fluctuations of the water-table in an adjacent flood-plain will largely depend on the composition of the flood-plain deposits. If these are coarse and gravelly, ground-water movement between the river and the flood-plain will be rapid and, consequently, variations in river-level will tend to be reflected in similar movements of the water-table. On the other hand, if the flood-plain deposits are fine and silty, variations in the river-level are not likely to be transmitted to the adjacent water-table except in a very modified form.

Apart from these major influences exerted by rainfall, evapotranspiration, and river level, water-table height can also be affected by other factors. For example, a rise in barometric pressure may, especially if the soil moisture content is high, cause a decline in water-table height. Furthermore, since the water-table is the upper surface of the zone of saturation, it follows that variations in ground-water flow will also be reflected in the height of the water-table.

However, changes in water-table height can themselves exert a considerable influence both on the amount of water lost by evapotranspiration since the smaller the distance between the ground-surface and the water-table the greater is the likelihood of ground-water extraction by plant roots and also on river-height, because when the water-table is higher than the river, ground-water flow into the river will tend to occur.

Finally, Fig. 2 indicates that both evapotranspiration and the height of the river are influenced by other factors. The former, for example, is governed principally by climatic factors such as temperature, solar radiation, humidity, wind-speed and rainfall, while variations in river-level are influenced by rainfall, by the amount of evaporation which takes place from the water surface, by ground-water flow through the flood-plain and by human control such as locks, weirs and sluices.

The relationship of a flood-plain to the complete drainage basin and, more particularly, the complex hydrological inter-relationships within the flood-plain itself, severely complicate any assessment of the quantity of water available for the needs of plants. This, in turn, means that the irrigation requirements of river meadows must be very carefully judged. The normal estimations, based solely on climatic and soil moisture data, are, in these conditions, insufficient since a rapid rise in water-table level can bring abundant supplies of moisture within reach of the plant roots. Thus, all the factors which can affect the height of the water-table should properly be taken into account when the water 'balance sheet' is drawn up, in order to prevent the possibly harmful results of over-irrigation.

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ULTRASONIC RELAXATION IN A MICELLAR SYSTEM: *n*-OCTYLAMINE–WATER

By J. F. McKELLAR* and J. H. ANDREAE†

Akers Research Laboratories, Imperial Chemical Industries, Ltd., Welwyn, Herts

In order to extend our investigation of hydrogen bonding in aqueous solutions of simple amines[1,5] to micellar systems, we have made ultrasonic absorption measurements on the system *n*-octylamine–water. The phase diagram[2] for this system shows, at 20° C, two regions of isotropic solution at low (0.00–0.03 mole fraction) and high (0.40–1.0 mole fraction) amine concentrations, suitable for sound absorption measurements. No attempt was made to make measurements in the mesomorphic and two-phase regions at intermediate concentrations because of the possibility of sound scattering.

We have found a correlation between sound absorption and micelle formation which is the subject of this article.

The ultrasonic techniques have been described[1,3]. The sound absorption measurements were made at frequencies between 1.5 Mc/s and 230 Mc/s, and the sound velocity was measured at 2 Mc/s.

As with the primary and secondary amines previously examined[1] the measurements of α/f^2 for *n*-octylamine–water in both isotropic regions could be described by the relation for a single relaxation,

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* Now at Imperial Chemical Industries, Ltd., Dyestuffs Division, Manchester 9.

† Now at Standard Telecommunications Laboratories, Ltd., London Road, Harlow, Essex.

$$\frac{\alpha}{f^2} = \frac{A}{1 + (f/f_c)^2} + B \qquad (1)$$

where A and B are constants and f_c is the relaxation frequency. Values of μ_{max}, the maximum absorption per wave-length defined by

$$\mu_{max} = \tfrac{1}{2} A\, f_c\, C \qquad (2)$$

were calculated. C is the sound velocity.

The main feature which emerges from the measurements on *n*-octylamine–water is the anomalous variation of μ_{max} at low amine concentrations, when it is compared with that found with the shorter chain amines[1]. It is seen from Fig. 1 that μ_{max} for *n*-octylamine exhibits only a very gradual increase with concentration of amine up to the phase boundary at 0.03 mole fraction. The variation of μ_{max} for *n*-amylamine at 20° C is included for comparison.

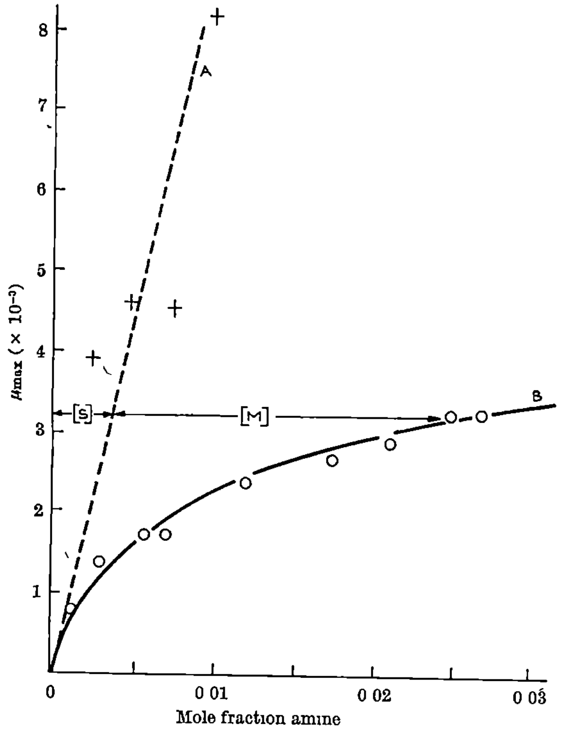

Fig. 1. Variation of μ_{max} with concentration. O, *n*-octylamine (20° C); +, *n*-amylamine (20° C)

Other properties also distinguish the foregoing two systems in this concentration range. The variation in shear viscosity is shown in Fig. 2. It is seen that an abrupt change in the viscosity concentration curve occurs at about 0.007 mole fraction for *n*-octylamine. This suggests that aggregation of *n*-octylamine into micelles occurs in this concentration region and that perhaps it is this property of the system which predominantly influences the variation of μ_{max} in Fig. 1. The viscosity behaviour for *n*-amylamine is similar to that found with diethylamine–water[4].

We shall consider that the mechanism responsible for the relaxation in *n*-octylamine–water is the same as that attributed to the higher frequency relaxation found with the shorter-chain amines (perturbation of an equilibrium involving the hydrogen bonding of unlike molecules[1,4]), and that the consequent sound absorption is due only to unaggregated molecules, as can be assumed for the shorter-chain amines. For the mechanism of hydrogen bonding between unlike molecules, the relaxation frequency f_c is determined by k_f and k_b, the rate constants of the forward and backward reactions[5]. For *n*-octylamine–water containing both unaggregated and aggregated amine (considering that the polar groups are orientated towards the water structure) it is likely that both these species will form hydrogen bonds with the water and give rise to sound absorption. It may be, however, that the rates at which these reactions occur will differ for the two species. This could lead, if the difference were significant, to two distinct relaxational processes, one being due to the association of water and aggregated amine and the other to water and unaggregated amine. This is illustrated in Fig. 3, $\mu_{max}(M)$ and $\mu_{max}(S)$ denote the absorption for the aggregated and unaggregated amine and similarly $f_c(M)$ and $f_c(S)$ denote the respective relaxation frequencies.

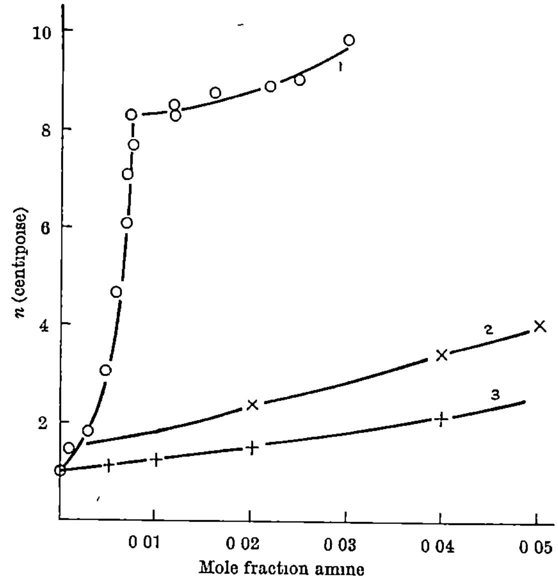

Fig. 2. Variation of shear viscosity with concentration. (1) *n*-octylamine (20° C); (2) *n*-amylamine (5° C); (3) *n*-amylamine (20° C)

From Fig. 1 it is seen that this hypothesis can be examined in a more quantitative manner. If we consider that the sound absorption indicates the concentration of amine, and that to a first approximation the absorption is the same for *n*-amylamine and unaggregated *n*-octylamine (indicated by the broken

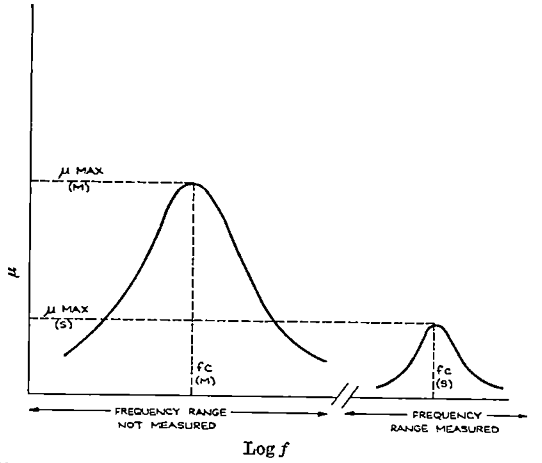

Fig. 3. Sound absorption per wave-length for two relaxing processes

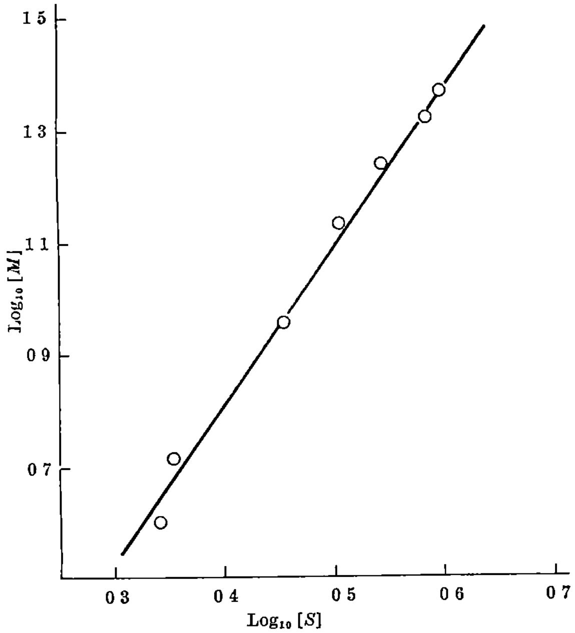

Fig 4 Plot of $\log_{10} [M]$ against $\log_{10} [S]$ for *n*-octylamine–water Temp = 20° C

line *OA*) we see that at a mole fraction of 0 025 the concentration of unaggregated and aggregated *n*-octylamine is given by [*S*] and [*M*] respectively Taking the aggregation reaction as

$$mRNH_2 \rightleftharpoons (RNH_2)_m \qquad (3)$$

where m = number of single molecules per micelle, and equating activities to concentrations, we obtain from the simple law of mass action for un-ionized molecules aggregating to uncharged micelles

$$K = \frac{[M]}{m[S]^m} \qquad (4)$$

In logarithmic form (4) gives

$$\log [M] = m \log [S] + \log [K m] \qquad (5)$$

A plot of log [*M*] against log [*S*] for the mole fraction-range 0 006–0 0275 is given in Fig 4 A straight line is obtained with $m = 3$ (approx) Substituting $m = 3$ in equation (4) an average value of $K = 1\ 35 \times 10^5$ is obtained Again, taking [*S*] as indicated by *OA* (Fig 1) and substituting in equation (5) the values for m and K obtained above we can calculate the variation of [*S*] and [*M*] with concentration of amine This is shown in Fig 1 by the line *0B* which gives a good fit with the experimental results Substituting the value for K in the relation

$$-\Delta G = R\, T \ln K \qquad (6)$$

we obtain $-6\ 9$ kcal per mole of micelles for the free energy of micellization at 20° C

The foregoing treatment gives a reasonably satisfactory explanation for the anomalous behaviour of μ_{max} in *n*-octylamine–water at low amine concentrations Unfortunately, there are few thermodynamic data available on non-ionic micellar systems and none for this system which may be compared with the above values of m and K to test the hypothesis Calculations[6] from diffusion measurements for the paraffin chain salts (which form ionic micelles) have indicated that salts containing 8 carbon atoms in the chain form micelles each containing about 12 molecules The above value of $m = 3$ derived from *n*-octylamine–water suggests, if the interpretation of the ultrasonic measurements is correct, an appreciable difference in size for the two types of micelle

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PARENTERAL COPPER IN COPPER-DEFICIENCY DISEASES OF ANIMALS

By A. ASHFORD and Dr S E. MICHAEL

The Crookes Laboratories, Ltd , London, N W 10

COPPER deficiency occurs in farm animals, particularly in cattle and sheep, in various parts of the world The incidence of swayback is a hazard in several sheep-farming areas in the United Kingdom, and hypocuprosis in cattle is also of growing importance, especially during the grazing season Copper-deficiency may be a limiting factor in the economy of sheep production in New Zealand and Australia and losses in fleece value due to this deficiency merit special consideration

Marston[1] has suggested that the most economic way of treating this condition is the parenteral application of copper in such dosage that only one injection per animal is normally required As ionized copper in high doses is toxic, it is necessary to apply the copper in a non-ionized form or in a form in which it is only gradually absorbed from the site of injection so that the level of copper in the blood remains low Examples of the first type which have been used in the past are the highly complexed copper-edetate and copper-hydroxyquinoline disulphonate[2], and of the second type are suspensions of copper glycinate (moderately soluble in water) in non-aqueous media, such as beeswax-oil, whereby absorption is retarded These types are clinically effective, but they are not free from toxicity and they may give rise to irritation and swelling at the site of injection

A somewhat different approach to the problem is, however, possible, and this has been followed in the work recorded here It has been known for a long time that the solubility in water of the very sparingly soluble copper salts of certain amino-acids is greatly influenced by the presence of other amino-acids Hofmeister[3] pointed out that copper glutamate is much more soluble in the presence of glycine than in water alone This increased solubility can be explained by assuming that the copper-ion can be exchanged between the two types of amino-acid

If this exchange can take place between different amino-acids, then a similar exchange might occur between the copper–amino-acid combination and peptides or proteins Assuming this to be so, then copper salts of amino-acids of very low solubility could be introduced into the animal organism by parenteral injection, and it might be expected that the proteins of the body fluids which pass the site of injection would remove some of the copper by exchange with the amino-acid, the copper would thus be transported from the site of injection in combination with protein As the copper in this transport system may never be in a substantially ionized form, the desired clinical effect might be brought about without the toxicity or strong irritation which are characteristics of ionized copper

The assumption of copper exchange between an amino-acid and proteins may be supported by a simple model experiment The copper salt of *dl*-methionine, prepared according to Mueller[4], was suspended in water, saline, pig blood serum or a 2 per cent aqueous solution of serum albumin The mixtures were agitated for 24 hr , the liquors were separated by filtration from the excess of insoluble copper salt, and the copper contents of the filtrates were determined colorimetrically with sodium diethyl dithiocarbamate[5]

Table 1 gives the results so obtained

Table 1

	Copper (μgm /ml)	Colour of filtrate
Water-filtrate	9 4	Colourless
Saline-filtrate	12 0	Colourless
Serum-filtrate	120	Olive-green
Albumin-filtrate	180	Olive-green
Albumin-solution (control)	6	

Inspection of these figures shows that the solubility of the copper salt of methionine is about 1 100,000 in water or saline, but is increased 10–15 times in the presence of serum or serum albumin.

A comparison of the toxicities and irritancies of some soluble and 'insoluble' copper compounds was therefore made, and this was followed by an examination of the metabolism of the copper of the least-toxic compounds Acute toxicity tests were carried out on female albino rats weighing between 70 and 130 gm Intramuscular injections of solutions or suspensions containing 30 mgm copper/ml were made into the right hind-limb Five rats per dose group were used, and they were observed for a period of five days The results of these tests are given in Table 2.

Table 2

Material	LD_{50} mgm Cu^{++}/kgm.
Copper sulphate in water	24–42
Copper salt of glycine in water	10
Copper salt of glycine in 30 per cent gelatine	24
Copper glutamate in aqueous suspension	> 60
Copper salt of methionine in aqueous suspension	> 120

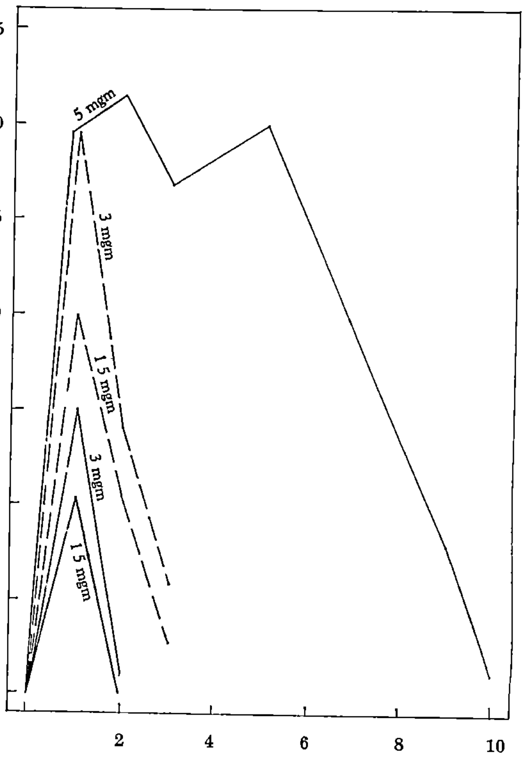

Fig 1 Irritant effects of copper after intramuscular injection into the hind leg of the rat
—, methionine complex, – – –, copper sulphate Doses are given in mgm copper/kgm body-weight

Rather surprisingly, the copper salt of glycine was the most toxic substance tested, and although the use of a delaying medium (30 per cent gelatine) reduced the toxicity to approximately that of an aqueous solution of copper sulphate, the glycine complex is still considerably more toxic than either the poorly soluble copper glutamate or the copper salt of methionine The LD_{50} of the copper salt of methionine also exceeded 100 mgm. copper/kgm when injected subcutaneously into male albino mice (Schneider) weighing 18–22 gm

On the basis of these results, the methionine complex was studied for irritant effects following an intramuscular injection into rats The clinical dose of copper in domestic animals is about 1 5 mgm Cu^{++}/kgm , and the effects of copper injections should be interpreted in the light of this dose Irritancy studies were carried out in female albino rats, the copper complex again being given intramuscularly into the right hind-limb The degree of swelling of the copper-injected limb was assessed by measuring

Table 3

Form and amount of copper injected	No of rats	Total copper injected (mgm)	Copper conc (μgm /gm wet weights) found in injected leg	Copper conc (μgm /gm wet weights) found in liver	Total copper (mgm) found in injected leg	Total copper (mgm) found in liver	Copper (per cent) absorbed	Per cent absorbed deposited in liver
Methionine 1 5 mgm /kgm	10	2 8	3	4	0 2	0 25	93	
Control	6			5		0 32		
Methionine 60 mgm /kgm	5	21 0	349	681	11 6	16 9	45	>100 ?
Control	2			24		0 6		
Glutamate 60 mgm /kgm	2	11 9	615	611	5 0	4 9	58	69
Control	4			20		0 16		
Glutamate 60 mgm /kgm	5	30 0	139	210	3 5	5 4	88	20
Control	3			7		0 17		
Glutamate 60 mgm /kgm	2	12 0	420	777	3 6	6 5	70	76
Control	2			16		0 13		

the increase in limb diameter of the injected leg, compared with the control hind-leg of the same animal. Diameters were measured with calipers. Doses of 1·5 mgm. and 3·0 mgm. Cu^{++} (as the copper-methionine complex) per kgm. body-weight caused a swelling of the limb which was greatest on the day after injection and which regressed completely by the second day. Equivalent doses of copper sulphate caused a bigger initial swelling which took up to 3–4 days to regress. Doses larger than 3 mgm. copper/kgm. caused a much more protracted irritation; for example, a dose of 5 mgm. copper/kgm., as the methionine complex, required 11 days for complete regression (Fig. 1). Histologically, the inflammation evoked by the copper salt of methionine showed a large cellular migration into the injured tissue and the lymph nodes in the limb were enlarged. These effects persisted for a period of at least 17 days. Occasionally, after a dose of 1·5 mgm. copper/kgm. (methionine complex), there was a residual fibrous deposit in the region of injection. Very large doses of the copper salt of methionine may cause necrosis.

The metabolic fate of the injected copper is, however, the most important feature of the problem. It is known that copper is preferentially concentrated in the liver, kidney and bone marrow of the rat, guinea pig, rabbit, dog, cattle and man[1]. We have examined the removal of copper from the site of injection in the rat and its accumulation in the liver. We have found that, after intramuscular injection of copper glutamate or the copper salt of methionine, the copper is largely removed from the site of injection and deposited in the liver. In the experiments summarized in Table 3, 1·5 mgm. copper/kgm. body-weight and 60 mgm. copper/kgm. respectively of the copper salt of methionine, or 60 mgm. copper/kgm. of copper glutamate were injected. Seven days later the rats were killed and the copper contents of injected leg, and of the liver, were determined. Table 3 shows that out of the total copper (of methionine salt) injected nearly all the lower dose and 45 per cent of the higher dose were removed from the site of injection. The absorption of the copper from the glutamate, given in the high dose, varied from 58 to 88 per cent of the amount injected. Varying, but considerable, amounts of the absorbed copper were found in the liver: 20–76 per cent from copper glutamate, apparently more than 100 per cent (probably experimental error) from the copper salt of methionine.

These experiments show that the insoluble copper salts of glutamic acid and methionine are less toxic than ionic copper salts, such as copper sulphate, or less highly complexed compounds, such as the copper salt of glycine. The irritancy also of the insoluble copper complexes is lower than those of the more soluble compounds. Both copper salts are removed from the site of injection and distributed in the body. After injection of larger doses, a considerable part of the copper was found in the liver, thus indicating that the copper is indeed taken up by the body fluids and transported to the target organ. It is important to point out that in these experiments the animals used were not copper-deficient.

On the strength of this investigation, the copper salt of methionine has been introduced with success for the treatment of copper deficiency of cattle and sheep.

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TIME-SEQUENCE OF HUMAN CHROMOSOME DUPLICATION

By C. W. GILBERT, S. MULDAL, L. G. LAJTHA and JANET ROWLEY*

Paterson Research Laboratories, Christie Hospital and Holt Radium Institute, Manchester

CHROMOSOME duplication occurs during a part only of the intermitotic cycle time: the period of synthesis of DNA (*S* period). Grain count distributions on autoradiographs with DNA label have indicated that the time sequence of events during the *S* period is largely determined by the fact that DNA is synthesized on discrete large structures—the chromosomes[1]. Consequently, a search has been started to investigate the detailed pattern of chromosome duplication in man, to find out whether it is of random nature or follows a definite time-sequence. Some of the preliminary findings are reported here.

Human peripheral leucocytes were cultured *in vitro*, using the phytohæmagglutinin method[2]: 20 ml. heparinized blood was mixed with 1 ml. of 6 per cent dextran solution and kept at 4° C for 1 h. The supernatant plasma was removed, diluted 1 : 5 with medium 199 (Glaxo). Extra plasma was obtained by centrifuging the sedimented red cells and also was diluted with medium 199. The white cell count was adjusted, by adding this extra medium, to about 1,200–1,400 cells/mm^3. Phytohæmagglutinin was then added; 0·2 ml. of a 1 : 50 dilution of the Difco '*P*' preparation per 10 ml. cell suspension. The suspension was then distributed in 4–5 ml. lots into a number of screw-cap universal containers, and incubated at 37° C for about 66–68 h.

The bottles were then divided into two groups, one labelled 4 h, the other 3 h, before terminating the culture. Labelling was performed by adding 0·2 μc./ml. medium of tritiated thymidine (Amersham, specific activity 2·3 mc./μM). Care must be taken that the cultures should not cool appreciably during the process of labelling. Finally, 'Colcemid' (Ciba) was added, 0·04 ml./ml. medium, 1½ h before terminating the culture by replacing the incubation medium with 4 ml. of 0·95 per cent sodium citrate.

Chromosome preparations were made by the air dry method; these were coated with Kodak *AR* 10 autoradiographic stripping film. The slides were exposed for 3–8 days, processed and stained with Leishman–Giemsa stain. The selected cells were photographed for record, the karyotype analysed,

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* U.S. Public Health Special Trainee Fellow at the Radiobiological Laboratory, Churchill Hospital, Oxford. Present address: Department of Medicine, University of Chicago.

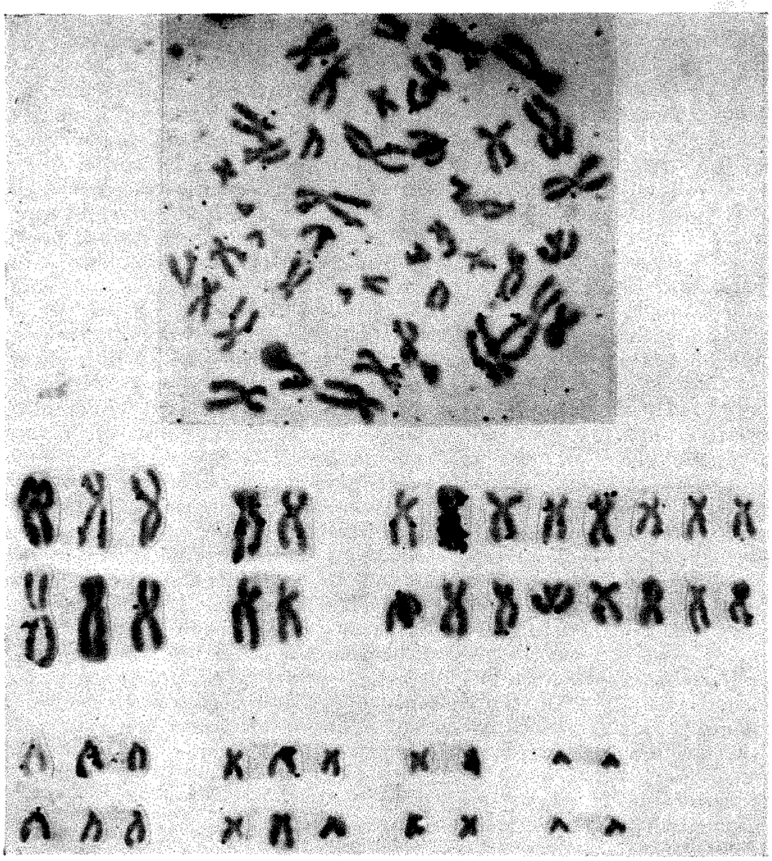

Fig. 1. Autoradiograph of karyogram of human female cell labelled at the end of the S period

and the grains over the individual chromosomes counted and their position noted. Only labelled cells with the best possible morphological picture of the chromosomes were selected for analysis. The selected cells were mostly in late prophase/early metaphase. Classification was carried out according to the Denver system, although we place more emphasis on homologous pairing than on chromosome sizes[3]. Consequently, we have assumed that one can often distinguish chromosome 4 from 5 in group II, and that 6, 10, 11 and 12 are not too often confused with X, 7, 8 and 9, the X chromosomes being more variable than the autosomes. In group IV, chromosome 13, apart from being the largest in the group, also has the longest 'centromere/satellite' region, and chromosome 15, the shortest, is also provided with a more definite euchromatic short arm. Fourteen will thus be left over when the other two are identified. We also assume that 19 tends to be separable from 20 in these early metaphases, although this has not been done with much confidence. Finally, group VII has been arranged on the principle that chromosome 21 has the larger size and satellite.

Like all mammalian cells, the dividing cells in this system also have a 2–3 h period (G_2 period) between the end of the synthesis of DNA (S period) and onset of mitosis. Hence in a 3–4 h incubation time the cells appearing in mitosis at the end of the incubation period must have been near to or just passed the end of the S period. This indeed was indicated by the large proportion of unlabelled mitoses (30–50 per cent). The total duration of the S period in these cells is not known, but grain counts over the labelled mitoses compared with those over the labelled resting nuclei indicated that the mean labelled mitoses contained only about one-fifth of the label, that is, the time spent in S period has scarcely exceeded 30 min. As the S period in most mammalian cells so far studied is of the order of 6–8 h, the labelling system used here represents the very end, the last 30–50 min of that period.

Several cultures were examined in this way, with similar results. The quantitative results presented here are from counts on 33 labelled mitoses in a culture from a normal female, labelled at minus 4 h. A typical autoradiograph and karyogram is shown in Fig. 1.

The grain distribution over the chromosomes of 33 cells was assessed. The arms of the chromosomes were divided into 1, 2, 3 or 4 equal segments depending on the length of the arms and the grains were counted over each segment. The mean density of the grains over each segment was calculated from the relation:

$$\frac{1{,}000s}{Nl}$$

where s is the total number of grains counted over the corresponding segments of all homologues (66 chromosome segments), N the grand total of grains over all chromosomes in all cells, and l the relative length of the segment (1,000 being the total length of all chromosomes in a female cell). The results are shown in Fig. 2 as histograms giving the distribution of grains along each chromosome. The X chromosomes are shown separately. Chromosomes 1, 3, 19 and 20 have almost central centromeres and so the mean distribution over the two arms is shown. In these diagrams the normalization is such that the chromosomes would have a uniform grain density (of unity) if at the period of labelling, the end of S, all the chromosomes had been synthesizing at the same rate per unit length. The distributions are significantly different from this and clearly show the existence of a regular pattern of synthesis.

The most noticeable feature of this pattern is the high number of grains over one of the X chromosomes ('hot' X). Apart from that the larger chromosomes show generally a greater density of labelling than the shorter ones, but there are exceptions. Group II is the most heavily labelled and the short chromosomes 17, 19, 20 and 21 are weak, though 13 has a heavily labelled long arm. Many of the chromosomes, notably 5, 9 and 16, also show marked differences between the two arms, and there is a general tendency for the shorter arm to be the more heavily labelled in the longer chromosomes and the reverse in the shorter ones. Many chromosomes show variations of density along the length of one or both arms. There is no general pattern, and examples of most of the possible patterns can be found. Chromosomes 1 and 13 show a significantly high grain count in the middle of an arm, chromosomes 4, 5, 9 and 17 show

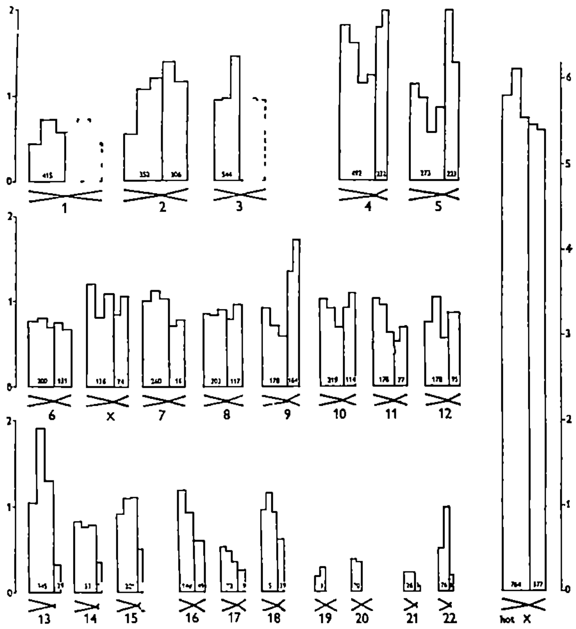

Fig 2 Distribution of tritiated thymidine over chromosomes of human female cell, labelled at the end of *S* period Ordinate shows the linear density of grains normalized so that the average density over all the chromosomes is unity The numbers show the total grain counts over each arm for 33 cells

a gradual increase of grains from the centromere to the end and chromosome 2 shows the reverse Although the short chromosomes have low grain counts, nevertheless the relatively high density of chromosome 22 compared with 21 is significant

The 'hot' *X* chromosome accounts for 14 4 per cent of all the grains on the cells However, there is a significant change in the proportion with the total number of grains over a particular cell This is shown in Fig 3 The points in the figure show the mean proportions of grains over the 'hot' *X* for the five groups of cells with total grain counts between 0–100, 100–200, 200–300, 300–400 and over 400 The line is the calculated regression line, and the slope of this line is significant at a probability of 000 3 The data are insufficient to distinguish a curved from a straight regression line

The total number of grains over a cell probably indicates how near that cell was to the end of the *S* period when the label was applied, the smaller the uptake the nearer the end Thus, as the end of the *S* period is approached the relative difference between the label on the 'hot' *X* and the other chromosomes becomes more marked This indicates that synthesis of the whole of the 'hot' *X* chromosome occurs at the very end of the *S* period

Only in the *X* chromosomes is there a marked difference (heterozygosity) between the two homologues, a detailed statistical analysis failed to show any difference for any other chromosome

An analysis was made of the variance of the grain counts over each chromosome pair For each the following quantity was calculated

$$Y = \frac{2N}{2N-S}\left[\frac{2N}{S}\sum_{i=1}^{33}\left(\frac{a^2+b^2}{n_i}\right) - S\right]$$

where N is the total grains over all cells, S is the total grains over the pair of chromosomes of all cells, a_i, b_i, the grains over the pair of chromosomes of the i-th cell, n_i the total grains over the i-th cell, and the summation is taken over all the cells If each grain were a statistically independent event, this would be a χ^2 of 65 degrees of freedom and its expected value would be 64 5, or about 1 per degree of freedom However, the average value of Y is 2 10 per degree of freedom Thus, the variance of the grain counts is 2 10 times as great as that expected from a Poisson distribution Something of this sort is to be expected, since each β-particle of tritium may produce more than one grain in the autoradiographic emulsion If G is the mean number of grains per β-particle, and if these have a Poisson distribution, then since the β-particles are random events, the variance of the grain counts will be increased ($G + 1$) times Thus, the assumption of a G of 1 1 will fully account for the observed variance The quantity $Y/2$ 1 will then be a χ^2 of 65 degrees of freedom and the calculated values for the 23 chromosomes (excluding the 'hot' *X*) scatter about the mean value of 64 5 in the range to be expected for a χ^2 This may therefore be regarded as some internal evidence of the consistency of pairing and identification of the chromosomes For example, if the chromosome pair 13 and 14 or 21 and 22 which show markedly different intensity of labelling had been interchanged frequently, then larger values would be expected for the χ^2 for these chromosomes

The real differences in the intensity of labelling of the different chromosomes or parts of the chromosomes, together with the constant variance of the grain count, suggest a very consistent pattern of replication of the chromosomes for all the cells of this culture

The same analysis of variance of the grain counts over the 'hot' *X* shows a considerably greater variance even after allowing for the fitting of the regression line This is probably a real biological variation and expresses the fact that the lateness of the 'hot' *X* chromosome varies somewhat from cell to cell

The most striking observation is the finding of an exceptionally heavily labelled chromosome in group III It was, of course, of crucial importance to decide on the identity of this chromosome, especially since the intensity of label could introduce some bias in classification, sometimes even obliterating morphology, a feature not affecting the other chromosomes Ideally, one might classify the chromosomes before putting on the film, but this obviously would involve a great deal of wasted effort in analysing afterwards unlabelled cells

The procedure adopted here was to pair up homologues[3], which resulted in two chromosomes being left over, one of which in each case was the 'hot' chromosome This chromosome pair with the

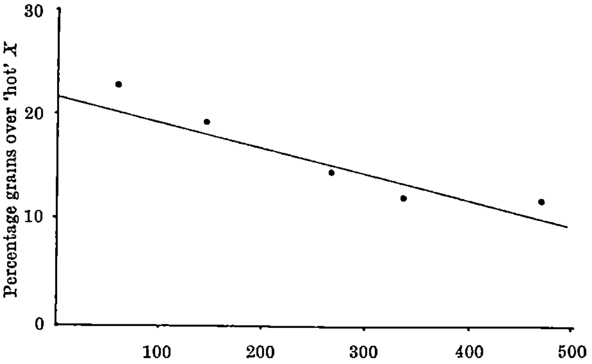

Fig 3 Change of relative intensity of labelling of 'hot' *X* chromosome with total labelling of the cell

expected size difference, fitted in between 6 and 7 in group III, that is, the position of the X chromosome

Furthermore, as the other chromosomes show a consistent pattern of label, it is reasonable to suspect that that chromosome which is exceptionally heavily labelled is the same chromosome in all cases

The sex chromatin in human females (the Barr body[4]) is now generally believed to be produced by the heteropycnosis of one of the X chromosomes[5] (or part of it)[6] while the other X remains isopycnotic like the autosomes This heteropycnotic behaviour is transmitted to the daughter chromosomes[7] from one cell generation to the next Heteropycnotic differentiation of the X is presumably established early in ontogeny[8,9], and may apparently involve either of the two X chromosomes in different regions of the embryo, resulting in a mosaic pattern when the two chromosomes carry different dominant genes[10] The general implication is that the heteropycnotic X chromosome is genetically inactive and that only one active X chromosome is required for the normal function of the cell Chromosome 'activity' is, however, expressed in two ways (*a*) genetic activity expressed in RNA coding, and (*b*) chromosome duplication (DNA synthesis)

Morphologically, the heteropycnotic state appears as a more tightly coiled chromonema, and it is possible that a chromosome in such a condition cannot reveal much surface for coding activity It would also follow that when chromosome duplication takes place such a chromosome may be delayed compared with isopycnotic chromosomes We found this X chromosome not only many-fold 'hotter' than the other chromosomes but that it often had a higher grain density than any chromosome region observed in interphase nuclei This must mean that the 'hot' X synthesizes DNA more or less simultaneously along its whole length and has, therefore, a very short S period, shorter than any other chromosome of similar size

Our findings are in good agreement with earlier reports on this subject in the literature Taylor, using embryonic tissue cultures of Chinese hamster, has already demonstrated such a delay in DNA synthesis of sex chromatin[11] More recently, Morishima, Grumbach and Taylor[12] have reported on a late synthesizing X chromosome in human female as well as on other late synthesizing chromosomes, one each in groups II, IV, VI and VII German[13] also reports a 'hot' chromosome in group III Although these authors do not present quantitative information, their conclusions are basically similar to those reported here

Structural heterochromatin is frequently associated with regions of spontaneous or chemically induced chromosome and chromatid breaks[14-16] By comparison with data collected of the spontaneous somatic breakage pattern in man, it appears that the peak spots fit in well with the highly labelled regions on the chromosomes in the late stages of DNA synthesis Owing to the relatively low grain counts in the present series, however, this cannot be statistically proved Labelled chromatid breaks were, however, observed, especially in the 'hot' X chromosome, and it seems that tritiated thymidine applied at the end of the S period provides a method of producing breaks in one of the X chromosomes with a high yield This could be checked simply by the study of variation in Barr body sizes in subsequent cell generations, which may also give further information about the structure of the Barr body

Since spontaneous chromatid breaks or gaps are more or less open, it may be that they are due to interference with DNA synthesis at the end of the S period when no further time is available for their restitution

Certain chromosome translocations are known to occur with an unexpected high incidence as compared with others It is difficult to imagine a translocation to occur between chromosome regions which are not synchronized in their DNA synthesis If we now consider the particularly 'cold' chromosomes at the end of the S in the present series (that is, 21, 19, 17 and 14) we find that three of these may be involved in translocations which are mechanically stable and viable for generations[17,18] The translocations usually associated with mongolism are 21/13–15 and 21/21 (refs 19 and 20) Although it is a fact that chromosome 21 causes mongolism when in trisomic state[21], it is doubtful that the morphology of the translocation chromosomes has been worked out satisfactorily From the labelling pattern reported here it appears that chromosome 14 may be the most likely one in group IV for a synchronized exchange with chromosome 21 The mechanical fitness of these translocation chromosomes may be due to their having arisen in early S, allowing sufficient time for a solid repair at the exchange points The association of satellited chromosomes on the same nucleolus must further increase the probability of translocations

In contrast to the heterozygosity in chromosome labelling in the human male reported by Lima-de-Faria *et al* [22], we found no evidence of such a phenomenon apart from the X pair in the female The degree of heterozygosity observed was no more than expected from the spread of grain count distribution As a matter of fact, the heterozygosity is so negligible that a reasonable correct classification of chromosomes based on their labelling properties seems to be a major feature of the present report Our results support well the Denver classification with emphasis on homologous chromosome pairing rather than on actual chromosome length It has been stated previously that the X chromosome is relatively more variable in size than are the autosomes in group III This is also borne out by the present series Of the two X chromosomes, the 'hot' X is usually the smaller, is often bent, and it may be found in a peripheral position suggestive of the Barr body In the more contracted metaphases the size difference between the two X's tends to disappear, which indicates that the cold X catches up in contraction with the 'hot' X

The choice of early metaphases has given a better grading of the small chromosomes, confirming the old observation that these contract relatively earlier than the longer ones This principle may now be linked with their generally earlier completion of duplication, with the exception of chromosomes 13 and 22

Differential grain counting along the chromosome emphasizes the asynchronous replication of different chromosome parts, but gives little support for a 'zipper' fashion pattern of chromosome duplication from telomeres towards centromeres If the centromeres with adjacent heterochromatin are the last to duplicate it is not a striking feature This may, however, be caused by the tapering of the chromonema in these constrictions Over-exposure of the autoradiographs of weakly labelled mitoses may be necessary to elucidate final localizations such as the significance of the radioactivity over the heterochromatic band in the 'cold' X chromosome

This work will be extended back into the earlier part of the S period in order to map out the complete time sequence of synthesis for the entire chromosome complement in man We have mentioned here some of the possibilities that this technique offers, and one may be justified in expecting some important findings with this 'functional' approach to chromosome physiology, findings possibly involving differences between normal and pathological cells, or minor differences between races of man

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TRANSFER OF DEOXYRIBONUCLEIC ACID ACCOMPANYING THE TRANSMISSION OF COLICINOGENIC PROPERTIES BY CELL MATING

By Dr SIMON SILVER* and Dr HAROU OZEKI†

Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass

MANY strains of Enterobacteriaceae produce bactericidal protein substances called colicines The genetic determinants for the synthesis of colicines (colicinogenic factors) behave, under some conditions, as autonomous particles which can be transmitted during conjugation independently of the bacterial chromosome[1-3]

There has been no direct evidence concerning the chemical nature of the colicinogenic factors Analogies with prophages[2,4] suggest that the factors consist of DNA Some colicinogenic factors are transducible by DNA-containing phages[3,5,6] Attempts to determine the DNA content of colicinogenic factor E_1 by phosphorus-32 suicide experiments, however, were unsuccessful[7] By combining the high-frequency colicinogeny-transferring (HFC) system[8] with a method of measuring material transfer[9] we have been able to measure DNA transfer accompanying the transmission of colicinogenic factors I, E_1 and E_2

Radioactive HFC donor preparations[8] of *Salmonella typhimurium LT*-2, mutant *cys D-36*, were prepared as follows tryptone broth containing ^{14}C-thymidine, ^{14}C-uracil or ^{14}C-leucine was inoculated with a mixture of 1 part of a culture of strain *cys D-36* (I) and 20 parts of either *cys D-36 col*$^-$ or *cys D-36* (E_2) or *cys D-36* (E_1) (E_2) After overnight incubation at 37° C , the cultures were diluted four-fold in fresh radioactive broth and incubated for an additional 2 5 hr (with aeration by shaking during the last hour) This procedure gave preparations which transferred either factor I, or factors I and E_2, or factors I, E_1 and E_2 respectively Radioactive cells of *cys D-36 col*$^-$ were prepared in an analogous way, leaving out the inoculum of *cys D-36* (I) An overnight culture of the recipient strain *Escherichia coli* *C*600/I, E_1, E_2 (resistant to all these colicines) was diluted 1 10 with fresh non-radioactive broth and incubated at 37° C for 2 5 hr with aeration by shaking The cultures were centrifuged, washed and re-suspended in broth at densities about 10^9 cells/ml Samples of 3 ml each of donor and recipient cultures were mixed in 500-ml Erlenmeyer flasks and incubated for 75 min at 37° C The mating mixtures were assayed for the frequency of transfer of the various colicinogenic factors[3], centrifuged and re-suspended in 4 ml of fresh broth

With duplicate samples, the *E coli* recipients were disrupted by the addition of a large excess of *T*6 phage (to which *S typhimurium* is resistant) in the presence of DNase After 15 min at 37° C , when lysis-from-without was complete, the lysates were filtered through 'Millipore' filters Any radioactivity which had been transferred from donor to recipient during mating would be released by lysis and could be measured in the filtrates Controls were run to test for spontaneous and phage-induced lysis of the donor strains Chromosomal transfer in these matings is either completely absent or at such a low level as not to interfere with measurement of colicinogenic factor transfer[10]

Thymidine (for DNA) transfer was measured with four different preparations of donor *S typhimurium* strains a non-colicinogenic strain, a mixture that transferred only factor I, a mixture that transferred factors I and E_2, and a mixture that transferred I, E_1 and E_2 The results of several experiments are shown in Table 1

By taking the differences between the levels of transfer, using paired experiments to eliminate some systematic errors[9], one can estimate the amount of DNA associated with the individual factors as I = 0 7 per cent, E_1 = 0 8 per cent and E_2 = 0 4 per cent of the DNA of the donor cells The lower limit of detectability in these experiments is about 0 3 per cent transfer per mating donor so these numbers can be considered only order of magnitude estimates

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* Predoctoral Fellow (*GF* 14, 956) of the Division of General Medical Sciences of the U S Public Health Service Present address Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London, W 12

† Present address National Institute of Health, Shinagawa, Tokyo, Japan

Table 1 ^{14}C-THYMIDINE TRANSFER FROM COLICINOGENIC DONORS

Factors transferred	Exp No	*A* Per cent radioactivity released by phage Mating	*A* Per cent radioactivity released by phage Control	*B* Per cent radioactivity transferred	*C* Per cent recombinants/input donor	*D* Per cent transferred/recombinant
(I) (E_1) (E_2)	10	0 96	0 27	0 69 ± 0 18	33	2 1
	11	1 34	0 06	1 28 ± 0 08	68	1 9
	12	0 78	0 08	0 70 ± 0 06	54	1 3
(I) (E_2)	10	0 69	0 20	0 49 ± 0 31	45	1 1
	12	0 55	−0 02	0 55 ± 0 15	100	0 55
(I)	10	0 29	0 07	0 22 ± 0 07	31	0 71
	12	0 32	0 30	0 02 ± 0 13	13	—
col$^-$'donor'	12	0 09	0 02	0 07 ± 0 02	—	—

A, difference between the average per cent of the radioactivity found in the filtrates with and without phage, *B*, difference between the values from col *A*, mating minus control, *C*, *col*$^+$ *E coli* after 75 min mating/*S typhimurium* at time of mixing, *D*, value in col *B* divided by value in col *C*

From the data of ref 11 for DNA per cell of *S typhimurium*, and assuming that only one copy of each factor is transferred, the estimates for the sizes of each factor correspond to $I = 6 \times 10^4$, $E_1 = 7 \times 10^4$ and $E_2 = 3 \times 10^4$ nucleotide pairs These estimates may be somewhat smaller than the true values if non-radioactive factors have been transferred during a second round of conjugation, or larger than the true values if more than one copy of each factor is transferred during mating

Colicinogenic factors E_1 and E_2 (but not I) can be transduced by phage P_{22} (refs 3 and 5) (which has a DNA content of 7×10^4 nucleotide pairs[12]) This sets an upper limit for the size of factors E_1 and E_2, which agrees with the order of magnitude estimates from the present work This agreement, along with the apparent correlation between the amount of DNA transferred and the number of types of factors transferred (3 > 2 > 1 > 0), suggests that the DNA transferred comprises the colicinogenic factors and that only one copy of each factor is transferred Lavallé and Jacob[7] have shown from phosphorus-32 suicide experiments that colicinogenic factor E_1 is very resistant to phosphorus-32 decay and estimated that it cannot contain more than 5×10^3 nucleotide pairs of DNA and possibly less If one takes both our estimate and Lavallé and Jacob's limit at face value, about 14 copies of the E_1 factor would have to be transferred in each mating pair

There are, however, several reasons why Lavallé and Jacob may have under-estimated the size of the colicinogenic factor Rapid multiplication of the colicinogenic factor after transfer and prior to freezing would result in a pronounced under-estimate (Preliminary experiments[13] indicate that this is the case) The suicide experiments measured the size of the colicine-producing gene while the colicinogenic factor might contain additional genes, including those involved in transfer by conjugation, induction of colicine formation and resistance to colicine

Experiments using ^{14}C-uracil (for RNA) and ^{14}C-leucine (for protein) did not show measurable transfer, but the resolution was such that genetically (or physiologically) significant transfer could have remained undetected The limit for the transfer of uracil-radioactivity was about 0 3 per cent per donor in each case However, since there is about six times as much RNA as DNA in the donor cells this limit represents about 3×10^5 nucleotides—the same as the amount of DNA transferred The limit for leucine transfer was similar, and there is more than ten times as much protein as DNA in the donor cells In conclusion, we have evidence for DNA transfer but no reason to exclude RNA and/or protein from the colicinogenic factors

This work was supported by National Institutes of Health (Allergy and Infectious Diseases), U S Public Health Service grant *E*–2028 to Prof C Levinthal and by National Science Foundation grant *G*–8808 to Prof S E Luria

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INFECTIVE RIBONUCLEIC ACID COMPONENT OF CELLS INFECTED BY ENCEPHALOMYOCARDITIS VIRUS

By A. J. D. BELLETT, A. T. H. BURNESS and F. KINGSLEY SANDERS

Virus Research Unit, Medical Research Council Laboratories,
Carshalton, Surrey

HUPPERT and Sanders[1] reported that infective ribonucleic acid (RNA) could be recovered from encephalomyocarditis (EMC) virus-infected mouse ascites cells maintained *in vitro*, or from the culture fluid into which such cells had released virus When the virus was deposited by centrifuging this fluid at 100,000*g* for 1 h, little infective RNA could be recovered from the sediment, though large amounts could still be obtained from the supernatant When the sediment was washed by a further cycle of centrifugation, no infective RNA could be obtained from it This result led to the idea of a component, smaller or less dense than the virus, from which infective RNA could be prepared[1–3] Experiments reported here suggest that, although a viral precursor may exist, the results which led Huppert and Sanders to postulate its existence can be interpreted in another way

Huppert and Sanders prepared RNA by the technique of Schuster *et al* [4], that is, by three extractions with water-saturated phenol at 4° C, followed by ether extractions of the aqueous phase to remove phenol, ether was removed by bubbling nitrogen through the preparation, which was injected into mice to estimate its infectivity We now use the technique described by Sanders[2], which differs in that nickel ions are present during preparation, and that instead of using ether to remove phenol, RNA is precipitated directly from the aqueous phase by

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BRITISH ASSOCIATION MEETING IN MANCHESTER

INVESTMENT IN SCIENCE*

By Sir JOHN COCKCROFT, O.M., K.C.B., O.B.E., F.R.S.
The Master, Churchill College, Cambridge
President of the British Association

ALMOST half a century has passed since the British Association met in Manchester under the presidency of Prof. Arthur Schuster. This was the end of the period which had been dominated in Manchester by Rutherford, Horace Lamb, 'Sammy' Alexander, the philosopher with beard and bicycle, Tout, the historian, Chaim Weizmann and other giants. I was fortunate in spending two terms in the University under the influence of some of these great men before I went off to the War.

The Physics Section of the British Association met in 1915 in Rutherford's Laboratory under the presidency of Sir Frank Dyson, and Rutherford wrote to Boltwood that they had had very good discussions on isotopes led by Soddy, on the classification of stars by Fowler and on thermionic emission by O. W. Richardson. The Braggs, father and son, talked about X-rays and crystal structure, and A. N. Whitehead spoke on time, space and relativity, so in spite of the War the physicists had a good feast.

Bohr was still working in the Laboratory, and by this time his theory of atomic structure was gaining acceptance, and Rutherford had taken over from Marsden experiments on the projection of hydrogen particles by α-particles. He wrote to Bohr in December 1917:

> "I occasionally find an odd half hour to try a few of my own experiments and have got, I think, results that will prove of great importance but a great deal of work will be required to make sure".

By June 1919, however, he was able to publish the result of his four years of work and to be pretty certain that nitrogen atoms were disintegrated by the impact of his α-particles. These experiments were to lead twenty-three years later to the first controlled release of nuclear energy in Fermi's atomic pile and fourteen years later again to the first large-scale nuclear power station in Britain—the result of half a century of work by physicists of many nations and by our engineers.

While it is natural for me to think of physics in Manchester, we should not forget the line of illustrious chemists and biologists who have worked here and moved on to fertilize and inspire other universities, especially the Universities of Oxford and Cambridge.

During our week here we shall be reminded by our programme that Manchester has long been famous for activities other than science. The Hallé Orchestra is to entertain us and *The Guardian*, true to the tradition of C. P. Scott, will, as always, be describing our discussions. I am speaking then in an environment which has a long tradition of pioneer work in science and technology so I am going to talk about "Investment in Science"—a subject which is more important to our country to-day than it has ever been. If we look back over the forty-seven years since the Manchester meeting we can see that our contribution to basic science compares well with that of any other country, while in technology we have had many notable successes, including radar, jet engines, antibiotics and polymers. In the next half-century, when we have to face the increasingly competitive world of the Common Market, what are the prospects of doing as well or better?

If we look forward to the prospects for pure science, we see that it is in an intensely creative phase. Rutherford's burning desire to learn the innermost secrets of the atom is shared by the generation of his grandchildren. The primitive nuclear particle accelerators of Rutherford's later years which produced hydrogen nuclei with energies of a few hundred thousand volts have been succeeded by enormous nuclear accelerators producing particles of 100,000 times greater energy at 10,000 times the cost. When these beams of very energetic protons collide with other nuclei, they create in the intense field of force of the collision many different members of the nuclear underworld, often known as elementary particles—particles which live for a very short time and often then change into other particles, which in turn may change again until we recognize a well-known particle such as an electron or neutron.

In the immediate post-War years, the invention of the synchrocyclotron producing protons of 300 or 400 million volts enabled us to produce large numbers of the so-called pi-mesons, and to study the forces they transmit, forces which stick the components of atomic nuclei together and are closely connected with the enormous energy stored in atomic nuclei. The invention and construction of the proton-synchrotron or 'Cosmotron' at Brookhaven in the early 1950's increased available proton energies to 3 billion volts, and these protons produced particles heavier than protons known as hyperons, always accompanied by the production of another variety of particles known as K mesons The hyperons and K mesons became known as 'strange particles', since a new classification known as 'strangeness' was introduced to specify their genetic properties A further increase of energy to 6 billion volts in the Berkeley Bevatron enabled us to create anti-matter and to study the physics of anti-matter Anti-protons are produced in pairs with ordinary protons and so are anti-neutrons and anti-hyperons These anti-particles live until they collide with a normal particle, when they annihilate each other, signalling the event to the observant physicist through showers of mesons So we are now able to study the physics of anti-matter by transmitting anti-protons in very large numbers several hundred metres through evacuated pipes to the so-called bubble-chambers full of liquid hydrogen waiting to study their behaviour

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* Presidential address delivered in Manchester on August 29 and to be published in the September issue of *The Advancement of Science*, 19 (1962–63).

A year ago we thought we might have discovered all the members of the elementary particle family, 17 in all with 17 anti-particles, and then, as so often happens, the veil of Nature was lifted again and a new variety of still more ephemeral particles was discovered by experiments in the giant accelerator at Berkeley Some of these ephemeral particles seem to be excited states of the previously known hyperons with masses up to twice the mass of the proton They live for times of about 10^{-22} sec, just long enough to escape from the nucleus and then break up into more familiar particles, which in turn can change Other similarly ephemeral particles have been found with masses between the proton and pi-mesons which seem to be assemblies of the mesons An understanding of these particles and events of the nuclear underworld, connected as they are with the basic forces of the Universe, present a tremendous challenge to the human intellect If we succeed, our understanding of the physical Universe will be greatly enriched, as it has been enriched in the past by Newton's theory of gravitation, Bohr's theory of the nuclear atom, and by wave and quantum mechanics The study of elementary particles is likely to continue to be in the forefront of physics for several decades and will continue to attract many of the world's best scientists There is, therefore, good reason for maintaining our present heavy investment in this branch of science

While this work with very-high-energy particles goes on, another group of nuclear physicists working with less energetic particles of very precisely defined energies are exploring the detailed structure of the nucleus and its vibrations and oscillations and are also exploring the nuclear processes which lead to the evolution of the elements in the stars So we now know how this evolution starts—by fusing together hydrogen nuclei in a sequence of processes to form helium—and how, much later in the life of a star, as it gets hotter, helium nuclei fuse together to form heavier elements, and this can go on with ever-increasing temperatures until the terrific nuclear explosion of the supernovæ occurs In these explosions the very heavy elements are born

At the Rutherford Jubilee Conference last year Prof W A Fowler reminded us that in 1929 Rutherford estimated the age of the Earth as 3 4 billion years from calculations on the relative abundance of uranium isotopes This was a very good estimate, for the most recent work now estimates the time at which the Solar System began condensation from interstellar gas as 4 7 billion years The age of our Galaxy must be longer to provide time for the synthesis of all the elements, and estimates of its age have now been stretched out to between 7 and 15 billion years The combined efforts of the radio and optical astronomers have within the past two years identified a far distant galaxy, the light and radio waves of which have taken between 5 and 6 billion years to reach us So there is evidently still scope to reach farther and farther out into the boundaries of the Universe In this new branch of astronomy, Lovell at Manchester and Ryle at Cambridge have been world pioneers, and we look forward with great interest to our visit to Jodrell Bank this coming week Optical astronomers are also moving out into space to avoid the interference to their measurements by the absorption of ultra-violet and X-rays in the atmosphere, so there will soon be an 8-in. diameter telescope circling the Moon, and later a 50-in diameter telescope circling the Earth in a 24-h orbit, stabilized to a second or so of arc and transmitting information back to earthbound astronomers by telemetric signals Astronomy from being a poor, neglected subject in Great Britain, reckoning its budgets in thousands of pounds a year has now combined with space science to rival nuclear physics, with budgets in Britain soon likely to approach £5 million a year, and like nuclear physics driven by economic necessity as well as scientific interest to collaboration first with the United States and in future with Western Europe

In Britain and in the world generally solid-state physics attracts as many research workers as nuclear physics, and their achievements, while less glamorous and less publicized, have been great and perhaps of more immediate practical use They have been helped, like the nuclear physicists, by the feed-back from technology producing ever more powerful and discriminating instruments

Electron microscopes of high resolution make visible imperfections in crystal planes of metals and especially those known as dislocations The ease with which dislocations move through metals determines their ductility, and we now understand why metals change their properties with increasing age, why they become more brittle at lower temperature and how impurities affect them Observations of dislocations in uranium metal have recently enabled us to understand why uranium metal crystals grow in one direction under irradiation and shrink in another, a phenomenon of considerable importance in nuclear power stations Recent developments have even made it possible to obtain photographs showing individual atoms in a crystal array and to observe the disturbances produced in the array by the impact of particles

Another branch of solid-state physics, the study of semi-conductors, is now in a rapidly developing stage Starting as an academic subject in the 1930's it has led to the development of transistors, to rectifiers of increasing importance in power generation, to detectors of infra-red radiation of great virtuosity, to devices for producing refrigeration, to solar batteries and many other products

The Earth and environmental sciences are in an equally lively state Geophysicists are preparing to drill the Mohole, a hole 6 km deep through the Earth's crust to the Mohorovičić discontinuity between crust and mantle The recent discovery that the Earth can oscillate like a bell but with a few vibrations an hour promises to throw new light on its inner-structure Oceanographers believe that more extensive studies of the nutrient content of the deep ocean and methods of increasing the turnover could lead to greatly increased fish protein production which at present provides only about 1 per cent of the Earth's food Recent studies of the ocean floor show vast reserves of minerals, especially nickel, cobalt, copper Meteorological science and forecasting will be greatly improved in the future with the advent of more powerful computers and by observations of cloud formations and hurricanes from satellites such as *Tiros*

Recent discoveries in the life sciences have been no less notable and have been greatly helped again by the developments in techniques such as high-precision X-ray crystallography, the electron microscope, chromatography, radioisotopes Among the most important developments of the past decade has been the discovery of the general chemical structure of the giant molecules, known for short as DNA, which determine our hereditary characteristics Watson and Crick, from X-ray data, concluded that the DNA molecule consisted of two intertwining helices containing several million atoms Connecting the two helices like rungs of a ladder are pairs of four kinds of side groups called bases The sequence of these bases provides the genetic code, the genes of classical genetic theory which are now thought to consist of several hundred of these bases Within the past year workers in the Molecular Biology Unit at Cambridge have suggested that a group of three bases provides the information required to fabricate one of the amino-acids in the twenty which constitute a protein molecule Nerenberg and Ochoa discovered which bases are involved in fabricating a number of these amino-acids Kornberg has gone further and actually synthesized DNA by putting the four fundamental monomers into a test-tube together with a little bit of DNA from a living cell and a catalyst This is the first step in the artificial synthesis of genes Biochemists can also induce mutations in the genetic material by specific chemical agents and geneticists can now relate a severe genetic defect such as congenital anæmia to one amino-acid out of place in a protein molecule This work is a remarkable example of inter-disciplinary collaboration which is becoming more common in science Prof P B Medawar has said that

> “ this work considered as a whole is one of the most glorious episodes in biological research In the very remote future this discovery puts it in our power in principle to exercise a primary and direct control over genetic makeup and even to secure a direct and fundamental cure of inborn disorder”

Another great biological development started with the work of Hevesy in 1922 when he used radioactive lead as a tracer to study biological processes The use of nuclear reactions to produce tritium, radiocarbon and a multitude of other tracers has greatly extended the range of these tools and enabled the biochemist to unravel the very complex processes of living organisms, to show that the whole of the components of our bodies are in a general state of flux and to show how the breakdown of a single step in the complicated chemical chain can lead to disease

One of the most important problems of the next half-century is the control of human fertility and the provision of an increasing supply of human foodstuffs to outstrip the doubling of world population, which seems to be regarded as inevitable in this period The biochemists seem now to be making progress in fertility control, and the application of well-known technology to increase agricultural production in underdeveloped countries may be supplemented by turning fishing from a hunting to a farming operation and by new approaches to providing protein for human food

Biology seems, in fact, to be in a rapidly advancing, almost revolutionary phase, and it is important that its full potential should be realized by increased support

While pure basic research provides the essential pool of new knowledge from which, sooner or later, practical developments follow, objective basic research, searching for applications of new knowledge and followed by applied research devoted to a specific project, are among the principal promoters of economic growth to-day So investment in such research should be regarded as of comparable importance to investment in plant and buildings

One of the most spectacular examples of economic growth achieved in this way has been the development of the transistor industry Twelve years or so ago I visited the Bell Telephone Laboratories in New Jersey and saw scientists drawing single crystals of very pure germanium from a molten mass These very pure crystals were seeded with small quantities of vital impurities and then cut up to form the transistors which are now most familiar to us in our portable wireless sets Their influence on the development of electronics has been very great Their great reliability and small power requirements has led to a reduction in size and a great increase in reliability of electronic equipment Without them, space science and space travel would scarcely have been possible They have also made possible the very much more powerful next generation of computers, such as the *Atlas*, the first model of which we are to see at the University this week—a remarkable example of a combined research effort between university scientists and industry Computers are playing a creative part in the development of sciences such as molecular biology, high-energy nuclear physics, the study of stellar evolution, plasma physics, theoretical chemistry and many other branches The University of Cambridge alone has 100 computer users They are becoming an indispensable tool for design of complex equipment such as aircraft, nuclear power stations, and are even penetrating ship design They are becoming important in the control of industrial organizations and they are being used in studies of our economy and may, one day, help to decide the arguments between the expansionists and the restrictionists—we hope to our great benefit

Transistors are only one of the numerous new products of research into solid-state physics Eight years ago, workers in the General Electric Laboratories at Schenectady converted graphite into diamonds by applying a pressure of 100,000 atmospheres and temperatures of 1,600° C together with a catalyst. To-day synthetic industrial diamonds are made on the scale of tons per annum while diamonds as large as one carat have been made So a single laboratory study produced a new product valued at more than 100 million dollars a year

Until very recently, the phenomenon of metals losing their resistance to the passage of electrical current at temperatures near absolute zero—the phenomenon of superconductivity—was an academic subject, pursued almost entirely in university research laboratories Within the past two years important practical applications of superconductivity seem to be emerging The Bell Telephone Laboratories discovered that an alloy of tin and niobium remains superconducting in magnetic fields of up to 120,000 gauss, and already superconducting coils have been built to produce magnetic fields of more than 40,000 gauss with practically no power requirements So the strong magnetic fields required for many experiments in nuclear physics, controlled fusion research and other subjects are likely, in future, to be produced in this way We can even think of the possibility of reducing the loss of power in transmission of electrical energy by using underground superconducting cables, though this would certainly depend on further reducing the cost of cooling to these very low temperatures The next generation of very fast computers may well use for their powerful memories 'cryotrons' containing a multitude of thin superconducting metal films in which current flow can be reversed in a thousandth of a millionth of a second, thus changing the information stored

Another important application of solid-state physics, the so-called optical 'maser', has also come from the Bell Telephone Laboratories during the past two years Maser is a short word for microwave amplification by stimulated emission of radiation The principle of atoms being raised to excited states by absorption of radiation, and afterwards being stimulated by radiation to give out their stored energy, has been known in science for a very long time The maser consists of a synthetic ruby crystal containing chromium ions which can absorb and store light in excited states of the chromium atoms After a time the stored energy is emitted in a powerful short flash, which in some cases has reached the equivalent of 1,000 kW in intensity The radiation is emitted in an extremely narrow frequency band, and the beam of radiation can have a divergence of less than one minute of arc Already this has been used to illuminate an area of two miles diameter on the Moon and reflexions observed The very narrow, powerful beam can pierce holes in refractory metals, since a volume having a diameter of a hundredth of a centimetre can be raised to a temperature of about 8,000° C There will be applications in biology and many other fields, so again new vistas open

Equally important developments are proceeding in the field of energy Modern prospecting methods continue to discover vast new deposits of fossil fuels Uranium, from being an extremely scarce commodity ten years ago, has now, for a time, become almost too abundant At the same time, the cost of converting heat to electricity falls year by year, perhaps stimulated by the coming competition between power stations fuelled by coal and oil, and power stations fuelled by uranium Nuclear power is a good example of the way a great industrial application draws on the knowledge created by forty years of basic research in nuclear physics, followed up with intensive applied research once a clear goal appeared Since our fuel requirements for electricity production are forecast to increase from the present level of 60 million tons of coal a year to the equivalent of 180 million tons by the end of the 1970's, and it seems unlikely that more than 100 tons of our home coal production can be used for electricity production, it seems likely that nuclear power developed in Great Britain will increase from the 5 million kW output forecast for 1968 to much higher levels in the 1970's

Several interesting researches into new methods of converting energy may come to fruition in the next decade The fuel cell promises to provide a method of converting chemical energy directly to electricity New electronic devices such as diodes filled with cæsium vapour or other plasma devices may provide a new method of converting heat to electricity to supplement our present methods The development of power by the fusion together of the light elements seems still to be in the research stage, and practical applications are somewhat far away Nevertheless, they do provide a clear goal and a tremendous challenge for the future

The contributions of chemical and biological research to industrial growth have, of course, been equally important to those of physical research We all know of the developments made possible by new fibres, new plastics, antibiotics, insecticides, selective weed-killers, etc We shall see some examples of this work during our visits this week

One of the important problems facing us to-day is whether the organization of applied research in Great Britain can be improved, and, in particular, whether Government help to industrial research can be improved in effectiveness

We have, I think, a good deal to learn from some American organizations who have a consistent record of success in developing new products by objective basic research followed by applied research and developments Some of our large industrial organizations have also been very successful in creating new products, but research activity is overwhelmingly concentrated in these large organizations There seems to be a critical size for creativity in industrial research

A recent inquiry by the Federation of British Industries showed that the total qualified research personnel in British industry has doubled over the past ten years while expenditure has probably quadrupled On the average about 10 per cent of this expenditure is devoted to basic objective research, 35 per cent to the development of existing processes, 35 per cent to new products and 20 per cent to general services The highest ratio of qualified research workers to total employees is in the specialist medium-sized pharmaceutical and electronic firms and is next highest in research-based industries such as chemicals and electrical engineering In traditional industries such as food, drink, building and textiles it is much lower

The Federation's inquiry shows that five-sixths of the projects undertaken have found commercial applications or are likely to do so—a remarkably high figure A large proportion of firms would expand their present research activity if adequate resources were available The main limitation on expansion is a lack of qualified research workers, and at present 13 per cent of their available posts are unfilled

The survey states that few small firms have any qualified men engaged on research or capable of applying it However, we can take some comfort from the address by Dr D W Hill to the Parliamentary and Scientific Committee on the work of the research associations which particularly help the smaller-scale firms Dr Hill calculated that, on a conservative estimate, the benefit to industry from the work of the research associations is now more than £100 million a year—a good return for an expenditure of £8 million a year The work of the Shirley Institute, which some of us will see, is a good example of this Apart from its direct contribution to the textile industry, the Shirley Institute, together with the British Iron and Steel Research Institute, was largely responsible for introducing operational research into industry The Wool Industry's Research Association invented the powerful tool of partition chromatography, the world-wide importance of which was recognized by the award of the Nobel Prize to the inventor The major problem in applying the work of the research associations seems to be in increasing the number of technically trained men in smaller-scale industry

From all this evidence, it is clear that our ten-year programme for doubling the output of scientists and engineers is well justified especially when we bear in mind the importance of getting a much higher proportion of scientists and engineers into managerial positions The shortage of highly qualified mathematicians, scientists, and technologists will be a major difficulty to the universities during their expansion of the next decade An Institute of Physics and Physical Society inquiry has shown that we will need to increase the input of physicists to university staffs by 2½ times if we are not to lower standards during the expansion At the present time we are losing too high a proportion of our best scientists to the United States—20 per cent of our PhD's in physics—and this proportion is likely to increase as the United States giant space-travel programme accelerates The best way to reduce this drain is to improve our research facilities at home There has been a welcome increase in the Department of Scientific and Industrial Research grants to universities for equipment and technicians during the past six years, and this has been very beneficial, but we need a further steadily continuing increase to match the growth of our research schools and the increasing complexity of research tools

Not only the physical and biological sciences need more support The Committee of Section N of the British Association which deals with sociology, social anthropology and social psychology reported that these three social sciences have suffered acutely from the lack of research funds to make it possible systematically to build up knowledge and valid generalizations in their sphere Grants from the United States foundations on which social sciences have relied rather heavily in the past are no longer as available as they were ten or fifteen years ago, since the United States foundations are directing their help increasingly towards the under-developed countries

These foundations argue, and justly so, that British social research should be financed to a far larger extent from British sources A more substantial provision of postgraduate scholarships is urgently needed, together with the complementary funds which would enable the students to undertake significant research Without assistance of this kind the Committee considers that it will be impossible to meet the demands for well-trained, responsible social research workers and university teachers

In considering our future investment in science, we have to take account of the increasing proportion of our funds for basic research which are going into international scientific organizations We have long been familiar with international conferences sponsored by the scientific unions and the International Council of Scientific Unions and with the International Geophysical Year, and other large-scale and world-wide activities The cost of these activities has been small relative to our overall scientific budget, and they have paid good dividends

We have now, however, reached the stage where several forms of science are becoming so expensive that only by international co-operation can smaller countries take part

The foundation of CERN, the European Council for Nuclear Research, devoted to the construction and operation of two large nuclear accelerators, at a capital cost of more than £15 million and an operating cost of £7–£8 million per annum, of which Britain pays a quarter, is a portent of things to come CERN has turned out to be extraordinarily successful both technically and scientifically, and has restored to Western Europe the possibility of working in the forefront of high-energy nuclear physics Physicists from Britain, France, Germany, Italy and other European countries now build in their own laboratories extremely expensive and complicated equipment such as liquid hydrogen filled bubble chambers, and move them to CERN where they are used by international teams This collaboration has been made possible by adequate Government grants for travel and assistance for university scientists We may, within two or three years, be faced with the problem as to whether to build a still more powerful nuclear accelerator at much greater cost to penetrate still deeper into the nuclear underworld If there turns out to be strong scientific reasons for this, it may need, for economic reasons, to be built as a combined United States–European project or even a world project

Space research is another expensive field of science and already collaboration between ourselves and the United States has enabled us to launch the first U K 1 satellite, using an American *Thor* rocket as a launcher Quite a number of experiments are proceeding well at the present time We are likely soon to see the coming into being of the European Space Research Organization, which may well have a budget three times as large as CERN We are therefore well within sight of spending about a quarter of our budget for basic research in international organizations We shall need to be very careful that, in periods of financial stringency, these investments do not reduce the funds available for basic research in our universities

We have so far been fortunate in not being tempted to enter the field of space travel, as distinct from space research The United States is already spending between 2 and 3 billion dollars a year on the programme of landing a man on the Moon, and the Russians are probably spending an equivalent sum According to *Izvestia*

> " a Russian-manned flight to the Moon will be followed by the establishment of a scientific station and ultimately by industrial-type installation Then will follow flights to Mars and Venus"

Although we can be excited by the prospect of landing on the Moon or Mars and collecting scientific information about their terrain and atmospheres and any evidence of life, there is no doubt that these ventures are being undertaken mainly for prestige reasons and as an instrument of power politics They may seriously distort the pattern of scientific development through the diversion of very large numbers of engineers and scientists to these objectives When we remember the statement of Paul Hoffmann, director of the United Nations Special Fund, that a contribution of 2 billion dollars a year to under-developed countries was required to increase the *per capita* income of these countries by 25 per cent in a decade, we must sadly conclude that world priorities have gone badly wrong

The final report of the Colonial Research Council reminded us that in recent years Britain spent about £2 million a year in supporting research in more than fifty establishments in Colonial Territories, mainly in the field of agriculture and medicine, and their contributions were great The need for continued help is clear if the battle against tropical diseases and tropical pests is to be overcome and agricultural production raised more rapidly than the increase of population—which is about 3 per cent per annum in African territories. The recent dissolution of the West African Intergovernmental Research Organization and reports of the exodus of professional workers show that the future is by no means clear

For all these reasons, we need to increase our investment in science just as rapidly as our human and financial resources permit Two years ago the United States President's Scientific Advisory Committee advised the President

> " . that simply in terms of economic interest our proper course is to increase our investment in science just as fast as we can to a limit not yet in sight"

The U S National Science Foundation now predicts that in their country investment in basic research in the universities alone will increase from 900 million dollars in 1962 to 2,700 million dollars in 1970 If we wish to maintain our position in the highly technological world of the future, we need to do as much and not only at least to double the proportion of our gross national product (now 0 2 per cent) devoted to basic research during the next decade, but to devote a much greater proportion of our applied research effort to furthering the development of new products and new industries and less to maintaining the position we held in the world of the past

The very great contributions which investment in science can make to human welfare in the future depend on achieving a reasonable degree of political stability in the world In his presidential address last year, Sir Wilfrid Le Gros Clark said that

> " the frightening question is beginning to present itself whether the civilization which mankind has slowly and laboriously built up over a period of many thousands of years can avoid disastrous dissolution as a result of uncontrollable struggles for political power or economic superiority and indeed whether the human species can avoid at least partial extinction by the misapplication of its own ingenuity"

The development during the past year of bombs which can destroy by heat alone everything within a radius of 20–30 miles below the point of burst has carried the world still further along the road to destruction

The dangers are so obvious and extreme that scientists and professional people who have special knowledge should make whatever contributions they can to solve the technical problems of the 'controlled and complete disarmament' which is now the declared objective of the United States, the U S S R and the British Commonwealth Soon after Sir Wilfrid spoke, an informal conference on Science and World Affairs met in Stowe, Vermont, and made some contributions to the solution of the most difficult of the technical problems—that of constructing an inspection system acceptable to both sides I hope that more can be achieved at the next conference, which is now in progress We must realize, however, that the great difficulties in achieving disarmament are political and not technical, and, if there was a real will on the part of all major powers to disarm, it could be achieved, releasing enormous resources for diversion to urgent needs of Britain and less-fortunate countries.

SUMMARIES OF ADDRESSES OF PRESIDENTS OF SECTIONS

LIGHTNING AND THE LONG ELECTRIC SPARK

SIR BASIL SCHONLAND'S presidential address to Section A (Mathematics and Physics) was preceded by an introductory film, made by Ferranti, Ltd , outlining the present knowledge of the generation and distribution of electricity in thunderclouds and describing the various processes in the lightning flash The typical flash to ground involves five separate processes an initial trigger discharge in the base of the cloud, a pilot and a following stepped leader lowering charge towards the ground, which, in the case of subsequent strokes or partial discharges, are replaced by single dart leaders, and finally in each case upward return processes starting when the leaders are close enough to some projecting object Interpretations of the detailed mechanisms of these processes are based on electric field and current measurements of high time resolution, on their velocities and on fast camera studies of their variation of luminosity with time

The most frequent value for the quantity of electricity carried down on a leader is 4 coulombs From recent measurements of the heights from which leaders start, the potential difference between cloud and ground can be re-evaluated as 10^8 V This provides only 350 V/cm as the potential gradient in the leader channel and leads to the conclusion that it has at its centre a narrow conducting core with arc characteristics This core is surrounded by a corona or ionization envelope of much larger diameter with glow characteristics At the front of the advancing channel the glow takes the form in virgin air of a controlled electron avalanche which is periodically caught up by the leader In subsequent dart leaders it makes use of pre-existing ionization

The breakdown processes in the long electric spark closely resemble those in lightning, and the step process has been identified in sparks in air, oil and water, on a scale 1,000 times smaller

Further investigations on sparks and lightning are required to provide fuller quantitative explanations of these phenomena on an atomic scale So far it can be deduced that there are two basic atomic processes governing breakdown in air—ionization, which sets a minimum electric field for advance (30,000 V/cm in air), and electron capture, which can only be avoided in the pilot channel behind the tip if the field there exceeds 4,000 V/cm The step process can be interpreted in terms of these fields

The radius of protection of Franklin's rod has now been explained quantitatively in terms of measured quantities in lightning leaders In the form of a horizontal earth-wire strung between the tops of power-line towers it is of crucial importance Recent unexpected failures in such protection, not necessarily involving interruption of electrical supply, but troublesome and connected with increasing heights of towers, require interpretation

An explanation of the occurrence of ball lightning in the Alps is offered in terms of two features special to this region The first is the recently discovered powerful ground flash of long duration from the tops of thunderclouds to mountain peaks, the second is the tendency of the ground currents supplying current to the return processes of such flashes to follow the beds of streams in Alpine regions of high rock resistivity Ball lightning may consist of bubbles of reactive gas occasionally produced by the heavy current in these stream-beds

CHEMISTRY OF TANNINS

PROF R D HAWORTH, in his presidential address to Section B (Chemistry), reviews the recent progress made in the elucidation of the chemical nature of vegetable tannin extracts which, until the advent of numerous modern techniques of isolation and analysis, has remained a difficult and therefore largely neglected field of study Although plant extracts may contain more than one type, vegetable tannins are classified as hydrolysable or condensed dependent on their action towards acids and enzymes The former group is conveniently sub-divided into gallotannins and ellagitannins, which give predominantly gallic acid and ellagic acid respectively on hydrolysis

The gallotannins, which are amorphous and probably mixtures of isomers and closely related substances, were originally investigated by Emil Fischer and have the general structure of polygalloyl carbohydrate derivatives, the carbohydrate is normally glucose, but several are now known, such as Tara tannin, which is a polygallolyquinic acid, in which the glucose is replaced by other carbohydrates or related substances The galloyl groups in the gallotannins are of two types, those linked directly to the carbohydrate and those linked depsidically to other galloyl groups, and although recent work has shown accurately how the former are distributed in Chinese, Sumach, Turkish and Tara gallotannins, the arrangement of the latter is probably random and remains unsolved

Closely related to the gallotannins are the ellagitannins, which are often crystalline, and elegant schemes for their derivation have been suggested. In general the ellagitannins are probably formed by oxidative coupling of adjacent galloyl groups on a simple galloyl glucose and the hexahydroxydiphenoyl group thus produced may then undergo modifications by oxidation, reduction and hydrolysis to give the many novel structures found in this group Compared with the state of knowledge of the structure of the hydrolysable tannins very little is yet known concerning that of the condensed or flavanoid tannins, which are amorphous and characterized by their conversion with acid to the insoluble highly coloured phlobaphenes They contain little if any glucose, but are thought to possess a common structural basis and to be derived by postmortal polymerization or polycondensation of the frequently occurring hydroxyflavan-3-ols and hydroxyflavan-3,4-diols Several dimeric products of these C_6–C_3–C_6 types have been isolated and characterized following acid-catalysed polymerization of the flavan-3-ol catechin, and these, it is suggested, are typical of the initial products formed in the complex process Other workers have suggested alternative oxidative pathways for the formation of these tannins, but this remains a field of study in which further work is clearly necessary

The developments made in the chemistry of the vegetable tannins concomitant with an increasing knowledge of the nature of the protein collagen chains in hide indicates the possibility in the near future of a greater insight into the chemical processes involved in the conversion, by the action of tannins, of animal skins into leather

PHYSICAL BACKGROUND OF BRITAIN IN THE PLEISTOCENE

THE "Physical Background of Britain in the Pleistocene Period" is described by Prof F W Shotton in his presidential address to Section C (Geology)

The fluctuations of climate in Britain during the Pleistocene may well have been more rapid than at any previous time in the country's geological history Associated with the waxing and waning of the glaciers must have been impressive shifts in the plant and animal populations, fed during the interglacials from the European mainland The argument is advanced that the Dover isthmus was established very early in the Pleistocene and thereafter remained unbreached until the well-authenticated cutting of the Straits about 7,000 years ago By contrast, it is suggested that there was no land link between Ireland and the rest of Britain until the last (Wurm) glaciation

There is much evidence available to the geologist by which he can evaluate climate It is almost certain that when the large ice sheets were established, dominant wind directions were greatly changed by this fact North-easterlies rather than south-westerlies would dominate Summers would be colder than now, but winters very much colder The very existence of glaciers down to the Bristol Channel and Essex demands a lowering of mean annual temperature in excess of 9° C, while the widespread frost structure soils (polygons, stripes, ice-wedges) entail a fall exceeding 7° C When these structure soils develop on a massive scale—polygons 20–100 ft across, stripes 20–50 ft apart—it can be assumed by analogy with the Arctic and Antarctic that 'permafrost' was present, that is, ground frozen winter and summer only a few feet below the surface One of the most spectacular features of air photography is the frequency with which corn crops, as they ripen in late July, pick out these large-scale patterns Other evidences of permafrost in Britain are pingoes, ice mounds, the dry valleys of limestone country and valley-bottom folds. There were certainly several separate episodes of permafrost and at each it may be argued that the mean temperature was depressed by 13° C or more.

A chemical method for evaluating temperature and humidity is provided by weathered soils (not common in Britain). In the Atlantic, Emiliani's work on the oxygen isotope ratios of foraminifera tests in deep sea cores suggests a damped down version of the more violent fluctuations on land, with an extreme variation of the annual mean of about 6° C.

The record of vegetational changes is of great importance and can be related to the physical conditions governing such features as the tree line, and the occurrence of the mixed oak forest. Some plants, like the holly and ivy, are indicators of winters which seldom went below freezing point.

Among vertebrate fossils, the musk ox, reindeer, arctic fox, lemming and a few others allow comparison with the arctic or sub-arctic, while hippopotamus and the fallow deer are equally indicative of mild conditions. Some extinct animals can now also be clearly related to cold or milder climatic conditions as well as to habitats, though exact estimations of climatic parameters are difficult.

The growing study of beetles is providing an ever-increasing number of opportunities of estimating climate as well as ecology. An assemblage of beetles can sometimes be placed closely within the series of plant zones which occur above and below the tree line, and these can again be translated into physical terms of temperature, particularly summer ones. In four English examples, all chosen from interstadials of the Last Glaciation and with ages ranging from 57,000 to 13,600 years, it is argued that the summer temperature was as little as 2½° and as much as 5½° below the present day.

FRONTIERS OF KNOWLEDGE IN THE STUDY OF SENSORY FUNCTION

In his review of recent progress in the field of comparative sensory physiology, Prof. O. Lowenstein, president of Section D (Zoology), deals with sense organs classified into three groups, according to the physical nature of the stimuli to which they respond. Electro-magnetic waves (light and radiant heat) are the appropriate stimuli for photo-receptors, mechanical deformation of parts of an animal's body are registered by mechano-receptors and chemoreceptors respond to changes in the chemical make-up of the external and internal 'environment' of the body. Up to fairly recently, sense physiologists were chiefly interested in exploring the range of sensory perception available to various animal types, and in an analysis of the range of functions of the various receptor organs. The focus of interest has now shifted to an analysis of the mode of function of sense organs on the cellular level. We are rapidly reaching a point where the ultimate understanding of the physicochemical processes involved in receptor function would have to be studied on a molecular level.

The chief topic of Prof. Lowenstein's address with reference to all three fields of receptor function is the question of transformation of the energy reaching the receptor organ in the form of its appropriate stimulus into the electrical energy of the final signal which is conveyed from the sense organ to the central nervous system in the form of propagated all-or-nothing action potentials.

All sense organs are incredibly sensitive. Their sensitivity thresholds lie in most cases near the limits of self-stimulation by molecular agitation. The energy content of stimuli at threshold is therefore minute. One quantum of light is sufficient to set up an excited state in a single visual rod in the retina of the mammalian eye. Such a rod presents a target of two thousand million photo-sensitive molecules of visual pigment to an incident light beam. A dent of half of a thousandth part of a millimetre in the nerve-ending of a tactile receptor and probably one molecule only of a scent are sufficient to set going the processes involved in the sensory perception of the stimulus. It is quite clear that the electrical signals which are generated by these stimuli and which are the carriers of coded information from sense organ to central nervous system have an energy content many powers of ten higher than the stimuli. A process of amplification must therefore be incorporated in the sensory mechanism.

One of the chief problems confronting the sense physiologist of to-day is to find out how an electromagnetic, mechanical or chemical stimulus is transformed into the electrical sensory message. This process of stimulus transduction can only be unravelled by an examination of the ultra-structure of the sensory cell and by a minute analysis of the physicochemical events going on in it.

The electron microscope has in recent years given us a chance to come to grips with this problem. The ultra-structure of visual cells, of sensory cells in the inner ear, of postural and chemo-sensitive receptors in vertebrates and insects, of the receptor cells in the mammalian nose and tongue has been investigated among others. A few tentative first steps have thus been taken in the elucidation of the fundamental receptor mechanism. Refined recording techniques based on the use of micro-electrodes and high-power biological amplifiers are being utilized in the attempt to analyse the mechanism of stimulus transduction. A few first victories in this offensive have been won and the fact has emerged that great fundamental similarities exist between the cellular processes in all the provinces of sensory function which have been studied by these methods. Exciting new information has also been gained in the field of the study of colour vision, especially in insects. Still great are the gaps in our understanding, and it has to be conceded that a real break-through will not happen until we are able to push forward the line of attack on to the molecular rather than cellular level of biological organization.

NUBIA: AN AFRICAN FRONTIER REGION

The building of President Nasser's High Dam makes Nubia of special interest to geographers.

First there are the physical effects of the Dam. Between November 1964 and November 1975, the Nile waters will rise by stages to some two hundred feet above maximum recorded levels, thus transforming the physical geography of Nubia. When the reservoir is full, a great lake will be formed, 300 miles long and 16 miles wide in places, bisected by the international frontier which divides Egyptian from

Sudanese Nubia This lake should not, however, be given two names—"Lake Nasser', for example, or "Lake Abboud" In memory of an ancient land and people, it should be called "Lake Nubia"

The High Dam is already beginning to disrupt the human ecology of Nubia, and the first of 100,000 Nubians are now being evacuated from their homeland Racially and linguistically, in their social organization and in their tribal traditions and beliefs, the Nubians, many of whom have lived for generations in comparative isolation, are a people of very ancient origins Much attention is being devoted to the ancient Egyptian monuments in Nubia, but little has been said or done about this human aspect of Nubian studies, about the need for research into the sociology and human ecology of modern Nubia The evacuation of the Nubians from their homeland, some to an environment as different as that of the Atbara River, is bound to result in some disintegration of the fabric and traditions of Nubian society Here a chance to study an ancient African community before dispersal has been missed

The High Dam also threatens to destroy an ancient cultural environment the geographical history of which forms the theme of Mr L P Kirwan's presidential address to Section E (Geography) The northern frontier of Nubia, racial and linguistic, lies in the neighbourhood of the First Cataract and is marked physically by the transition from limestone to sandstone and by the Pre-Cambrian rocks of the First of the Cataracts of the Nile The First Cataract was known to the ancient Egyptians and medieval Arab geographers as "The Gateway"—the gateway to the south It marks not only the northern boundary of Nubia but also the boundary between Near Eastern Egypt and an African frontier region leading to the Sudan

Nubia's traditional southern frontier lies near the Third Cataract This was the opinion of the Swiss traveller, John Lewis Burckhardt, the one hundred and fiftieth anniversary of whose first great Nubian journey falls this year Burckhardt, among many claims to fame, was the first, on this journey, to discover the Great Temple of Ramses II at Abu Simbel and five years later made the first excavations there in the drift sand then covering the temple He carried out his journey and these excavations on behalf of the African Association of London which was later absorbed into the Royal Geographical Society

Between these two frontiers, Nubia has been since about 2500 B C an almost rainless hilly desert region, with only a fringe of cultivation along the river The geographical value of the country can be summed up in terms of trade, mineral wealth, particularly gold, and the defensive advantages of Nubian terrain In ancient times, the shortest trade route linking the Mediterranean with Central Africa ran through Nubia, the key to it being the Third Cataract area from which trans-desert routes led to the Southern Sudan, cutting off a great bend in the Nile The Third Cataract area was reached by Egyptian explorers in the third millenium, among them Harkhuf, "the first explorer known by name in African history" Trading expeditions continued to use this route to the wealth of Central Africa in Graeco-Roman times One of these, launched by the Emperor Nero in the first century A D, reached to within some 500 miles of the Congo and Uganda borders, a feat of exploration unequalled until the nineteenth century

Nubia's principal mineral resources were stone, copper and, by far the most important, gold, the source of which was the rocks of the Basement Complex Possession of the gold deposits east of Lower Nubia induced the Pharaohs to occupy the country up to the Second Cataract about 2000 B C Four centuries later, gold, and the capture of the terminus of the trans-desert trade routes to the Central Sudan, were the motives behind the Egyptian colonization of Nubia, and of the Nile Valley between the Third and Fourth Cataracts

The key to defence in Nubia was the Second Cataract area This was Ancient Egypt's frontier for two centuries, and the disposition of the massive mud-brick forts there, now being excavated, shows how alive the Egyptians were to the advantages of Nubian terrain Conversely, in Graeco-Roman and in medieval times, the Second Cataract was the main line of defence for the Sudan

Valuable as Nubia was economically or as a defensive zone in ancient times, it was not then that she reached the zenith of her short-lived prosperity Even under the Pharaohs who have left so many monuments in Nubia, "the level of life, and of art, was never better than provincial" Much is now being written about Egyptian art in Nubia, but the Egyptian temples there owe more to the effects of an unusual and striking environment than to their qualities of sculpture or relief This is true even of the temples at Abu Simbel The Egyptian architects were very conscious of the need to relate architecture to environment Carved from the cliff-face of a sandstone plateau, "these massive memorials of arrogant, authoritarian power" are—in their present position—part of the geography of Nubia It is very doubtful whether they can ever be made to appear as their architects intended when they are lifted to their new position on the shores of "Lake Nubia"

It was not in Pharaonic but in Byzantine and medieval times that Nubia reached her zenith This was partly due to the introduction by the Romans of the ox-driven water-wheel which revolutionized agriculture and greatly increased the population It was also due to the importance of Nubia's geographical position in the strategy of the Byzantine emperors of the sixth century Nubia was brought within the orbit of the Empire through her conversion to Christianity in the middle of the sixth century by missionaries not from Christian Egypt but from Constantinople One of the most important and sensational discoveries made in Nubia in the past two years has been of a series of remarkable Christian wall-paintings at Faras in Sudanese Nubia These, discovered by an expedition sponsored by the Polish Academy of Sciences and the University of Warsaw, show strong Byzantine influence and confirm the direction from which Nubian Christianity came With them was discovered a memorial inscription in Greek which seems to commemorate the first Bishop of the Sudan

Christianity survived in Nubia until the fourteenth century, that is to say, for seven hundred years after the conquest of Egypt by Islam Early in the fifteenth century Nubia, following the invasion by the Ottoman Sultan Selim I, became nominally a frontier province of Egypt In fact, it was largely independent, a frontier zone, implacably hostile to all foreigners Even Napoleon's soldiers failed to enter Nubia, and it was not until Burckhardt's journey 150 years ago that a European succeeded in travelling the whole length of the Nubian Nile

ECONOMICS OF UNDER-DEVELOPED COUNTRIES

IN discussing the economics of under-developed countries before Section F (Economics), Mr W B Reddaway bases his presidential address largely on conditions in India, as analysed in his book *The Development of the Indian Economy*

There is no need for a different set of fundamental economic principles when studying under-developed countries, but the economic problems of these countries are normally different, at least in degree, and the background assumptions which one should make in studying any particular problem are also different These background assumptions do not correspond with those appropriate for analysing a Western economy on either of the traditional assumptions ('full employment' or 'recession')

The low level of output per head in under-developed countries is commonly attributed to lack of capital equipment and cultivable land, but much could be done by better methods of working to raise output per acre or per unit of capital These are usually better yardsticks of success than output per man

Nevertheless, it is most important to increase the stock of capital equipment of many kinds One great difficulty for under-developed countries is that most machinery must be imported unless there is a ready export market for things which they can easily produce, a development programme is likely to create an acute balance of payments problem, which cannot be overcome merely by willingness to save

Some useful capital assets, for example rural roads can, however, be created in the country by putting 'surplus' labour to work with simple tools and local materials, none of which presents supply problems Nevertheless, one cannot rely on the Keynesian multiplier process to generate the necessary savings to cover this expenditure the main secondary demand will be for food, the output of which is not limited by lack of demand A programme of additional investment in public works financed by credit creation would lead mainly to a balance of payments deficit or (if imports were controlled) to rising prices

The amount of rural road-making, etc , could, however, be expanded without curtailing other production if the expenditure were covered by taxation, or by aid from abroad in the form of surplus food-grains

An inflationary process is liable to be more rapid in an under-developed country because the profits which it generates do not typically accrue to companies (and so go largely into savings or tax provisions, with the remainder distributed later to shareholders), but rather to farmers and small traders, who may well spend them immediately

The development process renders the economy of a backward country very much more complex Each productive unit is more dependent on the proper functioning of many others, and on the marketing network Above all, the whole economy is dependent on the apparatus of Government, taken in the widest sense, attaining at least a certain standard of effectiveness

Developed countries can help by providing not only 'aid' (in the form both of loans, etc , and of technical assistance), but also trade facilities Admission of 'cheap Asiatic textiles' to their markets causes difficulties to particular producers, but these are as nothing compared with the problems which exclusion of such goods causes to the whole development of the poverty-stricken countries of Asia

TIME THE CATALYST : OR WHY WE SHOULD STUDY THE MATERIAL CULTURE OF PRIMITIVE PEOPLES

IN his presidential address to Section H (Anthropology), Mr Adrian Digby discusses this question

If we are to understand him properly, not only man but also his works should be the proper study of anthropology Thirty years ago this was recognized by the broad division between physical anthropology and cultural anthropology, which was itself divided into a major aspect, social anthropology, and a minor partner, material culture studies The common factor then was the primitive nature of the people to be studied But to have any practical utility, anthropology must extend its studies to more advanced societies, including our own, and to the reactions of these societies to new stimuli and to technological advance In pre-war days we regarded anthropology as, among other things, a convenient tool for the sympathetic and understanding administration of colonial peoples To-day, when more and more new nations are gaining their independence, and acquiring at a tremendous rate new technological processes and new political and social institutions from our own extremely complicated civilization, it is more important than ever to understand the effects of technological change on their social institutions, when we meet them as equals In particular the time taken in the new demands created by new techniques will have a profound effect on their social institutions

British social anthropologists, following the lead of Malinowski and of Radcliffe Brown, have concentrated intensively on the working of social institutions and their effect on the integration of society as a whole

These studies have not necessarily been limited to primitive communities, but are equally applicable to institutions in advanced communities The material and technological sides of man's activities seem to have been neglected, or in any event underestimated so far as their effects on social institutions are concerned

The historical approach to anthropology, exemplified in its extreme (and most unscientific) form by the writings of Elliot Smith and Parry, have been rejected as irrelevant

The students of material culture and primitive technology, tending to ignore social problems, and using techniques strictly comparable with those of the archæologists, have retained an evolutionary and historical approach, using the concept of diffusion, with caution, as a valuable historical tool Much of the work on material culture is relevant to archæologists if only by way of analogy and by the interpretation of the imperishable fragments of composite artefacts

But the relevance of technological studies to social anthropology, implied in the old term 'cultural anthropology', is relevant to modern social studies Social institutions must be influenced by the material needs of a community and by the time and labour involved in satisfying them Time and amount of labour will vary according to the techniques used

At a symposium of Section H in 1948 at Brighton, the effect of technique on social institutions was considered, but the conclusions suffered from lack of data on the time factor More recently McCarthy and McArthur made a careful study of the time occupied in the food quest by two groups of Australian aborigines, from which significant conclusions could be drawn Further, Manning Nash has recently published a study of pottery-making in a Mexican village in which he showed that the overall factor determining the output of pots was the incidence of fiestas He also drew general conclusions on the impediment to pottery-making caused by the presence in a family of young children and of the advantage held by families where there were enough women to introduce something akin to production line methods The value of these generalizations would have been enhanced if the time involved in the various processes of manufacture had been obtained

The method of timing operations will be difficult in large or complicated societies, but by careful sampling, combined with orthodox methods of anthropological research, valuable information should be obtained It is most important to obtain information on primitive techniques before it is too late

LIPID CO-FACTORS IN BIOCHEMISTRY

IN his presidential address to Section I (Physiology and Biochemistry), Prof R A Morton considers "Lipid Co-factors in Biochemistry"

Many enzymes have prosthetic groups, which may be detachable coenzymes These sometimes contain a water-soluble vitamin which is the locus of the substrate–coenzyme interaction

It is reasonable to postulate that fat-soluble vitamins may also act as co-factors in enzymatic processes The modes of action of lipid co-factors present difficult problems and progress has been relatively slow

The concept of a vitamin is now somewhat blurred Thus vitamin A has carotenoid precursors which in the animal organism yield vitamin A alcohol This is followed by ester formation and liver storage, and although the vitamin is mobilized as alcohol it may function as a derived compound Similarly, cholesterol is synthesized by the animal and dehydrogenated in the gut wall to yield provitamin D_3, which yields some cholecalciferol (vitamin D_3) under the action of ultra-violet light The status of vitamin D as a dietary essential is thus weakened although its efficacy under test conditions is not in doubt Vitamin E also has lost simplicity, the term tocopherol now refers to several natural substances varying in 'activity' The distribution of tocopherols in plant and animal tissues does little more than emphasize an antioxidant role

Vitamin K_1, with its phytyl side-chain, is found only in plant products and in fact has become somewhat elusive The numerous vitamins K_2 are 2-methyl-1,4-naphthoquinones with a polyisoprenoid side-chain at position 3 Vitamin $K_{2(20)}$ has a $C_{20}H_{33}$ side-chain and is peculiar to animals Vitamins $K_{2(30)}$, $K_{2(35)}$, $K_{2(40)}$, $K_{2(45)}$ are all found in micro-organisms Vitamin K-antagonists point to an enzymatic role and the lack of sharp specificity of chemical structure in relation to 'activity' raises difficult problems α-Lipoic acid leads to a useful linking of a fat-soluble co-factor with enzymatic processes catalysed by thiamine pyrophosphate

The blurring of the vitamin concept has been followed by the discovery of new endogenous lipid co-factors which are readily synthesized but which deserve to be called essential metabolites Thus plastoquinone (a 2,3-dimethyl-1,4-benzoquinone with a $C_{45}H_{73}$ side-chain at position 6) occurs in the chloroplasts of leaves and is perhaps more significant in photosynthesis than vitamin K_1 The alcohol solanesol ($C_{45}H_{73}OH$) occurs in plants and a cyclic isomeride of plastoquinone, solanachromene, is obtained from tobacco The ubiquinones are 5-methyl-2,3-dimethoxy-1,4-naphthoquinones with a polyisoprenoid side-chain $(C_5H_8)_n$-H, where *n* is 6–10

The compound ubichromenol (50) is isomeric with ubiquinone (50) and bears the same relationship to the latter as solanachromene does to solanesol

With regard to lipid co-factor functions real progress has been made, but baffling difficulties persist For example, retinene$_1$ (retinal or vitamin A aldehyde) is the key compound in vision, with special emphasis on one *cis*-isomer which combines with an amino group in a specific protein before a visual pigment can be formed Vitamin A acid (retinoic acid) can fulfil some of the systemic functions of vitamin A alcohol (retinol) but it cannot sustain vision, reproduction or some aspects of bone growth It may be that vitamin A alcohol is the precursor of a more powerful but very labile catalyst

The evidence on vitamin D suggests that in rats it tends to prevent rickets, when the causative low phosphate intake is marginal

Vitamin E occurs in animal tissues and its distribution in cell components does not exclude a major catalytic role, despite complicating evidence that synthetic anti-oxidants permit reproduction over three generations on a vitamin E-free diet The multiplicity of syndromes in different species and the response of some but not all, to catalytic amounts of selenite or organically bound selenium complicates an already difficult situation

The possibility that α-tocopherol, vitamin K and ubiquinone function in electron transport and oxidative phosphorylation has been much canvassed, and will be discussed by Dr F A Holton and Dr. E R Redfearn Plastoquinone is a key substance in photosynthesis and in photophosphorylation Vitamins K seem to function in plants and in many bacteria Some micro-organisms produce ubiquinones while others produce vitamins K, and a few produce both Tocopherols on oxidation yield quinones, and it seems that quinones may act as phosphate carriers via chromans or chromenes

Knowledge of the modes of action of lipid co-factors is in transition; most of the pieces of the puzzle are possibly to hand and bits of the picture are probably correctly assembled, but the major synthesis has still to be made The discovery of new quinones has transformed several topics and new techniques have radically altered the approach via enzymology and biosynthesis

IDENTIFICATION AND USE OF TALENT

TALENT in the sense of general mental efficiency, operational and latent, actual and potential, is identified and assessed by psychologists by means of tests This forms the theme of Mr Denis McMahon's presidential address to Section J (Psychology) These have certain advantages, but some scientific difficulties can arise which can lead to circular arguments From test evidence, which is backed by other evidence, it is clear that even before the 11-plus stage many able children have ‘opted out’ After 11, differences in performance in intelligence tests appear to reflect the goodness of the education and other environmental influences to a significant degree Home and social conditions affect even more markedly the ‘actual’ (as against the ‘expected’) school performance all the way through grammar schools and, in particular, the early leaving rate The social factors associated with these differences have now been identified It is now very clear that well before the university entrance stage a large proportion of the nation’s talent has dropped out—much of it sunk without a trace

It is complacently hoped that much of this talent is identified, trained and used in industry and commerce, Mr McMahon shows that there are no grounds whatever for such complacency

Individual variations of ‘vocational maturity’ are so great that all doors leading to careers should be left ajar as long as possible Examples are given of people “finding themselves” very late in their lives and, when given opportunities because of an open-door policy, making important contributions to the nation’s health, wealth, and happiness

Mr McMahon concludes with another aspect of developing latent talent and with an attempt to correct the tendency for the emergence of two nations in terms of culture and education A sketch plan is made of a scheme to use television programmes in the evenings in combination with tutoring provided by a local university—the aim and hope being the encouragement of an educated democracy

PLANT RESPIRATION AND MICRO-STRUCTURE OF PLANT CELLS

TO-DAY we realize that form and function are inseparable Organization is a convenient term to express the fusion of form and function, and the purpose of Prof W O James’s presidential address to Section K (Botany) is to consider that aspect of it which is represented by the respiratory–micro-structure continuum of plant cells

He describes respiration as covering the material changes undergone by complex cellular substances leading in the aggregate to a diminution of free energy and to an immense variety of intermediate products It can then be said that the structural unit associated with this function is the cell There are already known about fifty catalysed reactions which take some part in respiration, these reactions can be conveniently grouped under the headings mobilization, glycolysis, the pentose phosphate cycle and oxidation Both the energy and the materials which are the starting point for syntheses come overwhelmingly from aerobic oxidation, and so Prof James treats the oxidation stages as the core of his subject and the others as only ancillary to it

It is now without question that the mitochondria are the site of oxidations associated with the citrate cycle, but we have no great assurance that these are the only oxidations of significance to cell respiration

All living cells which have been examined contain mitochondria dispersed through the cytoplasm without any special orientation—in a lively cell to the number of probably several hundred They are often approximately spherical with a diameter of 0 5–1 0μ but are sometimes elongated or of more irregular shape The mitochondrion has a semi-permeable layered wall of lipoprotein which folds profusely into the central chamber which is occupied by an aqueous solution containing much soluble protein Mitochondria are extracted *en masse* by homogenization and differential centrifugation

The oxidations occurring in the mitochondria involve the following stages of electron transport besides others which are less clear

Substrate	e g Malic acid	Succinic acid
Dehydrogenase	Malic dehydrogenase	Succinic dehydrogenase
Dinucleotide	NAD (P)	
Flavoprotein	NAD-cytochrome *c* Reductase	Cytochrome *b*
Carriers	Cytochrome c_1 Cytochrome *c* Cytochrome *a*	
Oxidase	Cytochrome a_3 (P)	
Oxygen	O_2	

There are also further enzymes catalysing non-oxidative stages of the citrate cycle such as fumarase and aconitase and the kinases which catalyse the accompanying phosphorylations at (P)

Several lines of evidence indicate that cytochrome a_3 (cytochrome oxidase) is a firmly anchored constituent of the mitochondrial wall Moreover, the ability to oxidize succinic acid which is retained by broken mitochondrial walls indicates that the whole of the cytochrome system together with succinic dehydrogenase must be an integral part of the wall structure Further recent investigations in Prof James’s laboratory have failed to detect malic dehydrogenase in the sap, while of fumarase activity about one-quarter was in the sap and three-quarters in the wall It is therefore a reasonable guess that the entire enzyme outfit of the citrate-cycle and its associated electron transportation are a part of the membrane architecture There is also evidence that glutamic transaminase, which aminates the α-keto acids formed in the citrate cycle, is also included in the enzyme assembly of the mitochondrial wall It also appears probable that the soluble nucleotides NAD and NADP are firmly linked with their dehydrogenases on the membrane

The mitochondrion wall, like other cell membranes, probably consists of a lipid centre with protein layers on either side When the wall is disrupted, Dr Simon found with *Arum* mitochondria that the rate at which they would oxidize cytochrome *c* increased six-fold, probably on account of increase in the accessibility of cytochrome *c* to the internally sited cytochrome oxidase, but as six-to-one is a likely ratio for the internal surface/external surface of the infolded membrane we do not have to assume that the two surfaces have greatly different concentrations of the enzyme

We may reasonably suppose that the great bulk of the catalytic proteins are anchored to the inner surface of the lipid layer and we may think of the inner layer as possessing a mosaic of protein molecules cemented on to a lipid base In surface view the inner wall may be conceived as a repeating pattern of enzyme assemblies each containing all the enzymes necessary to put a succession of substrate molecules through the oxidation cycle and even to aminate some of its members, to transfer its lost electrons to oxygen and to phosphorylate ADP The internal surface of a moderate-sized mitochondrion could accommodate more than 100,000 such units

The enzymes of glycolysis and anaerobic respiration are highly soluble and do not appear to be attached to any particles so far recognized They are therefore supposed to be held in the ‘soluble cytoplasm , but it would be inadvisable to assume that the soluble cytoplasm is devoid of structure and electronmicrograms of cytoplasm *in situ* suggest that in many cells it may well be laced with an endoplasmic reticulum of membranes

While it would be premature to say that mitochondria are the only sites at which the production of transformable energy occurs, they are clearly the principal site of oxidative phosphorylation which provides the only mechanism of energy transfer in respiration that we know much about Neither chloroplasts nor ribosomes contain a respiratory oxidation system, nor do the nuclei appear to possess a normal oxidation equipment We have therefore the intriguing query whether the mitochondria can export their oxidatively accumulated energy to nuclei and ribosomes which appear to need it There certainly appears no reason why ATP regenerated by mitochondria should not be exportable, and experiments with nuclei and mitochondria isolated from the wheat germ have suggested that a sort of shuttle service ATP ⇌ ADP was being maintained by diffusion between mitochondria and nuclei, which used the ATP-bound energy to build peptide linkages

MARGINAL ASPECTS OF MODERN BRITISH FORESTRY

MR M V EDWARDS discusses the "Marginal Aspects of Modern British Forestry" in his presidential address to Section K (Forestry)

The practice of forestry in Britain, the forests and soils of which have been removed or altered by man, cannot be organized on the basis of methods devised in natural forests Afforestation has been planned for former woodlands and for grasslands, but difficulty has been experienced in fixing the limit to marginal land Experience has shown that greater success has been achieved if the site was modified and if exotic species were planted Recent Government policy changes emphasize a planned acreage programme concentrating on uplands and places where employment is deficient The re-assessment of afforestation limits on the basis of sustained volume of timber production and of manpower requirements is being made

The critical limiting factors are primarily climatic, of which precipitation (rain or snow), wind, and temperature are of first importance These must be known in general by regions, and also in particular for local areas and for micro-climates within the forest Soil factors of importance include the amount of soil available for rooting, its moisture balance and nutrient status, over the whole life of the forest All these factors have to be determined in relation to a range of different species of trees which are capable of establishment, and Britain is fortunate in having an extremely large number to work with

These factors result in a certain aspect of marginality over much of Britain's new forests Stresses between forest and habitat may be set up in Britain's new afforestation, and these have to be investigated as the forests develop The margins beyond which trees cannot be made to grow at all and those at which they can be established by modification of the site are gradually being supplanted by the marginal balance within the site and forest ecosystem

Problems are complicated by the long time-lapse which takes place between seed sowing and marketing of produce, and the difficulties of early determination of the qualities and quantities of mature produce It may also be difficult to determine the right scale by which to measure results, either as volume of timber or by alternatives such as dry weight or measures of quality Economic scales are difficult to apply in the absence of long-term data and they tend to operate against the use of degraded sites, the rehabilitation of which is one of the prime objects of afforestation The present scale of acres planted as used by the Government is a rough and ready approximation which should be replaced by a more definite appraisal of probable results

In natural forests the balance between habitat and crop has already been attained, but in artificial forests some form of human control is necessary Experience in forest nurseries has shown how artificial fertilizers can be used to replace organic manures, but only by maintaining a check on both the soil and the plant The same principles apply to forests Many possible future paths for progress in forestry now appear open, but probably the situation is in fact less unconstrained than it seems The future programme must be based on the investigation of the changes occurring in both habitat and crop as a result of new developments, and detailed recording of these changes is essential if undue risks are not to be run

THE UNITY OF KNOWLEDGE. A NEW DYNAMIC FOR THE SCHOOLS

MR PERCY WILSON begins his address to Section L (Education) with two quotations from Sir Julian Huxley's introduction to *The Humanist Frame* The first declares man's historic indebtedness to the arts and sciences, in all their variety and multiplicity, the second sketches Huxley's view of "evolutionary truth"—a process of enlightenment and liberation of mind to which, over a long process of time, we owe our culture, our security in life, so far as we have any, and our hopes for "the long future of our planet" These thoughts are linked briefly in the address with current fears about the fragmentation of the different branches of learning Mr Wilson's thesis is that, even at this late hour, the time is propitious for realizing Huxley's and Snow's hopes of a more united and dynamic national culture before the process of disintegration goes too far

These hopes—and their attendant problems—are then looked at in the schools, especially the fifth and sixth forms of the English grammar schools, where over-specialization is rife The mounting pressure needs to be relieved by an expansion and diversification of university education, by internal reforms in the schools and by modernizing the school curriculum This applies, for rather different reasons, to non-selective as well as to selective schools

Looked at from the wider perspective of the "Two Cultures", there are some alarming portents in contemporary culture These apply to arts and sciences alike, and examples are given from both sides But the most alarming is the arrogant mistrust of science and its contribution by those—and they are the majority of educated men—who are almost wholly ignorant of the physical sciences This ignorance cripples our understanding of the world we live in, as well as our ability to steer a rational course in our affairs

Apart from questions of internal administration, organization and curriculum in the schools and universities, there are important problems of the nature and inter-relation of the different disciplines

Some of these are examined, one by one in physical science, economics, history and literature Despite the growing narrowness of specialization, there is among the leading scholars and inventors more community of interest, more concern with mutual affinities, more attention to the junctions or interstices of the different subjects than there was fifty years ago This may be our last chance of an integrated culture, but it is probably our best for at least a hundred years "However the arts and sciences may differ in their methods and materials, they are only the dependent branches of the single tree of knowledge, and it takes more than one of them or even two or three of them, to make the tree comely and whole " It is for all school and university teachers to give their pupils some glimpse of the single tree of knowledge as this is seen by those great scientists, philosophers, poets, prophets and artists who have had, and still have, the synoptic view of learning

SCIENCE AND HUNGER

THE two basic facts of world hunger are first that half the present world population of 3,000 millions is hungry or malnourished, and, secondly, that the population is likely to increase to 6,000 millions by the year 2000, mostly in those areas where shortage of food is greatest Yet we could eliminate hunger and prevent its recurrence, if only we applied to the full the knowledge we already have With this as his basic text, Lord De La Warr summarizes the extent of this knowledge in his presidential address to Section M (Agriculture)

There is now a considerable amount of information about cultivation of soils, though much of it concerns temperate lands rather than the underdeveloped tropical lands Nevertheless, the soil conservationist, soil chemist, soil physicist, mechanical engineer and others are able to advise us both on soils and on irrigation The plant breeder can produce plants not only with greater yields, but also particularly adapted to special conditions, such as short or long growing seasons, drought and diseases and pests Herbicides and insecticides can greatly improve yields, which may be worth several times the cost of the treatment, they can also prevent loss during storage, which may amount to as much as 30 per cent of the harvest

Tools for cultivation can improve food production not only by making the labour force more effective but also by clearing, draining and preparing the land Mechanization need not be elaborate, for quite simple improvements can make a considerable contribution Our knowledge of animal nutrition allows far better yields of meat and milk In addition, we know now how to control many animal diseases, both nutritional and infectious Much infertility can be avoided, and animal genetics means that breeds can be improved so as to make them more suitable for a variety of environmental conditions

There is every possibility that fishing could be made to give us 50 per cent more food from the ocean, without danger of over-fishing It has also been shown that fish culture in fresh-water ponds can be a highly efficient way of producing food

It is important to remember that increased food production implies the simultaneous application of all these branches of knowledge Nevertheless, even if we produce enough food, we shall still find that there are many problems of ignorance and superstition which prevent proper nutrition

Thus the most important question which arises is how to put across this knowledge, especially in countries with low levels of training and education As with food production, the answer is again multiple Perhaps the first task is to ensure the training of indigenous advisers Farm institutes and agricultural and veterinary schools are necessary School gardens can be used not only for teaching how to grow food, but also for teaching what foods to grow for good nutrition Rural community development centres can also help, both in improving food production and in teaching nutrition In many parts of the world, co-operative farming, efficiently run, has been found to increase food production, and to make the best use of scarce materials, both human and mechanical Competitions between farms and communities, demonstration farms, and the use of modern techniques of instruction, have all proved useful methods of training

It is commonly believed that food surpluses in some countries could be used for relieving food shortages in others Such help can only be small in relation to needs, and in particular leaves the basic problem untouched, of improved food production where it is most needed Now that the scientists have given us the knowledge, it is up to Governments, educationists, and indeed all of us, to help rid the world of hunger and malnutrition

FACTS AND VALUES

THIS is the subject of Prof Morris Ginsberg's presidential address to Section N (Sociology)

The distinction between facts and values is often presented in the form of a sharp antithesis The philosophers tell us that from 'is' statements we cannot pass to 'ought' statements Social scientists in their turn say that they are concerned with means and not with ends, or that they can (with luck) tell us what we can do but not what we ought to do The impression frequently left is that values belong to a world of mystical intuition, or else have to be left in the end to individual choice or decision

The discussion of the issues thus raised has been greatly influenced by the work of Max Weber But his position is often misinterpreted He did not think that the value judgments implicit in our choices and preferences were incapable of being intelligently investigated On the contrary, it was of the greatest importance to bring out clearly the more remote ends and assumptions which they imply, to estimate the consequences, intended and unintended, likely to result from them and to consider the possibility of adopting new or different ends and assumptions He did not realize how closely this procedure resembles that advocated by those who believe in the possibility of a rational ethic In the end, his ethical outlook is sceptical and pessimistic It seems to be his view that when the process of analysis has been carried to its terminus, it would be seen that in the last resort values are incommensurable and irreconcilable No doubt he was led to this conclusion by the moral difficulties of power politics as he saw it in his own day The individual is left to choose between the demands of an "ethic of consequences" and the demands of an "ethic of ultimate ends" which has to be accepted or rejected in its entirety, presumably as a matter of faith Leaving this aside, as from a rationalist point of view unacceptable, it is important to inquire what bearing the sort of analysis outlined has on the problems of our own time, for example, on the differences dividing the communist world from the liberal-democracies It can be shown that as regards ultimate ends there is little or no disagreement The trouble is that the very loftiness of the ends removes them from the sphere of reality and opens the way to a dual morality, one for an ideal society the pattern of which is laid up in heaven and another in which what is called a realistic' policy is to be pursued regardless of moral standards Stripped of confusions arising from the intermingling of value judgments and judgments of fact the fundamental problem concerns the nature of the institutions most likely to curb the power of man over man and thus to make it possible to treat man as an end and not merely as a means There are signs that in recent decades the complexities of this problem are being increasingly realized in communist and non-communist countries alike and that, in this regard, we may expect that fuller knowledge of the relations between political and economic power and of the impact of modern technology on social relations may bring about some convergence of views now widely opposed or at least greater tolerations of divergence The problems involved are mainly factual, but the facts are distorted by implicit value judgments and these in their turn vitiated by an uncritical acceptance of supposed facts Indeed, it may well be that the problem is one for the psychology rather than the logic of morals From the philosophical point of view, the irreducibility of norms or values to facts is worth re-asserting in view of the persistence or revival of certain forms of legal or ethical positivism and, more particularly, of interpretations of Marxism which seek to justify communism by its supposed inevitability But this irreducibility does not mean that moral judgments have no basis in experience There is such a thing as moral experience Nor does the fact that there is an irreducible element in moral judgments make them mysterious there are irreducible elements in all knowledge

The practical difficulties of keeping questions of fact and questions of value distinct have been explored by the 'sociology of knowledge'—so far with very poor results There has been a tendency to assume that personal involvement or commitment necessarily distorts inquiry On the other hand, Marxist writers have urged that in social investigations we must give up the pretence of detachment and definitely take sides They thus make what might be called a pragmatic virtue of necessity As against both these views it is important to remember that detachment is a matter of degree, that in free societies bias does not remain undetected, and that, though a certain relativity of outlook is inevitable, it admits, in varying measure, of self-correction

ENGLISH AS AN INTERNATIONAL LANGUAGE

ENGLISH as an international language is discussed by Prof B Pattison in his presidential address to Section X (General)

Speech is an integral part of almost all human affairs, and languages develop and influence each other in response to their users' experience and contacts The novelty of the situation in the world at the present time is the density and range of communication Since the whole world is involved, there seems to be a need for a world language

Proposals for an artificial auxiliary language, to avoid invidious selection of the language of one community for general use, go back at least to the seventeenth century Recent ones have mainly been based on features common to a number of European languages The circumstances in which they were produced no longer apply with the emergence of new nations in Africa and Asia The solution of communication problems is being found by general use of a few natural languages of wide currency Of these English is the chief

The influence of the United States is one reason for the increasing use of English It has the technological expertise and the high standard of living most other nations want The English language has been the means of integrating people of diverse origins into one community It has endowed the Americans with the full European tradition The distinctively English contribution to that tradition was emphasized by the American War of Independence As a carrier of liberalism, English has struck deep roots in Asia and Africa

The strategic positions of English-speaking settlements have caused their lines of communication to encircle the globe Several European languages have established themselves on other continents, and French is as important as English in Africa But centres of English influence, such as those in south-east Asia, have tended in recent years to attract communities from other European languages Air lines have partly replaced shipping routes as carriers of English, and English is recognized as the international language of civil aviation

On attaining independence territories formerly governed by English-speaking powers are finding it difficult to change over to indigenous languages Many still need assistance from abroad, and English is the chief language of technical aid Education has been in English, which has given access to Western science and learning In many countries English is a force for national unity and a *lingua franca* of the educated from different speech communities It has lost its political associations and has been a working language for Afro-Asian conferences

There is no reason why any language should not eventually take over some of the present functions of English for local purposes, but it will take time for some to adapt themselves to new ways of thinking English itself took some centuries to become what it is to-day It is likely that by the time other languages have developed English will have established itself as the chief international language and will remain that Creolized forms of it, however, threaten mutual intelligibility among speakers of English from different parts of the world English-speaking countries have a great responsibility for helping to arrest a possible deterioration of standards of English which would deprive those to whom it is a second language of their present advantage in world affairs For historical reasons English has struck roots in many widely dispersed and diverse communities This explains its natural emergence as a world language and gives it an opportunity of providing a much-needed medium for international communication

THE MANCHESTER REGION

Manchester and its Region
(A Survey prepared for the British Association.) Pp. xviii+265+29 plates. (Manchester: The University Press, 1962. Published for the British Association.) 30*s*. net.

It can be said that the objectives of the annual meeting of the British Association for the Advancement of Science are essentially two-fold. The first is to present science in all its broadest fields to the world. The second is that the region in which the meeting is being held be given an opportunity to present itself. This second objective is made simpler if there is a means of gaining a preview of the area, and the more specialized knowledge gleaned at the addresses and excursions can be better appreciated. It was, in part, for this purpose that the survey *Manchester and its Region* was prepared. The result fulfils all in an admirable fashion and offers a most interesting and permanent record of Manchester up to 1962. The book of the survey consists of nineteen well-illustrated chapters prepared by thirty-three contributors, who are drawn mainly from the University of Manchester. There are also contributions from the Manchester Museum, the Civil Service and the Shirley Institute.

For the geographer visiting the region not only are the landscapes of 'Lancastria' ably described (the author here takes great care to define all technical terms used), but also excellent chapters have been prepared on the climate and the Manchester conurbation. Geologists are provided with a thirteen-page introduction to the area which is illustrated with bold maps styled after those presented by the Geological Survey of Great Britain in its *Regional Handbooks*. However, the authors of this chapter concentrate essentially on the geological history of the region, and, rather disappointingly, dismiss the 'structures' involved in just more than a page. For the botanist there is a chapter on "Interesting Features of the Local Vegetation and Flora", and here, on a very general ecological basis, the authors have chosen to describe only those features which they feel merit particular attention by the visitor. On the other hand, the fauna of the region is systematically described according to the various major classes represented. (An ample list of books for further reading is provided at the end of this chapter.)

Historians, including archæologists, are fortunate in that three chapters have been contributed. The first deals with Roman Manchester, the second with medieval and early modern Manchester, while the third covers "The Birth of Modern Manchester". Economists wishing to gain an insight into the structure of industry in Lancashire will find an interesting chapter which tackles such subjects as changes in population and the effects on industry, employment, the balance of industry and the location of growth industries. "Land Utilization and Agriculture in the North-West" describes such aspects as forest and woodland resources, urban development and other land uses, localized types of farming, the economic conditions of farming and the level of farm incomes. Those who take an interest in sociology will gain a good introduction from the chapter entitled "Social Statistics and Social Conditions of Greater Manchester". However, the authors are only able to deal in detail with the information that accrued from the *Census, England and Wales, 1951*, as only the preliminary results of the *Census* of 1961 have so far been issued.

Chapters 13–17 deal with the part that Manchester has played in the advancement of science, medicine, education (two chapters) and sociology, and this section of the book makes excellent general reading. For example, "The History of Science in Manchester" outlines the parts played in the rise of science and the Industrial Revolution by such scientists and engineers as Dalton, Frankland, Roscoe, Joule, Balfour Stewart, J. J. Thomson, Poynting, Rutherford, Schuster, Fairbairn, Whitworth, Osborne Reynolds, etc. This is indeed a formidable list on any account, and from this chapter one realizes the great debt that the world of science owes to such institutions as the Manchester Literary and Philosophical Society, Owens College, the Victoria University (of which the aforementioned was a constituent college) and the Manchester College of Science and Technology. The chapter dealing with Manchester's place in the history of medicine outlines the origins of the Medical School and systematically accounts for the work of such eminent people as White, Ferriar, Percival, Roberts, Whitehead, Jefferson, Dreschfeld, Barclay, Legge, Elliot Smith, Raper, etc., to mention but a few. This chapter deals with, as the author states, only those "who are no longer with us". Indeed, had the work of living medical men been included very much more space would have been required. The remaining three chapters of this 'section' are entitled, "Manchester and the Supply of Schools", "Adult Education in the Manchester Area" and "Some Social Pioneers".

The last two chapters of the book present the hopes for the future. Chapter 18 is entitled "The University of the Future", and Chapter 19 "Planning the City of the Future". These again make good general reading, but even more so for those who are participating in the 1962 Meeting of the British Association, who will be gaining much local knowledge and interest. Through this they will be equipped to judge for themselves the value of the development plans by which Manchester and its region can continue to play its part as one of the greatest industrial, commercial and cultural centres of the world.

The Local Committee who organized the survey and Prof. C. F. Carter, who edited the book, and his team of contributors are to be commended for the excellent way in which they have presented *Manchester and its Region*. So far as can be seen there are only two things that could possibly have improved it. The first is that not all chapters have lists of further recommended reading, and the second that a general index is not provided.

(*Continued from page 874*)

adding 2 volumes of cold ethanol, the precipitate is washed and redissolved in 0.2 M phosphate buffer *p*H 7.0. Alternatively, bentonite, prepared by the procedure of Fraenkel-Conrat *et al.*[5] is used instead of nickel ions. Preparations are titrated by the plaque technique described by Montagnier and Sanders[6]. When we repeated the experiment of Huppert and Sanders using these methods of preparing RNA, that prepared from the virus deposit was ten times as infective as that prepared from the supernatant (Table 1), the infectivity of the RNA preparations was abolished in 15 min at 37° C by 2 μg/ml. of ribonuclease. This experiment was repeated four times with the same result, which was not affected by the method of assay of infective RNA (Table 2). However, when the original method of extraction of Huppert and Sanders was substituted for the new method, the virus deposit again gave less infective RNA than the supernatant (Table 3). The loss of RNA titre in the deposit was thus due to the method used to prepare the RNA, and not to the infectivity assay.

Table 1

Sample	Infectivity of RNA prepared in the presence of nickel ions and assayed by plaque titration (p.f.u./ml. × 10³)			
Exp.	1	2	3	4
Tissue culture fluid	14	32	29	5
Supernatant 1 h at 100,000*g*	0.5	3	0.5	0
Sediment 1 h at 100,000*g*	11	41	22	1

Table 2

Sample	Infectivity of RNA prepared with nickel ions present p.f.u./ml. × 10³	Mouse LD_{50}/ml. × 10³
100,000*g* supernatant	0.5	1.0
100,000*g* sediment	6.0	5.0

Table 3

Sample	Infectivity of RNA (no nickel ions present during extraction) p.f.u. × 10³	Infectivity of RNA (nickel ions present during extraction) p.f.u. × 10³
Tissue culture fluid	60	43
100,000*g* supernatant	4	2
100,000*g* sediment	3	19

These results show that it is in fact possible to prepare infective RNA from EMC virus by extraction with cold phenol. Earlier[3], it was suggested that virus, unlike the 'infective RNA component', was resistant to the action of cold phenol by virtue of the protective protein capsid, but might be susceptible to the action of hot phenol as is ME virus[7]. Since infective RNA can now be obtained from virus using cold phenol, we looked for an increased yield of RNA infectivity with hot phenol, but found none, the susceptibility of both virus and infected cells to hot and cold phenol appears to be the same (Table 4). The reason for the original failure[1] to obtain infective material from the virus deposit was that the technique then used failed to inhibit traces of ribonuclease present in the final preparation, and that the enzyme destroyed the infectivity before it could be detected. That this may be so is shown in Table 3, where the main difference between the columns is the low infectivity of RNA prepared by the old technique from the centrifuge deposit. Preliminary results using the method described by Houck[8] showed that such deposits may contain more ribonuclease than the supernatants. Moreover, there was evidence that the tissue culture fluid and 100,000*g* supernatants contained an inhibitor of ribonuclease action which may protect the ribonucleic acid obtained from the virus in these preparations.

Table 4

Starting material	Infectivity of RNA obtained by cold phenol (4° C) extraction, nickel ions present during preparation PFU × 10³	Infectivity of RNA obtained by hot phenol (50° C) extraction, nickel ions present during preparation PFU × 10³
Cells 5 h after infection	23	13
Crude virus	12	4

Although there is now no evidence of a small 'ribonucleic acid component' in crude preparations of EMC virus it remains unlikely that virus is the only source of infective nucleic acid in infected cells. There is an eclipse phase during which the infectivity of extracted nucleic acid increases without a concomitant increase in virus titre[2], the maximum infectivity of RNA is reached about 3 h before maximum virus titre[2], and 5 μg/ml. of euflavin given at 4 h after infection suppresses the production of virus without affecting that of infective RNA[2]. However, our previous conclusions[3] as to the nature of the RNA component depended on the assumption that cold phenol extracts no infective RNA from virus, and may be incorrect.

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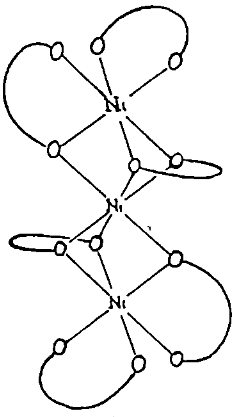
POLYMERIZATION OF TRANSITION METAL β-DIKETONE CHELATES

By Dr. D. P. GRADDON

Department of Inorganic Chemistry, University of New South Wales, Sydney

THE determination by Bullen, Pauling, and Mason[1] of the crystal structure of *bis*-acetylacetonato-nickel(II) showed that this green, paramagnetic compound, long regarded as an example of tetrahedrally 4 co-ordinated nickel(II), was in fact trimeric, each nickel atom being octahedrally 6 co-ordinated(I). That this structure is preserved in non-donor solvents is implied by Cotton and Fackler's observations of the spectra and magnetic properties; they also suggest that at high temperatures equilibrium is established between trimeric and monomeric forms, and observe that only diamagnetic, presumably monomeric and planar, forms occur when bulky substituents obstruct polymerization[2,3].

The preservation of the trimeric form in hydrocarbon solutions is confirmed by our own observations of the molecular weight in benzene and of the equilibrium of the reaction with 2,6-dimethylpyridine in toluene[4]. Fackler[5], studying the equilibrium with pyridine, also found the anhydrous compound to be trimeric, though disagreeing with us on the nature of the intermediate addition compound formed. It



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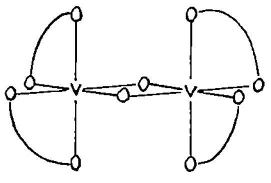

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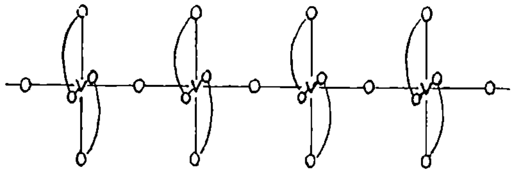

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Figs. 1–3. Preservation of octahedral co-ordination in acetylacetonates: trimer (Fig. 1) of *bis*-acetylacetonato-nickel(II) and suggested dimer (Fig. 2) and polymer (Fig. 3) of *bis*-acetylacetonato-oxo-vanadium(IV). To avoid confusion only the donor oxygen atoms of the ligands are shown

should be pointed out, however, that the bases used were not the same, and it is quite possible that different bases lead to different intermediate products.

Since divalent transition metal cations generally show a co-ordination number six towards oxygen donors, it seemed likely that this form of polymerization might be a common feature of apparently 4-co-ordinated species, such as the anhydrous β-diketone chelates; the results of molecular weight determinations on *bis*-benzoylacetonato-nickel(II), *bis*-acetylacetonato-cobalt(II), *bis*-acetylacetonato-oxo-vanadium(IV) and *bis*-acetylacetonato-zinc(II) are reported here. From the experimental point of view these substances are difficult to examine because of excessive sensitivity to water, low solubility in non-donor solvents or, if spectroscopic methods are used, very small spectral shifts. Their molecular weights have therefore been determined by the cryoscopic method in a variety of solvents which are solids at room temperature; the results are summarized as follows.

(1) *Bis-benzoylacetonato-nickel(II)*. The green, anhydrous compound was obtained by azeotropic distillation of the dihydrate with toluene. Like the acetylacetonate it reacts with water, alcohols or heterocyclic bases to form *bis*-adducts. The mean molecular weights observed were 1,195 in triphenylmethane, 1,100 in naphthalene, 1,187 in diphenylamine, and 392 in camphor (theory for monomer 381, trimer 1,143). This compound is clearly trimeric in non-donor solvents, but monomeric in camphor, which presumably forms a *bis*-adduct; attempts to isolate the camphor adduct were unsuccessful.

(2) *Bis-acetylacetonato-cobalt(II)*. This was made anhydrous by the same method; apparently extremely soluble in toluene, it was obtained as a plum-red powder by slow evaporation of the solution over sulphuric acid. This, like the nickel(II) compounds, readily formed adducts with water, alcohols or heterocyclic bases. The mean molecular weights observed were 766 in triphenylmethane, 816 in diphenylamine, and 257 in camphor (theory for monomer 257, trimer 771). This compound is thus trimeric in non-donor solvents, though apparently not isomorphous with the nickel(II) compound in the solid state[6]. The vapour is reported to be monomeric[7].

(3) *Bis-acetylacetonato-oxo-vanadium (IV)*, $VO(C_5H_7O_2)_2$. This was prepared anhydrous by a standard method[8] and is known to form 6-co-ordinated adducts with heterocyclic bases[9]. Molecular weights observed were 523 in triphenylmethane, 546 in diphenyl, 510 in diphenylamine, and 247 in camphor (theory for monomer 265, dimer 530). This compound probably has the dimeric structure (II) in which the co-ordination number is raised to six by sharing oxygen atoms. It was noticeable that solutions in molten hydrocarbons, though at first homogeneous, slowly deposited a blue solid, possibly a 6-co-ordinated polymeric species (III).

(4) *Bis-acetylacetonato-zinc(II)*. Despite previous claims that the zinc compound is readily obtained anhydrous[7,10], it was found that the product obtained from the reaction of zinc oxide with acetylacetone, and crystallization from 95 per cent ethanol, was the monohydrate $Zn(C_5H_7O_2)_2.H_2O$. Lippert and Rutter[11] also obtained this hydrate and showed by X-ray analysis that it was monomeric and trigonal-bipyramidal, with the water molecule in an equatorial site. The hydrate was found to be soluble and monomeric in triphenylmethane (observed mol. wt. 280, theory 281.5) and on reaction with 4-methylpyridine gave 6-co-ordinated *bis*-(4-methylpyridine)-*bis*-acetylacetonato-zinc(II). The anhydrous compound, obtained by azeotropic distillation of the hydrate with toluene, was very hygroscopic; observed molecular weights were 285 in triphenylmethane, 275 in diphenyl and 281 in diphenylamine (theory for monomer 263.5). *Bis*-acetylacetonato-zinc(II) thus differs remarkably from the other transition metal β-diketonates examined in being monomeric (presumably tetrahedral) and in forming a monomeric 5-co-ordinated hydrate. There appears to be no evidence for a dihydrate, though 6-co-ordination occurs if a heterocyclic base is used as donor.

Since the lower co-ordination number of zinc(II) as compared with nickel(II), cobalt(II) and vanadium (IV) is clearly not attributable to steric factors, we conclude that the zinc ion is unable to tolerate so large an extent of transferred charge as the others; this is in accordance with the observed changes in the co-ordination number of zinc towards oxygen donors of various types[12] and with the ionization potentials of the respective atoms. The ability of *bis*-acetylacetonato-zinc to form a *bis*-adduct with 4-methyl pyridine, despite the strong donor properties of the base, must thus be attributed to the possibility of 'back co-ordination' from the full 3*d* shell of the zinc ion.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Microwave Spectrum of Saturn

RADIO observations of Saturn have been reported previously[1,2] at wave-lengths of the order of 3 cm Observations at longer wave-lengths, combined with these earlier observations, would define the microwave spectrum of the planet, thus making it possible to determine whether Saturn also exhibits the striking microwave phenomena presented by Jupiter, evidently as a result of a vast system of radiation belts[3,4]

To provide some knowledge of the Saturn spectrum, observations of the planet were made for a total of 85 h on 35 days during the period March 10–May 10, 1962 The telescope used was the 85-ft telescope of the National Radio Astronomy Observatory, equipped with a 10 0-cm travelling wave tube radiometer The observation, calibration, and data reduction procedures were identical to those used in a programme of Venus observations[5], and, in fact, the Saturn observations were taken concurrently with the Venus observations A mean disk equivalent black-body temperature T_{BB} was derived from each day's observations as with Venus Inspection of the values of T_{BB} obtained showed that they were distributed in the expected gaussian manner, with the exception of one extreme value which was discarded on the basis of Pierce's criterion[6] The mean value of T_{BB} from the set of reliable observations is

$$T_{BB} = 196 \pm 44 \text{ (m e) } ^\circ \text{K}$$

where we have assumed that the rings of Saturn do not contribute to the observed radio emission This assumption is very likely correct since the value $T_{BB} = 106 \pm 21^\circ$ K, found at 3 45 cm[1], is very close to the infra-red disk temperature of 125° K[7], strongly suggesting that the rings do not radiate at 3 cm Since the rings are known to consist of small solid particles, they can only radiate even less at longer wave-lengths Thus their contribution at 10 cm should be negligible The random errors in the data are too large to permit a search for temporal variations in the radio emission

Combining this result with the accurate 3 45-cm data of Cook *et al* [1], and assuming that the flux density S follows the law

$$S = Kf^x$$

where K is a constant, f is frequency, and x is assumed constant over the range 3–10 cm, we obtain

$$x = 1\ 40$$

This is the most probable value of x from the two sets of observations If we calculate the values of x that obtain if we apply the quoted errors in the most unfavourable way, we find that it is highly probable that the true value of x lies in the range

$$1\ 03 < x < 1\ 83, \qquad 3\ 45 \text{ cm} < \lambda < 10 \text{ cm}$$

Thus it is highly probable, but without great statistical certainty, that the spectrum is not that of a black body, with which $x = 2$ As a comparison, the value of x for Jupiter over the same wave-length region is very nearly 0 75

Despite the apparent departure from a black-body spectrum, a possible interpretation of the radio emissions at 3 and 10 cm is that both are entirely of thermal origin As has been noted by Sagan[8], the atmosphere of Saturn should create a strong greenhouse effect, which, with the presence of an approximate adiabatic atmospheric temperature gradient, will lead to a large increase in temperature with atmospheric depth Temperatures of the order of at least 200° K appear possible at some depth in the atmospheres of the major planets The difference in disk temperature at 3 and 10 cm then could be due simply to the fact that we look more deeply into the atmosphere at 10 than at 3 cm With this interpretation, the present results do not require the existence of a non-thermal source of radiation, and do not necessarily suggest the presence of radiation belts

A second interpretation may be made by assuming that we are observing atmospheric levels of the same temperature at 3 and 10 cm As mentioned previously, nearly all the radiation at 3 cm must be of thermal origin Then, with the present assumption, about 100° K of brightness temperature at 10 cm must also be of thermal origin The remaining 100° K of brightness temperature at 10 cm will then be of non-thermal origin But the brightness temperature of Jupiter at 10 cm is often of the order of 600° K[9,10], of which only about 200° might be thermal, leaving a non-thermal brightness temperature of roughly 400° K With these data, the non-thermal components of Saturn would have about 1/6 the intrinsic luminosity of the Jovian non-thermal component, taking into account the difference in planetary disk areas

The two alternate interpretations given here are based on the extremes in assumptions that are presently plausible, the true situation, barring presently unknown complications, must lie somewhere between the two possibilities Therefore, the absolute non-thermal radio luminosity of Saturn appears to be less than about 1/6 that of Jupiter, at centimetre wave-lengths, and may be, in fact, much smaller This is an expected result, since it has been widely surmised that the rings of Saturn will capture radiation belt particles very rapidly, preventing the existence of any dense radiation belt system Further elucidation of this point will require observations at longer wave-lengths, or with polarimeters, or with sufficient resolution to measure the extent of the source of radio emission All these are difficult because of the very low flux density received from the planet

I thank Miss Ellen Gundermann and Mr C E Heiles for carrying out the reduction of the observational data

F D Drake

National Radio Astronomy Observatory*,
Green Bank, West Virginia

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* Operated by Associated Universities, Inc , under contract with the U S National Science Foundation

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10-cm Observations of Venus near Superior Conjunction

PREVIOUS extended observations[1] of Venus at 10-cm wave-length have given evidence for only a small variation in the mean disk equivalent black-body temperature T_{BB} with Venus phase angle i These observations were made during a period surrounding inferior conjunction, and did not extend to small phase angles Thus the disk temperature for small i could be inferred only from an uncomfortably large extrapolation of the uncertain function T_{BB} (i) derived from data containing significant random, and perhaps systematic, errors These circumstances have made it desirable to observe Venus, if possible. near superior conjunction This is difficult because Venus is then at maximum distance, leading to minimum signal-to-noise ratios, and the planet also appears close to the Sun, a powerful radiator which can contaminate the observations by introducing significant radiation into the antenna side-lobes

In an attempt to measure T_{BB} near superior conjunction, Venus was observed for 330 h on 44 days during the period March 9–May 13, 1962 The equipment used was the 85-ft telescope of the National Radio Astronomy Observatory and a 10 0-cm travelling wave tube radiometer[2] with digital data recording devices, this was the same equipment used to obtain the previous extended set of data[1] Observations consisted of 30-sec observations made with the antenna pointed directly at the planet, alternating with 30-sec observations made with the antenna pointed 30′ to one side of the planet These latter comparison positions were arranged symmetrically north, south, east, and west of the planet For purposes of calibration, observations of an argon noise tube were made at frequent intervals A directional coupler introduced the noise tube output power into the radiometer so as to avoid physical changes in the radiometer in the course of the observations The over-all telescope system was calibrated several times each day by observing the radio source 04$N$3$A$ in the same manner as Venus From these data, the ratio of the intensity of Venus to that of 04$N$3$A$ was calculated, which gives directly the Venus flux density, if the flux density from 04$N$3$A$ is known The adopted value of the 04$N$3$A$ flux density was 23 7 (10^{-26}) W/m^2/c/s[1,3] The standard black-body radiation formulæ and the ephemeris planetary semi-diameter then led directly to a value for T_{BB} An IBM 1620 computer, which read directly the punched-tape records prepared at the telescope, was used in the reductions These observation, calibration, and reduction procedures are identical to those used previously Thus the result obtained here is homogeneous with the previous data In particular, since the primary calibration standard is the flux from 04$N$3$A$, there should be no systematic differences in the calibration of T_{BB} between the present and previous sets of data

Inspection of the 44 daily mean values of T_{BB} showed that they were distributed in the expected random way about a mean value, except for (1) two values which deviated widely from the general run of values, (2) all data obtained during April 6–23, which deviated non-randomly from the mean by large amounts An analysis of these latter divergent data showed that values of T_{BB} based on observations using comparison points east and west of the planet differed significantly from the values obtained using north and south comparison points This can only be due to an interfering source of radiation, and the length of time over which this problem persisted shows that the source can be only the Sun Consequently, all points from this period were discarded The other two divergent points were discarded on the basis of Pierce's criterion[4] This left 36 of the original 44 days' observations, representing about 250 observing hours The mean value of T_{BB} obtained from these accepted data was·

$$T_{BB} = 610 \pm 55 \text{ (m e)}^\circ \text{ K}$$

The range of i spanned by the observations was $14^\circ < i < 37^\circ$, with the mean value of i being $i = 25^\circ$

The previous results gave $T_{BB} = 629 + 39 \cos(i{-}17^\circ)$ ° K for the range of i of concern here This relation predicts $T_{BB} = 660^\circ$ K at $i = 25^\circ$ Thus the present and previous results are mutually consistent They are strong evidence that the 10-cm phase effect is very small, nearly that given previously, and that there is little surface temperature difference between the illuminated and dark hemispheres of Venus

I thank Miss Ellen Gundermann and Mr C E Heiles for carrying out the data reductions

F D Drake

National Radio Astronomy Observatory*,
Green Bank, West Virginia

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The Lyman-α Problem and the Geocoma Hypothesis

IN a recent review article, Donahue[1] has discussed the various hypotheses put forward to explain the observed Lyman-α radiation[2,3] in the night sky The observed radiation is apparently solar Lyman-α radiation scattered by neutral hydrogen There are three hypotheses concerning the location of the hydrogen namely, the interplanetary hypothesis[3]; the geocorona hypothesis[4,5] (origin in a thin, essentially spherical shell with multiple scattering important), and the geocoma hypothesis[6,7] (origin in a comet-like distribution at distances greater than some 5–10 R_E, single scattering important) It is felt that the review[1] is generally not unfavourable to the geocoma hypothesis, but several important points were raised by Donahue The purpose of this communication is to discuss briefly the general status of the geocoma hypothesis in the light of recent observational and theoretical evidence and, in particular, to discuss the problems noted by Donahue

The first question raised by Donahue concerns the needed density of neutral hydrogen and its distribution as reflected in the observed and computed isophotes The calculations of Thomas and Donahue[8] indicate that a spherically symmetrical geocoma has too much Lyman-α radiation at large zenith distances Thus, it appears necessary to take account of the asymmetry which was a part of the original geocoma hypothesis[6,7] Spherical symmetry is often assumed for simplicity and because of the lack of a suitable model The asymmetry arises because neutral hydrogen in the exosphere and outside the Earth's shadow is subject to radiation pressure and because neutral hydrogen outside the geomagnetic cut-off is moved in the anti-solar direction by the drag of the expanding interplanetary plasma Thus, we need a detailed knowledge of the Lyman-α flux, the configuration of the geomagnetic field boundary, and the velocity and density of the solar wind The geomagnetic field boundary may be quite important as it may extend to many tens of Earth radii on the night side[9] This fact clearly aids the asymmetry, and it should be noted that the asymmetry will tend to bring together the observed isophotes and the isophotes computed on the basis of the geocoma hypothesis[8] The fact that the geomagnetic field has an appreciable downwind extension favours the existence of neutral hydrogen in this location, this means that there probably will not be a deep minimum in the anti-solar direction on the geocoma hypothesis, as was anticipated by Donahue The calculations of the neutral hydrogen density in the exosphere are difficult as again there are many uncertainties The densities outside the geomagnetic cut-off are limited by the collision time-scale for charge exchange with the solar wind protons The current parameters for the solar wind have been used by Donahue[1] in a recalculation, roughly the correct amount of hydrogen is obtained, but the amount is perhaps a little too low The needed amount is well within the uncertainties, and the effects of the asymmetry (in particular, the appreciable downwind extension of the geomagnetic field) could well make up the difference Thus, there does not appear to be any serious difficulty with the observed quantities and the density distribution expected on the geocoma hypothesis

Donahue[1] also points out that the observed night sky Hα radiation turns out to be an argument against the geocoma hypothesis (that is, single scattering) instead of an argument for it as had been previously supposed[6,7] The Hα radiation observed originally[10] was thought to correspond to the Hα ($4\pi\mathscr{J}_{32} \approx 6R$) predicted by Brandt and Chamberlain[11] using preliminary values for the flux and width of Lyman-β

The Hα is found to be of galactic origin[18], hence, the lack of an atmospheric component of Hα could be used as an argument against the geocoma hypothesis If we assume the Lyman-β originates in the same portion of the solar atmosphere as Lyman-α, it comes from a region[12] with $T \approx 1 \times 10^{5}$° K and then has a full width at half-intensity of about 0 3 Å This width is 1/3 the width used originally[11] The latest measurements by Hinteregger[13] give a flux in Lyman-β some 17 times less than the value used originally Thus, the Hα intensity is some 3/17 ≈ 1/6 the value formerly found Since the effects of line broadening due to opacity or turbulence and the effects of a central reversal are to further reduce the intensity, we conclude that $4\pi\mathscr{J}_{32}$ (geocoma) $\lesssim 1\ R$ This is a very low value and indicates that the geocoma hypothesis is consistent with the Hα observations when more recent parameters concerning Lyman-β are used

There is now general agreement that virtually none of the night sky Lyman-α radiation comes from the interplanetary medium, its contribution[6,14] is certainly ≲ 1 per cent The theoretical calculations of Thomas and Donahue[8] taken together with the *Javelin No 3* observations[15–17] argue strongly against the geocoronal-multiple scattering hypothesis[1] I note, however, that some 20–30 per cent of the observed radiation could arise from the geocoronal hydrogen Since the problems concerning the geocoma hypothesis are not necessarily severe, it seems that the major portion of the observed night sky Lyman-α radiation should be considered as coming from the geocoma, this view is supported by the Doppler temperature of the radiation of 8,000–10,000° K[6,7,19], which implies velocities ≈ 12 km/sec

I thank Dr T M Donahue for correspondence concerning the night sky Lyman-α problem

JOHN C BRANDT

Berkeley Astronomical Department,
University of California

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PHYSICS

Conductivity of Photographic Emulsions

ATTEMPTS to explain the origin of the latent image in sensitive photographic layers often take into consideration the close conjunction of processes (electronic and ionic) present during the formation of the latent image with those concerning photoconductivity or luminescence in the same materials It is therefore possible to presume that a mutual connexion exists between the so-called photochemical sensitivity of the photographic layer on one hand and photoconductivity or luminescence on the other. This is so, because that part of the photoelectrons which will participate in the formation of the latent image[1] cannot take part in, for example, luminescence and at the same time will influence, depending on the sensitivity of the layer (that is, depending on the number and energy-levels of the active trapping centres for photoelectrons in silver halide crystals), the size and course of the growth of photoconductivity of a definite photographic layer Similar problems were investigated by Vacek[2], West and Carroll[3] and by Gross[4]

We have examined the connexion between the photochemical sensitivity and the course of the total conductivity (electronic and ionic) in the dark and under illumination for two types of photographic layers, namely, commercial light-sensitive materials and laboratory-prepared nuclear emulsions All materials were kept at 58 per cent relative humidity prior to measurement The measurement of conductivity was made by a direct-current method at room temperature Determinations of the light sensitivity was made by a method used for the determination of sensitivity to the total radiation effect in nuclear emulsions

From the measured curves we evaluated the ratio of the maximum values of conductivity in the dark (i_1) and at constant illumination (i_2) The sample was illuminated symmetrically between the electrodes by the sensitometric lamp of 450 W from a distance of 0 79 m All pairs of measurements were made on separate samples

Fig 1 shows the characteristic curves of those photographic layers and Table 1 gives conductivity measurements, which only shows typical cases from a series of measurements being presented

Table 1

Photographic material		i_1/i_2	Curve in Fig 1
Light-sensitive emulsion	Gradual-ortho *Foma* (17/10 *DIN*)	6	1
	Dia U Foma (3° Sch)	2 3	2
Nuclear emulsion	Sensitized with gold	3	3
	Unsensitized	0 8$_6$	4
Silver chloride single-crystal	Crystals prepared according to ref 5	0 4$_7$	

By comparing the values from Table 1 and the course of the characteristic curves in Fig 1 it is evident that for each type of photographic material (nuclear and light-sensitive emulsions) the higher sensitivity corresponds to higher value of the ratio between the total conductivity in the dark and under illumination This result can be quite easily explained The measured conductivity in the photographic layer at room temperature consists of electronic (or photoelectronic) and ionic (mainly interstitial silver ions) constituents The higher the sensitivity of the photographic layer the higher the concentration of the deep trapping centres in silver halide crystals, that is after illumination of the photographic layer most of the freed photoelectrons are trapped The trapped photoelectrons on the trapping centres attract interstitial silver ions and neutralize them $Ag_0^+ + e^- \rightarrow Ag_0$ Apart from that, part of the electrons are trapped on shallow trapping centres or may non-radiatively recombine with holes The conductivity under illumination is lower in comparison with conductivity in the dark, for the number of Ag^+ ions which can take part in the conductivity is lowered for above reasons

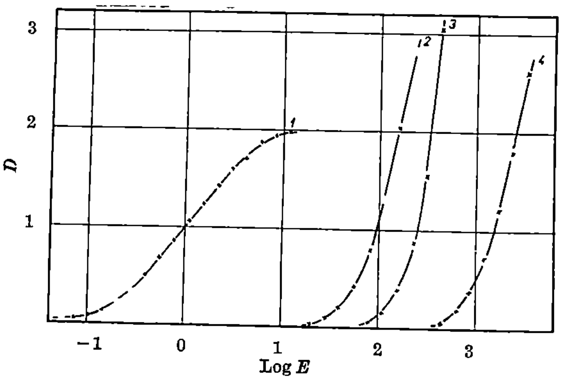

Fig 1

In materials of low sensitivity, where the concentration of trapping centres is much lower, most of the photoelectrons are not trapped This means that for a time they can take part in the conductivity (before they non-radiatively recombinate) and therefore the conductivity of the layer rises under illumination That is why the ratio of these materials is less than one

All these considerations confirm the measurements of conductivity made on single crystals of pure silver chloride, where the concentration of trapping centres in comparison with the photographic layer is a minimum That is why the ratio is smallest for these pure crystals (Table 1)

The conductivity measurement of a fogged photographic material ($D_0 > 3$) was also made The result of the measurement is interesting because the ratio was less than one in all cases

J Beneš

Department of Dosimetry,
Nuclear Research Institute,
Na Truhlářce 100,
Prague 8

A Fořt
K Vacek

Faculty of Mathematics and Physics,
Charles University,
Ke Karlovu 5,
Prague 2

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Transition Theory of Elastic-Plastic Deformation, Creep and Relaxation

The classical macroscopic treatment of problems in plasticity, creep and relaxation has to assume semi-empirical yield conditions like those of Tresca, Von Mises and creep-strain laws like those of Norton, Odqvist, Kachanov and others[1] This is a direct consequence of using linear strain measures, which neglect the non-linear transition region through which the yield occurs and the fact that creep and relaxation strains are never linear Tresca[2] pointed out that a transition state, which he called the mid-zone, existed, and Todhunter and Pearson have supported him[3] In this state the whole of the material participates, and not simply a line or selected region, as is assumed by classical theories Sir Lawrence Bragg[4] expresses this view when he points out that an element of a crystal, embedded in the matrix, should be pictured as being strained together with the whole mass of the metal, and hence the slip must take place by a whole intra-atomic distance A recent numerical investigation[5] on flow and deformation theories also shows that a continuous approximation through a transition state leads to a satisfactory convergent solution

A non-linear strain measure of the form[6]

$$s=(1-ne^{1/m})^{-1/n}, \quad m>0 \qquad (1)$$

includes a number of measures When $m=1$, we get the measures due to Cauchy, Green, Hencky, Alamansi and Swainger for values of n given by $n=-1, -2, 0, 2, 1$ No strain corresponds to $n \to \infty$ This measure readily gives the creep laws, both theoretical and experimental For example, Norton's law may be obtained by putting $m=1$, $s=\alpha t$ and differentiating the total strain s with respect to t, α being the average rate of creep-strain In like manner if we put $m=1$, $n=0$ (Hencky's measure) and $m=3$, $n=1$ in succession, and add the two rates obtained by differentiating $s=\alpha_1 t$ and $s=\alpha_2 t$ we get Andrade's very accurate experimental law[7]

$$l=l_0(1+\beta t^{1/3})e^{kt} \qquad (2)$$

This includes the transient creep flow represented by β and the ductile flow given by the exponential term Andrade's experiments show that this law holds good for a number of metals By using a functional in place of e in equation (1) we get Kachanov's law[8]

We may now satisfy the equation of equilibrium by using the measure in equation (1) and a quasi-linear stress-strain relation, which is found sufficient for most metals This avoids the use of complicated constitutive equations, which have remained mostly of theoretical interest Non-linear differential equations are obtained The concept of a transition state may be identified analytically with the asymptotic representation at the turning points of these differential equations Such representations have been used in a number of problems which involve quick transitions, discontinuities and non-uniformities[9] For elastic-plastic deformation the values $m=1$ and $n=2$ are found quite sufficient in equation (1) We get two turning points, which separate the regions of contraction and extension A number of other interesting results, which are generally assumed, come out of the equations and are now under publication[10] No yield condition is assumed It is contained in the solution

In like manner for creep rupture and relaxation the transition theory combined with the generalized measure (equation 1) does not require any yield condition or creep law[10] For a hollow tube subjected to internal pressure we get the same creep-stresses as obtained by Kachanov Results so far obtained suggest that the transition concept combined with that of a generalized strain measure can give a satisfactory basis for explaining a variety of irreversible phenomenon

B R. SETH

Mathematics Research Centre,
University of Wisconsin,
Madison, Wisconsin

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GEOLOGY

Glacial History of Beerenberg, Jan Mayen Island

CERTAIN aspects concerning the glacial history of the Beerenberg mountain, described by F. J Fitch in a recent article[1], are in need of amplification and comment

In the original article the suggestion was made that the most recent minor re-advance of the Island's glaciers was probably near its climax This is the view of one of us (F J. F), and is based on the overall geological considerations regarding the moraine sequence on Jan Mayen, and not on any definite indications shown as yet by the glaciological and meteorological observations Undisputed evidence of a glacial surge was recorded during studies of Sorbreen in 1959 and 1961 This surge has very probably reached its climax The trend of increasing precipitation on Jan Mayen (with which can be associated the latest re-advance of the glaciers), is still continuing, and on this account it seems to the other authors (D J. J. K and J W S.) unreasonable to postulate that the post-1954 re-advance as a whole is near its climax. In other regions around the Norwegian and Greenland Seas a general fall in the level of precipitation has followed the trend of lower temperatures noticeable since about 1940 It will be of interest to see when the precipitation at Jan Mayen begins to fall off, if indeed this is going to occur

At Sorbreen, only three clearly defined modern moraine systems can be discerned, and by comparison with other North Atlantic areas these have been provisionally dated The outer series, by far the largest and best developed, probably date from about A D 1750 An inner, less well developed, series probably dates from the mid-nineteenth century (A D 1850–70) and the innermost, ice-cored series from about A D 1910–20 Certain other glaciers of the south-east and east coasts of Beerenberg (for example, Wardbreen, Fotherbybreen and Griegbreen) exhibit four distinct sets of modern lateral, and in the case of Wardbreen, terminal moraines In some cases these sets are themselves subdivided into a multiple ridge system Weak evidence of a four-fold concentric moraine sequence can be seen elsewhere (for example, at Smithbreen and at the ice-field margin by Ryggvarden), and it appears certain that a re-advance period, or periods, not easily recognizable at Sorbreen or Kerckhoffbreen, occurred at some time between the production of the large outer laterals and the smaller 1850–70 moraines The suggestion, made in the original article, that this re-advance occurred about A D 1810–20, must be regarded as only one of a number of possibilities until further comparative or absolute evidence is available More detailed descriptions of the glaciology of Jan Mayen will be published elsewhere[2–4]

The statement concerning the breaching of the crater rim of Beerenberg needs amplification There have been several suggestions as to the origin of this feature They fall into two categories (*a*) those which advocate a primary origin such as volcanic explosion or lava outflow, (*b*) those advocating a secondary origin, by erosion Even if the former is correct, the initial feature has undergone profound modification by subsequent glacial erosion The entrenching of the deep glacial valleys of the north coast has proceeded hand in hand with the breaching of the crater rim, and has extended over the period since the Joris Ice Advance[5] (2,500 years B P), when it can be shown that the glaciers occupied only shallow valleys

D J J Kinsman
Geology Department,
J W. Sheard
Botany Department,
Imperial College of Science and Technology,
London, S W 7
F J Fitch
Geology Department,
Birkbeck College, London, W C 1

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METALLURGY

Characteristics of Fatigue-activated Creep in an Aluminium–Magnesium Alloy

Experiments have been conducted on thin-walled tubes of an aluminium–magnesium alloy (3 08 per cent magnesium, 0 3 iron, 0 44 manganese, 0 18 silicon) under conditions of simple shear Two tubes aligned on a common axis are used for each test, the torque being applied through a disk connected to both tubes The assembly is mounted in a thermostatically controlled enclosure, and to avoid any possible errors due to inertial effects associated with the dynamic stress component, the torque is applied through a spring system A simple electrical device is used to maintain a constancy of torque (within 1 part in 5 × 10^3) as the disk rotates during creep The creep curves are recorded continuously by a photoelectric follower and associated recorder (a 'Graphispot') which traces the movement of a spot of light reflected from a mirror mounted on the shaft of the torque disk

Creep and creep recovery have been investigated with the equipment over ranges of stress and temperature In Fig 1, the continuous curves (which are accurately reproducible) represent the creep under a constant torque of 7 9 × 10^3 lb /in ^{2} at the temperatures indicated Similar sets of curves for creep-recovery have been obtained for clarity, only that for 160° C is here shown Recorded results of this kind enable critical comparisons with the theoretically predicted behaviour to be made In particular, the plastic component may be established by unloading after various creep-times (t_1, t_2,) and recording the residual strain (p_1, p_2,) remaining after a prolonged period of unload A plot of t against p represents the 'plastic' creep component such a curve for the case of creep at 160° C is shown in Fig 1 If the creep-time is long enough for the creep-rate of the anelastic, or recoverable, component to be negligible, then, on unloading, the form of the creep-recovery curve should be a reverse replica of the forward component (provided Boltzmann superposition applies) We can thus check the deductions directly by subtracting such a curve (that for 160° C is shown dashed in Fig 1, and marked 'recoverable component') from the total creep We find the plastic strain so deduced to be accurately in agreement with the curve derived by the first method Creep-recovery obeys an equation of the form $\varepsilon = k \log t$, and not $\varepsilon = k\,t^m$, and the values of k are linear with temperature above a critical temperature ($\sim$ 120° C in the present case) The plastic strain is linear with time except for the first few minutes, when a small transient (possibly best represented by $\varepsilon \sim t^m$, where $0 2 < m < 0 3$) is certainly evident The plastic component of the creep is strongly temperature dependent, and the rate increases critically above $\sim$ 140° C The linear rate of plastic creep, $\dot{\varepsilon}_p$, does not correspond with a single activation energy over the range studied, as will be evident from Fig 2, where the curve A demonstrates the temperature-dependence of this component This relationship should. of course, be given by a straight line if the simple rate-process equation is obeyed For the higher temperatures the slope yields an activation energy of 63 kcal /mole (which happens to be close to that for recrystallization in aluminium), while for the lower temperatures the energy derived is about 15 3 kcal /mole Clearly the temperature-dependence of the frequency factor in the rate equation cannot be ignored and detailed deductions from the data as they stand could be misleading

However, curve A (Fig. 2) can be used as a basis for comparing the creep under a superimposed fatigue stress Previous work[1] has, of course, demonstrated that both creep and recovery can be accelerated In the present experiments the alternating stress was applied electromagnetically, through the torque disk, at a frequency of 50 c /s The fatigue stress-level is about 1 per cent of the static stress (this can be estimated from the increased apparent width of the recorded light beam) The acceleration of the creep is evident from the dashed curves drawn in Fig 1 (the creep-rates for 170° C are so much greater (Fig 2), that they would require a much smaller scale drawing, are are not included here) Curve B (Fig 2) demonstrates the effect Rates are significantly increased above the critical temperature for plastic creep which for this stress is about 140° C The close parallelism of curves A and B suggests that no new process is being activated, but that the basic process (or processes) operative are merely accelerated The results are, then, consistent with those theories which regard fatigue-accelerated creep as resulting from an increased point defect concentration, and hence an increased rate of dislocation climb No new process need be postulated Whatever operations are

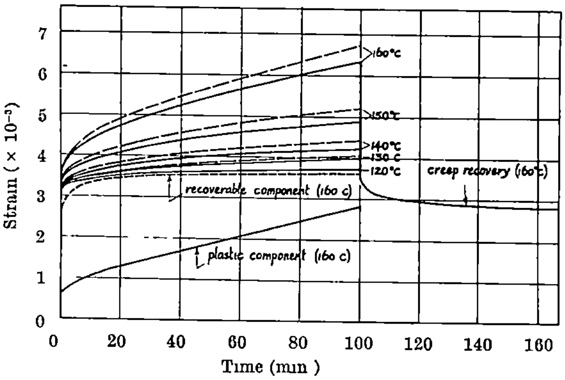

Fig 1 Creep curves of an aluminium–magnesium alloy under constant shear stress at the temperature indicated The dashed curves represent the creep when a fatigue stress with an amplitude of 1 per cent of the static stress is superimposed The creep recovery curve is shown for the case of the test at 110° C , and the analysis described enables the forward creep to be separated into the components drawn All the creep curves are continuously recorded

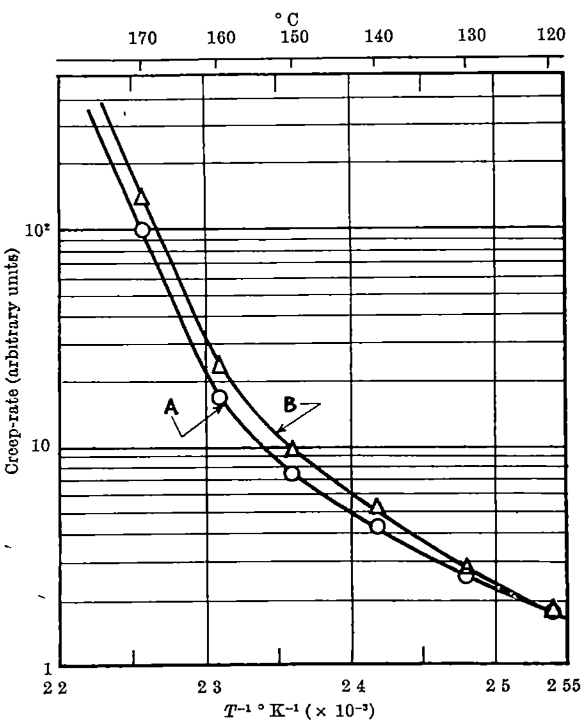

Fig 2 The logarithm of the steady-state plastic creep-rate plotted against the reciprocal of the absolute temperature for creep under a constant shear stress of 7 9 × 10^3 lb /in ^{2} (curve *A*), and also for creep under the same basic conditions, but with a 1 per cent fatigue stress superimposed (curve *B*)

carried out on the curves of Fig 2 to extract activation energies, the values would be the same for *A* or *B*

These investigations are being extended to alloys of special interest in connexion with the realization of a supersonic transport aircraft, as combined creep and fatigue conditions at skin temperatures in the region of 130° C will necessarily occur It is evident that relatively small-scale tests of this type can yield very definite information of significance to design The creep-rates imposed in the present experiments (10^{-4} hr $^{-1}$ at 140° C , for example) are higher than those acceptable, as an average, for the supersonic transport, but useful deductions can nevertheless be drawn about the nature of the effects to be expected, their characteristics and their possible importance

I thank Mr E W Wilson and Mr R C Whitbread, who carried out the experimental work, and the Department of Scientific and Industrial Research for a research grant

A J KENNEDY

Department of Materials,
College of Aeronautics,
Cranfield

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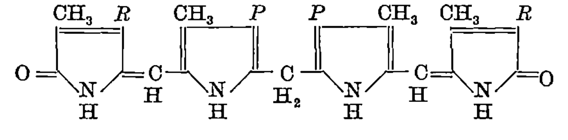
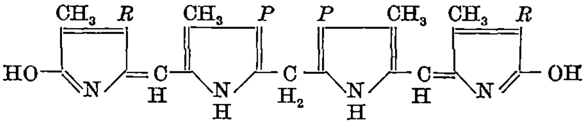
CHEMISTRY

Zinc Complex Salt Formation by Bilirubin and Mesobilirubin

ONE of the characteristic properties of most bile pigments is their ability to form complex salts with zinc ions, many of which display brilliant and distinctive fluorescence It seems to be generally accepted, however, that rubins do not form zinc complexes[1-4], mainly on the strength of Fischer's observation[3], that mesobilirubin gave no fluorescent zinc salt, and no further relevant work appears to have been published This belief has been used in support of the theory that the rubins exist in the *bis*lactam (I) rather than in the *bis*lactam form (II), as the latter contains two pyrrolenine nitrogen groups which should be capable of complexing with zinc ions, whereas the former does not[1,2,5,6]



(I)



(II)

$P = CH_2\ CH_2\ COOH$ Bilirubin $R = CH\ CH_2$
Mesobilirubin $R = CH_2\ CH_3$

I have recently obtained some evidence which indicates that bilirubin and mesobilirubin do form zinc complex salts

Bilirubin (10^{-5} M) in neutral 1 1 chloroform-methanol was yellow, with an absorption maximum at 452 mμ (Fig 1, curve 1) Addition of a drop of 2 per cent methanolic zinc acetate per 5 ml caused an immediate change of colour to saffron, the absorption maximum shifting to 476 mμ (curve 2) This solution was non-fluorescent under ultra-violet light When a drop of concentrated hydrochloric acid, or a few drops of glacial acetic acid were then added, the colour changed back to yellow, the absorption spectrum reverting to that of the pigment prior to the addition of the zinc acetate (λ_{max} 452 mμ)

Similar results were obtained in the case of mesobilirubin The absorption maximum of this pigment in 1 1 chloroform-methanol lay at 430 mμ (curve 3) On addition of a drop of 2 per cent zinc acetate in methanol, the absorption maximum shifted immedi-

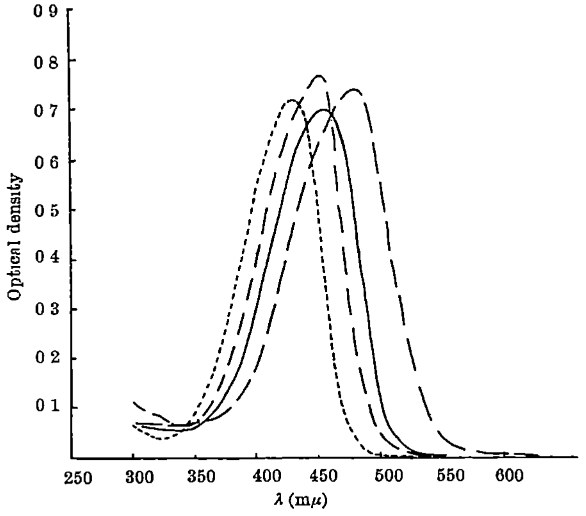


Fig 1 Absorption spectra ——— Bilirubin in 1 1 chloroform-methanol , — — —, same + zinc acetate , - - - - - , mesobilirubin in 1 1 chloroform-methanol , —— —— , same + zinc acetate

ately to 451 mμ (curve 4—solution non-fluorescent), and on subsequent addition of a drop of hydrochloric acid it reverted to 430 mμ In this case the spectral shifts were barely detectable visually

Since only acid-reversible reaction of the zinc ions with the rubins could be responsible for the foregoing spectral shifts (control experiments having shown that the acetate ions were not), this evidence indicates that bilirubin and mesobilirubin form non-fluorescent zinc complexes which, in common with the zinc complexes of other bile pigments, are readily decomposed by dilute acid, the original pigments being released

The zinc complexes of many bile pigments are oxidized rapidly on addition of a few drops of a solution of iodine in chloroform, whereas the free pigments are oxidized very much more slowly Iodine did not oxidize free bilirubin or mesobilirubin rapidly in chloroform-methanol solution, but on addition of a drop of zinc acetate in methanol, oxidation by iodine took place rapidly (giving mixtures of purpurins and choletelins) However, when zinc complex formation was prevented by addition of a drop of acid with the zinc acetate, rapid oxidation by iodine did not take place

This additional evidence seems to support the conclusion that the rubins form non-fluorescent complexes with zinc ions The structures of these complexes are being investigated

I thank Dr C Ó hEocha for his advice and for providing facilities for this work, which was supported by the U.S Air Force, through its European Office (contract No *AF* 61(052)–409) I also thank Prof C H Gray and Dr D C Nicholson for a sample of mesobilirubin

PÁDRAIG Ó CARRA

Department of Chemistry,
University College,
Galway, Ireland

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Acetone Isotherms

Casado, Massie and Whytlaw-Gray[1] measured the density of five organic vapours at 22° C with a quartz-fibre microbalance and obtained results which permit the calculation of second virial coefficients However, with acetone, the data imply a contribution over the experimental pressure range (26–136 mm) of a positive third virial coefficient sufficiently large to cause a two-fold change in the slope of the *PV*/*P* line, a finding quite at variance with Lambert's[2] linear isotherms Work on acetone by Pennington and Kobe[3] and Zaalishvilli and Kolysko[4] was conducted by methods which do not provide direct evidence on the *PV*/*P* curvature Recent *PVT* determinations at sub-atmospheric pressure for other organic compounds boiling near 50° C indicate that any supposed curvature is scarcely distinguishable from observational errors

To clarify the position we have recently examined acetone using the differential compressibility apparatus described previously[5] and offer a comparison with previous determinations (Table 1)

Table 1 SECOND VIRIAL COEFFICIENTS, CM^3/MOLE

	Present work	Lambert	Zaalishvilli	Pennington
50° C	−1,439	−1,560	−1,535	−1,480
22° C	−2,111	−1,960	—	−2,166

We have used $PV = nRT\ (1 + nB/V)$, Lambert and Zaalishvilli used $PV = nRT + nBP$, the final column is the value interpolated from the Stockmayer parameters given by Pennington and Kobe

Our technique, which permits measurement of the several *PV* products in one expansion to 1 in 10^5 shows that, as with other organic vapours similarly examined if there is any significant departure from linearity after the slight corrections applied for adsorption on mercury and glass surfaces, it is less than 2 or 3 parts in 10^5 for the pressure ranges examined (100 mm at 22° C, 200 mm at 50° C), at least ten-fold less than claimed previously, and favours a negative third virial Whytlaw-Gray's acetone results by the microbalance method, which is particularly sensitive to the purity of materials, decomposition of the acetone, and accidental contamination of the balance parts by minute particles of grease, we believe to be in error

To obtain further information about the general curvature of isotherms at reduced temperatures below 0 80, where the Stockmayer intermolecular potential indicates that the third virial coefficient becomes negative, it will be necessary to increase the available precision at least one order of magnitude In a very limited number of high-precision experiments, T_r = 0 5–0 7, recorded in the literature there is just an indication of such a curvature

G A BOTTOMLEY
T H SPURLING

Department of Chemistry,
University of Western Australia,
Nedlands

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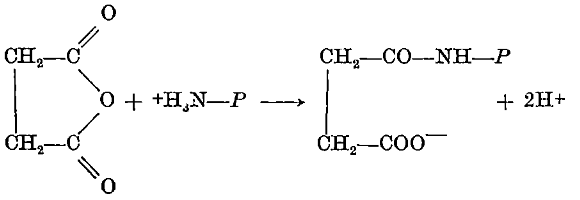
Dissociation of Proteins into Sub-units by Succinylation : Hæmerythrin

IN the course of investigation of the molecular weight of the marine oxygen-carrying pigment hæmerythrin (obtained from *Golfingia gouldii*), indications arose that the macromolecule of about 105,000 molecular weight might be constituted of several smaller units A few sedimentation experiments in solutions of sodium dodecyl sulphate, following a suggestion of Schachman[1], did reveal sub-units, but complete dissociation could not be obtained and the presence of detergent created a number of difficulties in experimental procedure Since the effectiveness of the detergent in splitting the macromolecule probably depends in part on the electrostatic repulsion produced by the large negative charge of the bound ions, it seemed worth while to examine the effect of the introduction of additional negative charges covalently linked to the macromolecule Reaction of protein with succinic anhydride[2,3] should replace each cationic ε-NH_3^+ charge by an anionic carboxylate group Since hæmerythrin contains about 80 lysine groups, complete succinylation would change the charge on the 105,000 molecular weight macromolecule at *p*H 7 by about 160 units



Succinylation was carried out very simply To the protein dissolved in dilute salt solution was added small quantities of solid succinic anhydride in small increments, the reaction mixture being stirred constantly The solution tended to become acidic as the reaction proceeded, the *p*H was maintained at 7–8 by the addition of dilute ammonium hydroxide

Samples were removed after the addition of various amounts of succinic anhydride and were examined in the ultracentrifuge The area of the *Schlieren* peak of the native macromolecule, with an S_{20} of about 6, decreased progressively and a new peak appeared with an S_{20} near 2 With increasing amounts of succinic anhydride, the fast peak was converted entirely into the slow one

To determine S^0_{20w} of the subunit accurately it was necessary to overcome any electrostatic effects on the sedimentation coefficient Sedimentations were carried out at high ionic strength, 0 2μ and 0 5μ borate buffers The sedimentation-rates in both buffers were identical, within experimental error, when corrected for differences in viscosity and density of the buffer solutions Thus charge effects are damped out at least at 0 2 ionic strength for this protein system Sedimentation coefficients were determined thereafter as a function of concentration and S^0_{20w} was found by extrapolation to zero concentration Combined with a measured diffusion coefficient, and with a partial specific volume assumed the same as that of the undissociated macromolecule, the sedimentation data led to a molecular weight of about 14,000 for the sub-unit Osmotic pressure measurements give 13,500 It seems clear therefore that hæmerythrin is composed of eight sub-units

Furthermore, it seems likely that other proteins composed of sub-units not linked covalently should be dissociable by succinylation The large negative charge thereby introduced is not a hindrance in characterizing the sub-unit by hydrodynamic measurements since the electrostatic repulsions can be damped out with aqueous electrolyte solutions of only moderately greater ionic strength than commonly used in analytical ultracentrifugation measurements

This work was supported in part by a U S Public Health Service research grant (*H*-2910) and a predoctoral fellowship from the National Heart Institute and the Division of General Medical Sciences, U S Public Health Service

I M KLOTZ
S KERESZTES-NAGY

Department of Chemistry,
Northwestern University,
Evanston, Illinois

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BIOPHYSICS

Conformation of Synthetic Polypeptide and Protein Monolayers at Interfaces

SYNTHETIC polypeptide monolayers at the air–water interface are generally thought to be in extended-chain conformations[1] though Bamford *et al* [2] have pointed out that the experimental data can often be interpreted as indicating the presence of the α-helix[3] Similarly, protein monolayers are usually considered to be denatured with the polypeptide chain fully extended[4]

To investigate the possible presence of the α-helix at the air–water interface, deuterium-exchange in N-deuterated synthetic polypeptide monolayers has been studied In solution peptide deuterium exchanges for hydrogen rapidly when the peptide groups hydrogen-bond to solvent molecules, but exchange is very slow when the polypeptide is in the α-helical conformation[5] Slow exchange in monolayers might similarly be an indication of the presence and stability of the α-helix The polymers used were poly-D-α-amino-*n*-butyric acid, poly-DL-leucine, poly-γ-methyl-L-glutamate, poly-γ-ethyl-L-glutamate and poly-γ-benzyl-L-glutamate, all prepared by Mr W E Hanby, of Courtaulds, Ltd , Maidenhead, to whom I am indebted All the polymers were spread from dilute solution in chloroform containing 8 per cent v/v O-deuterated dichloroacetic acid, with the exception of poly-DL-leucine, where benzene was used in place of chloroform

After being spread as monolayers on N/100 hydrochloric acid at 20° C for 10 min the films were swept off the surface, dried and examined by infrared spectroscopy The spectra in all cases showed very little deuterium-exchange and were typical of polypeptides in the α-helical form Exchange was observed in similar experiments using a substrate of N/100 sodium hydroxide though it was not always complete, and again the spectra were consistent with the presence of the α-helix A series of experiments with poly-γ-methyl-L-glutamate has shown that the exchange depends on the time the film is on the substrate and it is favoured by a high *p*H and temperature The spectra show that these same conditions also eventually lead to the formation of the extended β-conformation These results are all consistent with the general presence of the α-helix which becomes less stable on alkaline substrates so that ultimately the β-conformation may be produced

Measurements of the area per residue on a Langmuir trough supported this conclusion and agreed within 10 per cent of the areas calculated from the closest packing distances obtained by X-ray diffraction observations of the polymers in the α-helical form The surface potentials observed varied from 340 mV to 590 mV on the acid substrate and were somewhat less on the alkaline The α-helix can have no appreciable dipole-moment at right-angles to its axis unless the side-chains are polar and are arranged asymmetrically on the water surface If the conclusions presented here are correct this must mean that, at least for the polymers with hydrocarbon side-chains, the surface potential arises mainly from adsorption of water molecules on to the helix

Since the α-helix is probably the stable conformation of these polymers in a non-hydrogen-bonding environment, a further conclusion is that this will also be their probable conformation at the oil–water interface Doty[6] has shown that the helical content of proteins in solution is increased by decreasing the hydrogen-bonding capacity of the environment In the light of the results given here, this suggests that at least the regions of proteins where the side-chains are mainly hydrocarbon will be in a helical conformation at the lipid–water interface. Quite possibly the unfolding observed with proteins at interfaces is often a loss of the tertiary structure

The possibility that the α-helix is stable at the lipid–water interface is of considerable importance in relation to the biochemical activity of interfaces In contrast to extended chain conformations, the all-round arrangement of side-chains in relatively well-defined positions could lead to specific interactions with adjacent protein, with the components of the lipid and with the aqueous phase

B. R Malcolm

Department of Biophysics,
University of Edinburgh

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BIOCHEMISTRY

Alkaline Phosphatase in Human Eccrine Sweat

In the course of histochemical investigations on the human eccrine sweat gland alkaline phosphatase reactivity in the luminal portion of the ampulla was observed[1] This observation has been repeated frequently, and it has been noted that this reactivity may continue into the proximal part of the coiled duct. Dr. G H. Findlay of Pretoria suggested that this phenomenon might represent deposition of alkaline phosphatase, if the enzyme were present in sweat We have found no reference to enable us to answer this question A random sample of sweat was accordingly collected from a normal male volunteer half an hour after the onset of profuse sweating in a 'hot box' It contained 2 9 King–Armstrong units of alkaline phosphatase

Further estimations were therefore made in the sweat of 5 normal adult males and one suffering from miliaria rubra ('prickly heat') The subjects performed light exercise under simulated tropical conditions (temp wet bulb 90° F ; dry bulb 93° F) and hourly sweat samples were collected from the interscapular region after washing with distilled water

Table 1 Alkaline Phosphatase Content of Sweat Samples in King–Armstrong Units

	Hours				
Subject	1	2	3	4	5
Ku	0 1	1 2	5 3	2 2	2 7
Mo	0 7	1 4	*	2 9	*
He	1 4	1 0	1 6	1 5	1 9
Be	3 2	1 4	2 4	2 1	1 3
Br	1 8	2 9	2 0	1 6	2 8
Na (miliaria)	5 0	0 8	1 6	6 1	10 6

*Insufficient sweat available

The method of estimating the levels of enzyme activity was that described by King and Wootton[2]

The source of this enzyme in sweat is presumably the secretory cells of the sweat coil, the cytoplasm of which shows marked acid and alkaline phosphomonoesterase activity We cannot say whether it is present in microsomal particles, as in milk[3] , if this were the case it might help to elucidate the mechanism of eccrine secretion Alternatively or additionally it could indicate increased permeability of the secretory cell membrane, analogous to that occurring in the kidney under experimental conditions[4] , the high figures obtained in a case of miliaria—a disease of the eccrine sweat unit—are of interest in this connexion and warrant further investigation

The work was performed with the aid of a grant from the Rand Mutual Assurance Co , Johannesburg, and is published with the permission of the Director of the South African Institute for Medical Research

L J A Loewenthal
W M Politzer

South African Institute for Medical Research,
Johannesburg

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Uridine Diphosphate Glucose-Glycogen Synthetase Activity in the Chick Embryo

In a recent paper[1], it was shown histochemically that glycogen synthesis began earlier than the appearance of phosphorylase in some tissues of the chick embryo In skeletal muscle, in particular, glycogen synthesis preceded the appearance of phosphorylase by about ten days[1,2] Because of that finding, it was suggested that the chick embryo, like the adult mammal, may be synthetizing glycogen through the uridine diphosphate glucose-glycogen (UDPG) pathway

In the investigation reported here fresh and frozen tissues of the chick embryos of white leghorn fowls were homogenized in sucrose and tested for the activity of UDPG-synthetase by the method of Leloir and Goldemberg[3]

The results indicate that in the heart, liver, skeletal muscle and brain UDPG-synthetase activity occurred earlier in development than phosphorylase and paralleled the appearance of glycogen in the tissues Fig 1 shows the activity of UDPG-synthetase in skeletal muscle of the chick embryo It can be seen that UDPG-synthetase activity was detected from the fourth day when glycogen synthesis was reported to begin[4,5], and about 10 days earlier than the date when phosphorylase was detectable histochemically[1] The evidence supports the theory that early glycogen metabolism of the embryo proceeds without insulin, for that hormone was not found in embryo pancreases until the 12th day[6]

These findings may help in the future understanding of such live and myopathic glycogen storages diseases which indicate the ability of these tissues to synthesize glycogen without being able to break down the carbohydrate

We thank Prof Lepkovsky, Department of Poultry Husbandry, Berkeley, United States, for his

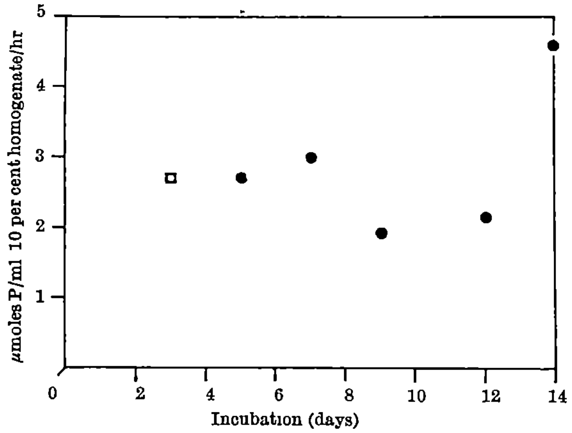

Fig 1 Glycogen synthetase activity. ●, Muscle, □, total embryo.

suggestions and the supply of eggs. This work was done during the visit of one of us (T A I G) to California

T Adesanya Ige Grillo
Kenji Ozone

Department of Anatomy,
University College,
Ibadan, Nigeria
and
Department of Poultry Husbandry,
University of California,
Berkeley, California

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Zinc in Bone

The role of zinc in biological systems has been the subject of many investigations during recent years Addink[1] suggested a possible correlation between the zinc content of blood and liver and the presence of cancer No clear confirmation of this correlation has been obtained Zinc has been found to be an integral portion of several enzymes by Vallee[2] The high levels of zinc present in the prostate, in leucocytes and in sperm have been observed by a number of investigators[3] In general, the understanding of the role of zinc in biological systems is still largely reduced to a description of concentration differences rather than of metabolic interactions

In a recent article Taylor[4] infers that the zinc content of rat bone increases with age, for example, 77 p p m to 136 p p m to 200 p p m at 56, 313 and 679 days respectively If this is so, this finding would be quite useful in ageing studies and in the general understanding of bone structure. This suggestion prompted a re-examination of bones from several previous strontium studies for their zinc content

The stable zinc-levels were determined by means of an X-ray fluorescence method and confirmed by emission spectrographic analysis The satisfactory recovery of zinc under the conditions of ashing (12 h at 600° C) was verified using metabolically incorporated zinc-65

In Table 1 are shown the results from a group of Wistar-strain rats reared on a diet which had a constant zinc to calcium ratio of 2 75 mg zinc/g calcium This experiment was terminated at 414 days, as compared with the 679 days of Taylor's experiment No elevation of zinc in the bone was observed In fact, since the ends contain approximately 30 per cent more zinc than the shaft, one might expect that the femur zinc-level would go down with age as the ratio of compact to cancellous bone increases, if it should be expected to change at all

Table 1 Zinc Content of Rat Femur

Age (days)	Zinc (p p m of ash) Shaft	End	Femur
New-born	338	430	399
57	362	431	402
67	339	412	375
145	323	419	371
169	327	426	380
262	302	369	336
314	278	398	353
365	—	—	384
374	319	451	393
409	340	455	412
414	306	407	370
		Mean ± *S D*	380 ± 24

In Table 2 are shown data from human ribs selected at random from two sets of samples previously analysed for strontium[5] The results are tabulated according to increasing zinc-level in order to observe any possible correlation with age or locality. No such correlation is apparent, however, the six-fold increase in variance for the human samples, as compared with the rats on a controlled diet, suggests a definite correlation with the zinc and calcium ratio of the diet It seems likely that the level of zinc in bone is directly related to the zinc to calcium ratio of the diet, as was found to be the case for strontium. The inferred retention factor for zinc in rat bone would be 0 40 as compared with 0 28 for strontium

Table 2 Zinc Content of Human Rib

Age (yr)	Location*	p p m. Zn (ash)	Age (yr)	Location*	p p m Zn (ash)
40	E	106	14	F	155
16	B	122	31	A	155
15	C	123	35	C	170
22	A	131	17	B	171
16	G	134	13	E	180
9	B	140	75	B	188
17	A	141	47 ±	B	202
5	B	143	38	G	209
34	B	144	51	B	300
47 ±	B	147	0 7	B	306
47 ±	B	149	63	B	330
30	D	150	38	G	372
11	D	153	Mean ± *S D* = 181 ± 70		

* A, Fresno and Oakland, California, B, Los Angeles, California C, Phoenix, Arizona, D, Tulsa, Oklahoma, E, Madison, Wisconsin; F, Minneapolis, Minnesota, G, Philadelphia, Pennsylvania

This work was supported by contract *AT*(04-1) *GEN*-12 between the U S Atomic Energy Commission and the University of California at Los Angeles

Geo V Alexander
R E Nusbaum

Laboratory of Nuclear Medicine
Radiation Biology,
Department of Biophysics and
and Nuclear Medicine,
School of Medicine,
University of California,
Los Angeles

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Effect of Phosphoric Acid on Digitalis Compounds

IT has been shown by Cox[1] that pregnane-3α,17α,-20α-triol (pregnanetriol) and related compounds react with 70 per cent ortho-phosphoric acid to produce a brilliant fluorescence Herman and Bruton[2,3] have shown that pregnanetriol and related compounds and digitalis compounds react intensely with antimony pentachloride, which thus can be used as a paper chromatographic detecting agent Since pregnanetriol reacts with both phosphoric acid and antimony pentachloride, by analogy, those steroids which react strongly with antimony pentachloride could be expected to react with 70 per cent ortho-phosphoric acid Heftmann *et al* [4] have noted briefly that aniline-phosphoric acid is useful as a detecting agent for cardiac glycosides

We have applied ortho-phosphoric acid to digitalis compounds and other steroids and have found that the natural fluorescence of the digitalis compounds under ultra-violet light is markedly enhanced Each steroid was dissolved in chloroform and applied to Whatman No 1 chromatogram paper in 50-μgm amounts or less The paper was dipped into 70 per cent orthophosphoric acid and placed on a sheet of tinfoil The paper was examined in visible and ultra-violet light (366 and 254 mμ) before and after heating at 80° C for 5 min

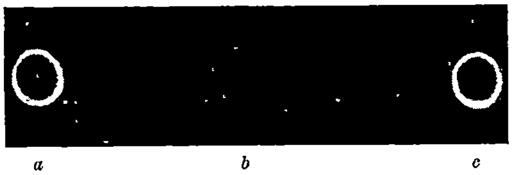

Fig 1 Fluorescent photograph of digitalis compounds treated with 70 per cent orthophosphoric acid The compounds are *a*, digitoxigenin, *b*, digoxin, *c*, digitoxin

Table 1 indicates the results obtained with digitalis compounds and other steroids and related compounds Fig 1 is a fluorescent photograph of the digitalis compounds treated with 70 per cent ortho-phosphoric acid The Whatman No 1 paper containing the treated digitalis compounds was placed on type *IF* contrast 5 Haloid photographic paper (Xerox Corp , Rochester, New York) and exposed for 5 sec at approximately 18 in using a 254 mμ ultra-violet light[5] Both digitoxin and digitoxigenin fluoresce with a bright yellow colour and are reproduced well on the Haloid photographic paper The digoxin fluoresces with a bright blue colour but appears much less intensely in the fluorescent photograph Haloid photographic paper is insensitive to wave-lengths of light beyond 450 mμ so that fluorescence at wave-lengths beyond 450 mμ can be recorded as an unexposed area

We were able to detect a minimum of 10 μgm of each of the three digitalis compounds in visible light using 70 per cent phosphoric acid With ultra-violet light (366 mμ) we could detect a minimum of 5-μgm with the fluorescence being more brilliant after heating

This technique should be useful as a detecting method for digitalis compounds

JOSEPH BRUTON
ROBERT H HERMAN
LLOYD E CLAYTON

Department of Metabolism,
Division of Medicine,
Walter Reed Army Institute of Research,
Washington 12, D C

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Table 1 EFFECT OF PHOSPHORIC ACID ON DIGITALIS COMPOUNDS AND OTHER STEROIDS AND RELATED COMPOUNDS

	Before heating			After heating		
Compound	Visible light	366 mμ	254 mμ	Visible light	366 mμ	254 mμ
Digoxin	yellow colour	bright blue fluorescence	bright blue fluorescence	green-yellow colour	bright blue fluorescence	blue fluorescence
Digitoxin	faint yellow colour	bright yellow fluorescence	dark yellow fluorescence	green-yellow colour	bright yellow fluorescence	yellow fluorescence
Digitoxigenin	no colour	bright yellow fluorescence	dark yellow fluorescence	green-yellow colour	bright yellow fluorescence	yellow fluorescence
Ergosterol	yellow colour	yellow absorbance	purple absorbance	pale-yellow colour	yellow absorbance	orange absorbance
7-Dehydrocholesterol	no colour	no colour	purple absorbance	no colour	yellow absorbance	orange absorbance
Vitamin D_2	no colour	no colour	purple absorbance	no colour	no colour	no colour
Vitamin D_3	no colour	no colour	purple absorbance	no colour	no colour	no colour
Adrenosterone	no colour	no colour	purple absorbance	no colour	no colour	purple absorbance

O-Methylation in Fish

DURING an investigation of the fate of some foreign organic compounds in fish, the metabolism of 3,4-dihydroxyphenylacetic acid (homoprotocatechuic acid) was investigated with the purpose of obtaining some information on methylation reactions in these animals DeEds *et al* [1] have noted that this compound is methylated at the 3-hydroxyl group in rats and rabbits A later report by Scheline *et al* [2] showed that homoprotocatechuic acid labelled with carbon-14 fed to rabbits at a dose-level of 100 mgm /kgm resulted in about 85 per cent of the radioactivity being excreted in the urine within 44 hr While the main excretory product was the original acid, nearly six per cent of the dose was found as 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid)

In the experiments described here 12 cod, *Gadus callarias*, weighing 420–1,370 gm (average 740 gm) were given homoprotocatechuic acid dissolved in saline The dose-levels ranged from 35 to 120 mgm / kgm (average 72 mgm /kgm) and the compound was injected intraperitoneally (nine animals) and intramuscularly (three animals) The method used for identifying metabolites was similar in part to that of Wood[3] and consisted of placing the treated fish in a small plastic tank containing 5 l of water The water was aerated and maintained at 10° C by keeping the tank in a constant flow of cold water After 20 hr the fish was removed, the water acidified, concentrated, and extracted with ether to obtain an extract suitable for chromatography Single dimension chromatograms of the extracts and reference compounds were made using three different solvent systems and the compounds visualized by spraying with solutions of brentamine fast blue *B* salt or 2,6-dichloroquinone chloroimide followed by 2 per cent sodium carbonate solution These chromatograms clearly showed the presence of 4-hydroxy-3-methoxyphenylacetic acid as well as homoprotocatechuic acid in the extracts While the relative amounts of these two substances varied somewhat from animal to animal, both were observed in all the experiments except one when, for an unknown reason, neither compound was found

Since the report by Axelrod[4] that the principal pathway in the inactivation of adrenaline and noradrenaline in man is one of *O*-methylation, considerable attention has been given to the subject of biological *O*-methylation The enzyme catechol *O*-methyl transferase which carries out this reaction is found in many mammalian species[5] and can methylate a wide variety of catechol compounds, including homoprotocatechuic acid[6] It has been noted that polyphenols such as pyrogallol inhibit the methylation of catecholamines[7,8] by acting as a competitive substrate for this enzyme[9]

Euler[10] has shown that visceral organs as well as chromaffin tissue of fish contain adrenaline and noradrenaline One might therefore expect catechol *O*-methyl transferase, or a similar enzyme system, to be present in these animals and for pyrogallol to inhibit the methylation of its catechol substrates With this in mind, six cod were given by intraperitoneal injection a solution containing both homoprotocatechuic acid and pyrogallol A dose-level of 40 mgm /kgm of homoprotocatechuic acid was used as this had been shown to give easily detectable amounts of the methylated metabolite The doses of pyrogallol were 40 mgm /kgm in two fish, 30 mgm /kgm in three fish, and 20 mgm /kgm in one fish and the extracts were prepared as before The results from these experiments clearly demonstrated that pyrogallol inhibited the methylation of homoprotocatechuic acid At the two higher dose-levels no 4-hydroxy-3-methoxyphenylacetic acid was observed on the chromatograms and only small amounts were found when 20 mgm /kgm of pyrogallol was given In all six experiments, the amounts of homoprotocatechuic acid on the chromatograms were increased over that usually seen

Also observed on these chromatograms was a spot differing in R_F value and colour reaction from pyrogallol or the methylated or dehydroxylated derivatives of homoprotocatechuic acid Archer *et al* [9] believe that pyrogallol first undergoes methylation on the 1-hydroxyl group before final methylation to form the 1,2-dimethyl ether The unknown compound differs from the 1-methyl ether of pyrogallol, however, in that its R_F value is slightly less and it gives a different colour after spraying with brentamine fast blue *B* salt In addition, it seems unlikely that it is the dimethyl ether as this compound would be expected to have a greater R_F value than pyrogallol monomethyl ether in the solvent systems used The identity of this compound is therefore not at present known although it is conceivable that it may be the 2-methyl ether of pyrogallol A similar reaction has been reported by Booth *et al* [11], who have shown that gallic acid is methylated to form 3,5-dihydroxy-4-methoxybenzoic acid

This work was supported by a U S Public Health Service fellowship No *EF*-8328 from the National Institute of Allergy and Infectious Diseases I thank Dr G Rollefsen and Mr G Sundnes for their help in providing facilities for this work

RONALD R SCHELINE

Institute of Marine Research,
Directorate of Fisheries,
Bergen, and
Pharmacology Institute,
University of Bergen,
Bergen, Norway

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Carotenoid Pigments in Rotifera

THE Rotifera are remarkable among the Metazoa for their small size, often smaller than the largest ciliated Protozoa Many rotifers are colourless or at most show some slight colour in the gut wall, clearly due to pigment taken in with food A few species may have pigment spread throughout the body, others may have red eyespots and the fat globules of resting eggs may be bright orange in colour This combination of small size and general lack of colour makes investigation of the pigments of this group difficult and so far as we are aware no rotifer pigments have previously been identified

We have been fortunate in obtaining, more or less by accident, two species of rotifer in sufficient numbers to extract pigments from and apply chromatographic and spectrophotometric techniques

A dense population of *Philodina roseola* Ehrb was found in a dish in which the anostracan crustacean *Chirocephalus diaphanus* was being cultured The rotifers formed dense pink patches on the sides of the dish, and when these patches were scraped off with a clean scalpel they were found to consist of thousands of individuals of *P roseola*, together with even greater numbers of pink eggs intermingled with a few yeast cells which had been added as food for *Chirocephalus*

The food available to the rotifers was almost entirely yeast, but as the culture dish was placed near a window a few algal cells may also have been present There were no other species of rotifer in the material scraped from the dish and a few yeast cells would not interfere with the analysis since no carotenoid pigment is detectable in the bakers' yeast which we used[1]

Carotenoid pigments were extracted from *Philodina* in acetone and transferred to light petroleum (b p 40°–60° C) after dilution of the aceone with water The solution in light petroleum was washed with water to remove traces of acetone and dried over anhydrous sodium sulphate The absorption spectrum of this solution showed that a mixture of carotenoids was present The solution was poured on to an alumina column and the chromatogram developed by adding increasing amounts of acetone to the light petroleum used to wash the column Four fractions were separated

(1) A yellow fraction passed through the column with light petroleum and showed a maximum absorption at 449 mμ The position of this absorption maximum together with the chromatographic behaviour of the fraction is in agreement with the presence of β-carotene This fraction did not form more than 10 per cent of the total carotenoid pigments

(2) This fraction was eluted with 30 per cent acetone and showed a single absorption maximum in light petroleum at 468–470 mμ It formed about 35 per cent of the total pigment and appears to be an ester of astaxanthin

(3) A third fraction was eluted with 30 per cent acetone and came off the column after the astaxanthin ester The amount of pigment was too small to examine spectroscopically and formed less than 5 per cent of the total carotenoids

(4) The final fraction formed a deep orange band at the top of the column It was resistant to elution with acetone but moved rapidly down the column when a trace of glacial acetic acid was added to the acetone The pigment was taken into light petroleum, washed well to remove the acetic acid and acetone and dried over anhydrous sodium sulphate The pigment was hypophasic when partitioned between light petroleum and 90 per cent methanol even when the hypophase was further diluted, but on the addition of a drop of glacial acetic acid the pigment moved to the epiphase The absorption spectrum in light petroleum showed a single maximum at 468–470 mμ, which, coupled with the characteristic phase behaviour, indicates that the pigment is astacene derived from astaxanthin. This pigment formed about 50 per cent of the total, so together with the esterified fraction, astaxanthin formed about 85 per cent of the total carotenoids in *Philodina*

These results suggest that *Philodina* is able to accumulate carotenoids from food and convert these into astaxanthin. Observations on another rotifer, *Brachionus calyciflorus* Pallas, further support this suggestion This rotifer was collected in large numbers from the overflow water at the east end of the Long Water in Hampton Court Park The plankton sample consisted mainly of *B calyciflorus* together with a very few other rotifers and small crustaceans. The rotifers were separated from the crustaceans by making use of their reactions to light The rotifer sample was then placed in a Petri dish and warmed until the animals became immobile When the sample was examined under a binocular microscope it was found that only a very few other rotifers were present and counts showed the sample to contain 99 8 per cent *B calyciflorus*.

The carotenoid pigments were extracted from *Brachionus* in acetone The extract was much more dilute than from *Philodina* and so the absorption spectrum was measured in acetone before trying any further treatment Since the gut of these rotifers probably contained algal cells taken in as food, it was decided to compare the absorption curve of an acetone extract of *Brachionus* with that of a sample of phytoplankton taken from the Long Water on the same day as the rotifers were collected This would show whether the rotifers were accumulating carotenoids or whether the carotenoids in the rotifer extract were from algal cells in the gut These curves are shown in Fig 1 and it can be seen that they differ markedly in shape The curve from phytoplankton (Fig 1*a*) shows the absorption maximum of chlorophyll *a* at 432 mμ but in the curve from *Brachionus* (Fig 1*b*) this maximum has shifted to 414 mμ, suggesting that the chlorophyll has been converted to phæophytin *a* in the gut of the rotifers The curve from *Brachionus* also shows a region of maximal absorption over the range 450–480 mμ not present in the phytoplankton curve This is the result of the accumulation of carotenoids by the rotifers The shape of the curve from *Brachionus* suggests a mixture of carotenoids and this was confirmed by chromatography of the extract in light petroleum on a very small alumina column. Two bands of carotenoid separated on the column, a lower yellow band was eluted with 60 per cent acetone and an upper pink band was eluted with acetone to which a few drops of glacial acetic acid were added Too little of the pigments was obtained to examine their spectral properties but the behaviour of the upper band suggests the presence of astaxanthin.

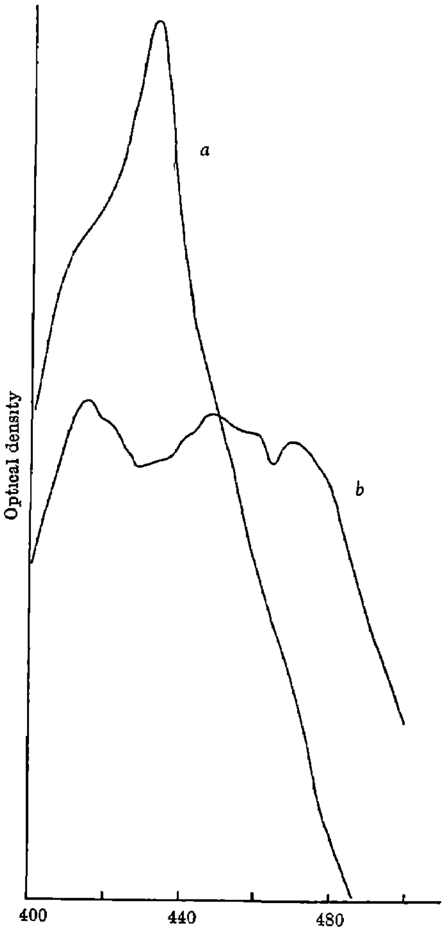

Fig 1 Spectral absorption curves of acetone extracts of (*a*) phytoplankton and (*b*) *Brachionus calyciflorus* from the Long Water, Hampton Court

An additional feature of interest in *Brachionus calyciflorus* is that the eggs are coloured pale blue and on treatment with acetone or dilute mineral acids they become pale orange This change in colour, coupled with the suggestion of astaxanthin on the alumina chromatogram, indicates that the eggs contain a carotenoprotein, thus showing remarkable similarity to the eggs of many Crustacea

These results add another phylum to those which include animals known to accumulate astaxanthin,.

and since *Philodina* belongs to the Bdelloidea and *Brachionus* to the Ploima, show that the ability to accumulate carotenoids is present in the two major subgroups of the Rotifera

B M Gilchrist
J Green

Zoology Department,
Bedford College,
Regent's Park,
London, N W 1

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A Solvent-soluble Fraction of the Fleece of Sheep associated with Suint

Suint has been defined as the water-soluble material in greasy wool[1], and recent work has shown it to be a complex mixture of salts of organic acids, polypeptide material, urea and colouring matter[2–4] The woolwax fraction in the fleece is usually determined by Soxhlet extraction with the appropriate solvent, but no importance has been attached to whether the Soxhlet extraction is carried out before, or after, the removal of suint[1] In addition, some workers have stated that the choice of solvents is not important, polar or non-polar solvents extracting about the same amount of material[5]

I have found that, with Romney cross-bred wools, quite different amounts of extract are obtained depending on whether wax determinations follow, or precede, suint determinations and in addition, differences between solvents are significant Table 1 gives typical results for some solvents, each result being the mean of six determinations made on wool from the same batch

Table 1 Results of Solvent Extractions

Solvent	Greasy wool		De-suinted wool*	
	Extract† (per cent)	C V (per cent)	Extract† (per cent)	C V. (per cent)
Petroleum ether 40/60	7 53	5 3	5 23	9 1
Diethyl ether	5 30	9 2	4 26	9 8
Benzene/methanol	10 88	5 1	5 22	8 7
Dry ethanol	8 65	6 1	5 94	11 5
Methylene chloride	8 28	5 2	4 35	10 3

* Suint removed by cold water
† Expressed as a percentage on clean oven-dry weight of wool

These results are not surprising when the heterogeneity of suint and wool wax is considered, and they are confirmed in recent work reported by Harker[6] They suggest that an 'overlap' material might exist, soluble or dispersible in water and also soluble in organic solvents Since other workers investigating the composition of suint have obtained their starting material after a preliminary solvent extraction of greasy wool[4,7–9], or by centrifuging and filtering the suint solution[1], it is possible that such an overlap fraction might have escaped isolation

Four lots of 50 gm of 50*s* quality greasy cross-bred fleece were gently stirred one after the other in 400 ml of cold water 200 ml of the resulting suint solution, after centrifuging to remove dirt, were evaporated to dryness and extracted with hot petroleum ether 40/60, yielding 290 mgm of a dark waxy material This possessed the unusual property of emulsifying spontaneously when shaken with cold water Table 2 shows its properties as gained from a preliminary investigation For comparison, the properties of the wool wax obtained from the same wool sample after removal of suint are also given

The material gave reactions similar to wool wax with the Liebermann–Burchardt and Salkowski tests, while micro-analysis revealed only a trace of nitrogen On the limited evidence available the material would appear to be a complex mixture of alcohols, a conclusion which is supported by its infra-red spectrum and high hydroxyl value

Table 2 Properties of Overlap Fraction and Wool Wax

	Overlap fraction	Wool wax from same wool sample
Colour	dark brown	pale yellow
Free fatty acid as oleic (per cent)	0	10
Slip point (°C)	42	40
Saponification value	60	91
Hydroxyl value	110	47

It is interesting to note that Anderson and Truter[10] have recently discussed the discoloration of wool wax recovered from both solvent and emulsion scouring in the presence of suint They ascribe this in part to the formation of an interfacial complex between wax and peptide components which darkens on heating The 'overlap' material described here could also be expected to contribute to a darkening of the recovered woolwax and like suint, would be removable by a cold water wash

I thank Dr E Corbett of the Department of Chemistry, University of Otago, for infra-red spectrographic analysis of the material

R G Stewart

New Zealand Wool Industries
Research Institute (Inc),
444 George Street,
Dunedin

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Soybean Phenoxyesterase

Esterases of blood plasma have been classified as *A*- and *B*-esterases on the basis of their responses to organophosphorous inhibitors[1] This classification has been extended to include esterases obtained from other animal tissues *A*-esterases (arylesterases) are not, whereas *B*-esterases (aliesterases) are, inhibited by organophosphorous chemicals Bergmann *et al* [2] isolated a *C*-esterase from hog kidney extracts which behaved like an *A*-esterase, but was activated by *p*-chloromercuribenzoate (PCMB) at concentrations which inhibited *A*-esterases Plant esterases have yet to be included in this classification An esterase from citrus[3] and wheat germ esterase[4] were originally described as acetylesterases Since inhibition was obtained with diisopropylfluorophosphate (DFP), they can be classified as acetyl-*B*-esterases

While we were characterizing plant enzymes which catalyse the hydrolysis of esters of phenoxyacetic acid, an enzyme which expressed considerable specificity for 2-naphthylphenoxyacetate (NPOA) was found in soybean seedlings This enzyme has properties different from those previously described Hence, the name phenoxyesterase is suggested

Acetone powders were prepared from the aboveground parts of 5-to-7-day-old soybean (*Glycine max* (L) Merr 'Lee') seedlings and extracted with cold

(3° C) distilled water The slurry was filtered through cheesecloth and the filtrate centrifuged at 12,000*g* for 15 min The supernatant constituted the crude enzyme preparation The *p*H of the supernatant was adjusted to 5 3 with 10 per cent acetic acid and the precipitate which formed was removed by centrifugation and discarded Alumina gel[5] was added to the supernatant at a ratio of 1 mgm gel/mgm protein After 30 min the gel was removed by centrifugation and the supernatant discarded The gel was washed with the original volume of cold distilled water and the enzyme was eluted with 0 01 *M* potassium phosphate buffer *p*H 7 0 Sufficient solid ammonium sulphate was then added to give 45 per cent saturation The precipitate was removed by centrifugation and suspended in 1/10 the original volume of 0 2 *M* potassium phosphate buffer *p*H 7 0 By means of the described procedure a 10-fold total purification, with 11 per cent recovery, was obtained when the enzyme was assayed with NPOA as substrate

The various 2-naphthyl esters used in this investigation were synthesized by the procedure of Gomori[6] Hydrolysis of 2-naphthyl esters was assayed by the method of Ramsey[7] 'Brij 35', a polyoxyethylene lauryl ether (Atlas Powder Co , Wilmington, Del) was used as an emulsifier at a concentration of 0 83 per cent (w/v)

The purified phenoxyesterase had an optimum *p*H of 6 0 (phosphate buffer) and the reaction followed first-order kinetics over the first 20 min During purification, enzymes which hydrolysed 2-naphthylacetate and 2-naphthylbutyrate were largely eliminated (Table 1) 2-Naphthylphenylacetate, -benzoate, and -caprylate and 1-naphthyl-*N*-methylcarbamate were not hydrolysed by the purified phenoxyesterase preparation Hence, phenoxyesterase hydrolyses neither esters of fatty acids nor esters of pure aromatic acids When the ethereal oxygen of phenoxyacetic acid is replaced by a methylene group as in 3-phenylpropionic acid, the corresponding 2-naphthyl ester was hydrolysed at a rate of only 0 7 per cent of that of NPOA Thus, phenoxyesterase shows a very high degree of specificity for NPOA

Table 1 RELATIVE ACTIVITIES OF CRUDE AND PURIFIED PREPARATIONS OF PHENOXYESTERASE ON DIFFERENT 2-NAPHTHYL SUBSTRATES AT *p*H 6 0 AND 37° C

Substrate	Relative activities Crude preparation	 Purified preparation
2-Naphthyl-		
phenoxyacetate	100	100
acetate	32	4
butyrate	19	3 6

Phenoxyesterase was inhibited slightly by 10^{-3} *M* copper sulphate (19 per cent) The enzyme could be protected against this inhibition by histidine, but not by glycine This behaviour suggests that an imidazole ring may be present at the active centre[2] PCMB had no effect on phenoxyesterase between 10^{-3} and 10^{-6} *M* Limited stimulation (17 per cent) was obtained with 10^{-3} *M* DFP This enzyme, therefore, appears to have properties different from *A*-, *B*-, and *C*-esterases[1,2]

Ethylphenoxyacetate (EPOA) was hydrolysed rapidly and at approximately the same rate as NPOA by both the crude and the purified preparations (Hydrolysis of EPOA was followed by an adaptation of the procedure of Hestrin[8]) Activity of the purified preparation was slightly inhibited (28 per cent) by 10^{-3} *M* DFP with EPOA as substrate Hence, an enzyme other than phenoxyesterase also possibly participated in the hydrolysis of EPOA

One of us (J v d W J) is grateful for support extended by the South African Wheat Industry Control Board and the South African Department of Agriculture Technical Services We thank Dr G C Klingman for help

JASPER VAN DER W JOOSTE*
DONALD E MORELAND

Department of Crop Science,
North Carolina State College
and Crops Research Division,
Agricultural Research Service,
U S Department of Agriculture,
Raleigh, N C

---

* Present address Stellenbosch-Elsenburg College of Agriculture, Stellenbosch, South Africa

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PHYSIOLOGY

Effect of Hydrogen Ion on the Rates of Migration of Sodium and Potassium Ions

BECAUSE of its high mobility, H^+ is superior to other cations as a source of diffusion potentials From the differences between the mobilities of Na^+ and K^+ it could be anticipated that in the simultaneous diffusion of these three cations the effect of the diffusion potential of H^+ on the rates of migration of Na^+ and K^+ would not be equal As shown, for example, by Vinograd and McBain, the diffusion potential of H^+ has a distinct effect on the rate of migration of K^+ in the system of simultaneous diffusion of H^+ and K^+ [1] The effect of diffusion of H^+ on ionic distribution in the system of steady state has been examined by Teorell[2] The fact that little attention has been paid to the effect of H^+ on the selective migration of Na^+ and K^+ may be due to technical difficulties in the determination of these cations in the same fluid before the era of flame photometry

It was found in the present work that the rates of migration of Na^+ and K^+ are selectively changed depending on the direction of migration of H^+ in the diffusion system Furthermore, it was found that heparin selectively changes the rates of migration of Na^+ and K^+ and that this selectivity could be changed by simultaneous migration of H^+

A U-shaped diffusion cell (Fig 1) was first filled with 1 ml of the fluid into which the diffusion occurs Then 0 5 ml of the initial fluid of known concentrations of Na^+ and K^+ was carefully pipetted into the left limb After a diffusion time of 24 h at room temperature, 0 7 ml of the diffusate fluid was pipetted out from the right bulb and its concentrations of alkali metal ions was determined with a flame photometer The methodical error calculated from the 10 duplicate determinations was found to be 13 per cent for the diffusate Na^+ concentration, 12 5 per cent for the diffusate K^+ concentration, and 3 5 per cent for the diffusate K^+/Na^+ ratio The results in the tables are the means of triplicate experiments

The approximate potential difference between the initial fluid and the diffusate was measured by immersing calomel electrode KCl bridges in the fluids

Table 1 DIFFUSION OF Na^+ AND K^+ WITHOUT AND WITH SIMULTANEOUS DIFFUSION OF H^+

	Exp	Initial fluid (mM/l)		P d (mV)	Diffusate (mM/l)		
		Na^+	K^+		Na^+	K^+	K^+/Na^+
1	Diffusion of NaCl and KCl into water	100	10	+12	2 30	0 32	0 139
		50	50	+4	1 10	1 51	1 37
2	Diffusion of NaCl and KCl with 0 01 N HCl into water	50	50	−18	1 06	1 03	0 97
3	Diffusion of NaCl and KCl into 0 01 N HCl	50	50	+20	1 30	1 90	1 46

Table 2 DIFFUSION OF Na^+ AND K^+ FROM HEPARIN SOLUTION INTO WATER AND INTO 0 01 N HCl

	Exp	Initial fluid (mM/l)		P d (mV)	Diffusate (mM/l)		
		Na^+	K^+		Na^+	K^+	K^+/Na^+
1	Diffusion of NaCl and KCl from heparin solution into water	100	10	−34	0 75	0 065	0 087
2	Diffusion of NaCl and KCl from heparin solution into 0 01 N HCl	100	10	+4	1 30	0 18	0 138

in the two bulbs The potential difference between the electrodes (p d) was measured with a compensation potentiometer The symbol − or + indicates the sign of the charge of the initial fluid

In the first experiments (Table 1) the aqueous solution of sodium chloride and potassium chloride was diffused against water The second and third experiments (Table 1) show what happens when dilute hydrochloric acid diffuses in the same direction and against the direction of simultaneous diffusion of sodium chloride and potassium chloride Owing to the high mobility of H^+, the initial fluid was negatively charged when H^+ diffused in the same direction as Na^+, K^+, and Cl^- This decreased the rate of migration of both Na^+ and K^+ However, the migration of K^+ was retarded more than that of Na^+, resulting in a decrease in the diffusate K^+/Na^+ ratio Opposite changes were seen when sodium chloride and potassium chloride diffused against hydrochloric acid

Thus in the simultaneous diffusion of Na^+, K^+, H^+, Cl^-, and water, the relative concentration of K^+ with respect to that of Na^+ increased in the fluid having the higher concentration of H^+

It is obvious that the changes in the K^+/Na^+ ratio were temporary and dependent on the continuous migration of H^+ It appears that this condition is not far removed from what happens in the living cell H^+ is a metabolic product in the intracellular fluid Thus these results might be of interest in regard to the problem of selective accumulation of K^+ in preference to Na^+ in the intracellular fluid

It is generally believed that the selective distribution of alkali metal ions is the effect of a specific cation pump system in the cell membrane[3] The chemical basis of this hypothesis is imperfectly understood, however As pointed out by Ussing, other possibilities should also be considered[4]

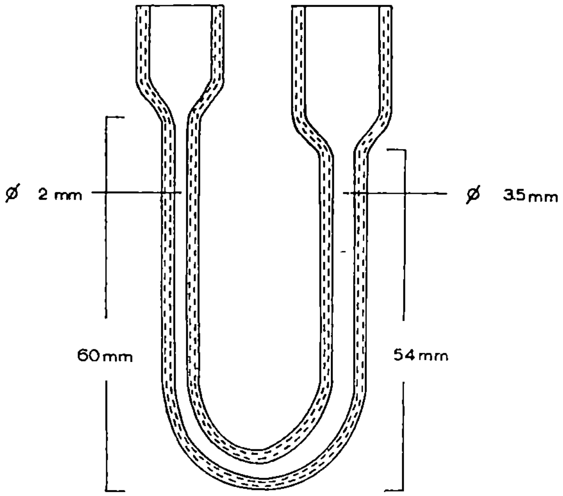

Fig 1 The diffusion cell made of 'Pyrex' glass

Ling, and Joseph *et al* suggested that negative charge of organic anions might be one of the primary reasons for the selective ionic distribution[5,6]. The present experiments with heparin solution are shown in Table 2 The composition of the initial heparin solution was as follows 100 mM/l of Na^+, 10 mM/l of K^+, 35 mM/l of Cl^-, and 2 4 g/100 ml of heparin Its *p*H was 7 3 (The original sodium heparin solution, 'Orion *OY*', Helsinki contained no K^+, and therefore potassium chloride was added to give a K^+ concentration of 10 mM/l)

Owing to the slow mobility of the heparin polyanion, the heparin solution was negatively charged when Na^+, K^+, and Cl^+ diffused from the heparin solution into the water The diffusate K^+/Na^+ ratio was lower than that of the heparin solution, suggesting that heparin acts as a cation exchanger immobilizing K^+ in preference to Na^+ This is in agreement with previous experiments carried out by the ultrafiltration through a semipermeable membrane[7] When the negative charge of the heparin solution was cancelled out by dilute hydrochloric acid diffusing against the heparin solution, the Na^+ and K^+ diffused from the heparin solution according to their free diffusion ratio, and the selectivity of heparin for alkali metal ions was thus abolished

Meyer and Bernfeld suggested that the origin of the resting potential of the intracellular fluid is the diffusion potential of H^+ diffusing out from the intracellular fluid[8] This hypothesis is not generally accepted The reason may be that it does not take into account the selective distribution of alkali metal ions It seems that this weakness in the hypothesis of Meyer and Bernfeld is partly overcome by the present finding, suggesting that the migration of H^+ may be one factor in the selective distribution of Na^+ and K^+

I thank Profs Pentti I Halonen, Eero Tommila and Veijo Wartiovaara for advice

SIMO SALMINEN

Wihuri Research Institute,
Helsinki

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Temporal Summation of After-Hyperpolarization following a Motoneurone Spike

When recorded intracellularly, the soma-dendritic spike potential of cat spinal motoneurones (*SD* spike[1]) is followed by a brief phase of depolarization which reverses to a prolonged hyperpolarization (duration, 60–250 msec.)[1,2]. Investigation of after-hyperpolarization has yielded three experimental results which conjointly led to the hypothesis that it is due to a prolonged increase in permeability to potassium ions[3]: there is an increased membrane conductance, when the membrane potential is changed by applying current intracellularly, the size of the after-hyperpolarization is increased by depolarization and decreased by hyperpolarization, being reduced to zero at about 20-mV hyperpolarization, this equilibrium potential is shifted in the depolarizing direction by an electrophoretic injection of sodium ions into the motoneurone, which would of course result in a decrease of intracellular potassium ion concentration. In the present investigation on after-hyperpolarization, successive spike potentials of a cat motoneurone resulted in a considerable summation of after-hyperpolarizations and there was a corresponding increase in the membrane conductance.

Under 'Nembutal' anæsthesia the cat spinal cord was transected at the L_1 or L_2 segmental level. Single micro-electrodes were filled with 3*M* potassium chloride solution and inserted into motoneurones at the L_7 or S_1 level. The antidromic spike potential was induced in impaled motoneurones by a stimulation of cut ventral roots and also by passing a short depolarizing pulse (duration, 0.1–0.2 msec.) through an intracellular micro-electrode[4,5].

Rectangular currents of a small intensity (2×10^{-9} amp.) were applied intracellularly for monitoring the membrane conductance. The potential change in the membrane produced by the current was observed after the voltage drop across the electrode resistance was compensated for by inphase-rejection at the differential input stage of a d.c. amplifier[6]. These potentials were displayed on two beams of the cathode ray oscilloscope, one with a low d.c. amplification and the other with a high a.c. (t.c., 1 sec., Fig. 1 *A–D*) or a high d.c. amplification (*G–L*). The records in Fig. 1 *A–D*, *G–L* were formed by the superposition of about ten traces at a repetition-rate of 3 per sec.

In Fig. 1*A* the lower trace shows a spike potential in a motoneurone obtained by an antidromic stimulation, and the phase of the after-hyperpolarization is illustrated in the upper trace with a high amplification. In Fig. 1*B* a stimulating current pulse was applied through the microelectrode at an interval of 5.7 msec. after the antidromic stimulation and was adjusted critically so as to induce a small spike from the initial segment of the motoneurone, the *IS* spike[4,5], in about half the trials, as shown in the lower trace. It is seen in the upper trace of *B* that this direct pulse, and the thereby induced *IS* spike, did not make any appreciable change in the after-hyperpolarization following the antidromic spike.

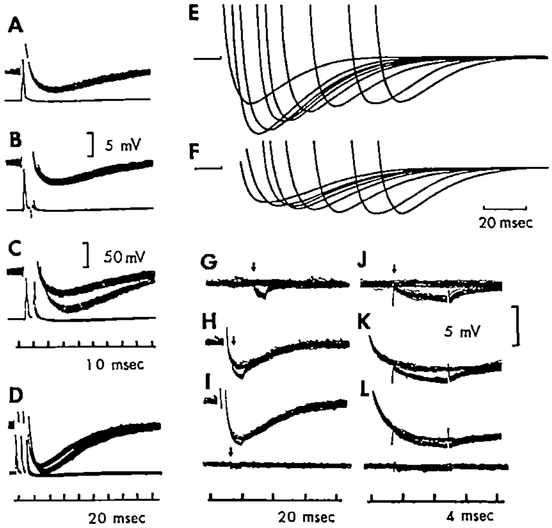

Fig. 1. *A–D*, antidromic spike of a cat motoneurone was followed by single (*B*, *C*) or double (*D*) intracellular stimulation. Voltage calibration of 5 mV applies to the upper traces and that of 50 mV to the lower ones. Time-scale of 10 msec. is for *A–C* and that of 20 msec. for *D*. *E*, tracings of after-hyperpolarizations following double antidromic spike potentials are superimposed after different intervals of ventral root stimulation. *F*, graphical illustration of the after-hyperpolarization that was added to the initial response at the various stimulus intervals in *E*. *G* and *J*, showing, with different sweep velocities, a potential change produced in a motoneurone membrane by applying square pulses of 2×10^{-9} amp. The onset of the pulses is indicated by arrows. Same square pulses as in *G* and *J* were applied at the summit of the after-hyperpolarization following single (*H* and *K*) and two successive (*I* and *L*) antidromic spikes. Time-scale of 20 msec. applies to *G*, *H* and *I* and that of 4 msec. to *J*, *K* and *L*. Below *I* and *L* are shown the artefact potentials in recording *G–I* and *J–L* respectively, being obtained in isolation in the extracellular position by passing the same current as in *G–L* through the microelectrode. Note that records *A–D*, *E–F* and *G–L* were obtained from three different motoneurones.

A slight increase in the intensity of the intracellular pulse (Fig. 1*C*, lower trace) caused the *IS* spike to be set up in every trace and in about half the *SD* spike was also elicited and is seen in the upper trace to result in an after-hyperpolarization which was superimposed on that following the antidromic spike in *A*. When a second current pulse generated an *SD* spike so that there were three *SD* spikes in succession (Fig. 1*D*), there was a further addition to the after-hyperpolarization. It was usually impossible to generate *SD* spikes by antidromic invasion of the motoneurone at brief intervals because the excitability of the *SD* membrane was depressed by the hyperpolarization following the first invasion[1]. However, on occasions where the safety factor in the antidromic conduction was high enough, it was possible to observe the same summation phenomenon of the after-hyperpolarization as described here, merely by using the antidromic stimuli.

In Fig. 1*E* after-hyperpolarizations following two successive antidromic volleys at various intervals are superimposed, and the amount of hyperpolarization added by the second volley is reproduced in Fig. 1*F*. There is seen to be a progressive decrease as the volley interval is progressively shortened. This depression might occur because the equilibrium potential for the after-hyperpolarization is only 20 mV below the resting potential-level. Fig. 1*G*, *J* shows a potential change in the membrane caused by passing a small hyperpolarizing pulse current. In *H* and *K* this pulse current was superimposed on the summit of the after-hyperpolarization that followed an antidromic *SD* spike. It can be seen that the magnitude of the potential change produced by the pulse current in *K* is about 80 per cent of that in *J*, being measured at the end of the pulse current.

In *I* and *L*, when another antidromic invasion occurred at 3 msec. before that in *H* and *K*, the peak size of the after-hyperpolarization was increased by 49 per cent of that in *H* and *K* and there was a marked reduction in the effect of the test hyperpolarizing pulse, the magnitude of the potential change thereby produced in *I* and *L* being 40 per cent of that in *G* and *J* By applying the test pulse at various phases during the single or summated after-hyperpolarization, it was shown that the depression of the test hyperpolarization or depolarization approximately paralleled the amplitude of the after-hyperpolarization

The prolonged after-hyperpolarizations following impulses in mammalian *C* fibres[7] and frog myelinated nerve[8] show also a temporal summation The ionic process of these after-hyperpolarizations has been related to the active transport of sodium and potassium ions that would hyperpolarize the membrane by depletion of the extracellular potassium[7,8] or by driving an electrogenic sodium current[9] In this respect, the after-hyperpolarization in motoneurones, which is generated by the increased potassium conductance, appears to be a unique phenomenon The special significance of the motoneurone after-hyperpolarization has already been discussed[2,10]

M Ito
T Oshima

Department of Physiology,
John Curtin School of Medical Research,
Australian National University, Canberra

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Mechanism of Calcification : Inhibitory Role of Pyrophosphate

The mechanism of calcification has been considerably clarified recently[1] Collagen fibrils were shown to possess *in vitro* the property of nucleating hydroxyapatite precipitation and of triggering mineralization[1–3] 'But why does only part of the collagen present in the organism calcify ? Recently, we suggested that the activating sites of collagen and crystal growth could be blocked in the organism by a plasma inhibitor[4] Indeed we showed plasma to contain one or several substances highly inhibitory to apatite precipitation The technique used was to determine *in vitro*, at physiological conditions, the minimum ion product (Ca) × (P) necessary for hydroxyapatite precipitation After addition of plasma, the rise of this minimum product revealed the presence of inhibitors As the inhibitor was shown to be destroyed by alkaline phosphatase, and the polyphosphates to have a strong inhibitory activity at concentrations as low as 10^{-7} M, we suggested this inhibitor to be a polyphosphate However, to our knowledge, no such compound has so far been demonstrated in plasma or in urine

As the concentration of plasma of this hypothetical polyphosphate was bound to be very low (10^{-5} M), we first searched for it in urine, where it was implied to exist, according to our test, in a much higher concentration After isolation, its nature was determined as inorganic pyrophosphate[5,6] The mean 'daily excretion of pyrophosphate in healthy males was found to be 2 16 mg expressed as phosphorus (ε = ±0 20), in adult young women it is below 1 mg, but it increases with age By its highly inhibitory action, the urine pyrophosphate accounts for the supersaturation of urine in calcium and phosphorus Our finding that its mean daily excretion falls to 1 06 mg (ε = ±0 17) in urolithiasic men, suggests that its action is present both *in vivo* and *in vitro*

The presence of pyrophosphate in urine suggests that the plasmatic inhibitor is of a similar composition. Indeed, in experiments recently completed, we could isolate from plasma a phosphorus compound highly inhibitory of hydroxyapatite precipitation which, after purification, migrates on paper chromatography in the same way as inorganic pyrophosphate, with three different solvents (Solvent I isopropanol, 70 , water, 30 , 20 per cent ammonium hydroxide, 0 3 , trichloroacetic acid, 4 g Solvent II methanol, 70 ; 2 N ammonium hydroxide, 30 Solvent III *n*-propanol, 30 , ethanol, 30 , water, 39 , 25 per cent ammonium hydroxide, 1) Its concentration, although very low (10^{-5} M), easily inhibits apatite precipitation Its action at this level cannot be explained by a lower ionic calcium concentration due to the formation of a complex, but it is probably a 'poisoning' of crystal growth

The following mechanism of calcification is therefore suggested Hydroxyapatite precipitation can occur at a physiological concentration of calcium and phosphorus. due to the nucleating function of collagen This property, together with crystal growth, is inhibited by plasma pyrophosphate, which protects the collagen that is not to be mineralized For collagen to calcify, pyrophosphate must be destroyed *in loco* by the enzyme pyrophosphatase, which was shown to be present in mineralizing tissues[7–9]

We thank Prof. H. Pfaendler, interpreter, World Health Organization, for translating this manuscript

This work was supported by the grant *A*-4909 of the National Institute of Arthritis and Metabolic Diseases. U S Public Health Service

Herbert Fleisch
Sylvia Bisaz

Institut de Physiologie,
Université de Lausanne,
Switzerland

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HÆMATOLOGY

Accelerated Lysis of Blood Clots

In a recent article[1], we described a class of compounds which specifically inhibited cross-linking of the sub-units of the clot network The possibility arose that clot lysis by the proteolytic enzyme system of blood (plasmin) might be greatly enhanced if clotting were allowed to take place in the presence of the foregoing inhibitors, of which glycineamide is a simple example (Of eight glycyl dipeptides so far examined, only those of L-isoleucine, L-valine and glycine showed appreciable effect, emphasizing the specific nature of the inhibition of cross-linking of the clot (unpublished data by L Lorand and K Konishi)) In order to test the effect of this substance on clot lysis, the following experiment was performed 0 8 ml aliquots of freshly drawn (cubital vein) human blood was mixed in glass tubes (*a*) with 0 1 ml of 0 2 M neutral glycineamide and 0 1 ml of purified urokinase (a gift from Abbott Laboratories, North Chicago, Illinois) containing 90 units of the plasminogen activator in 0 15 M neutral sodium chloride, (*b*) with 0 1 ml each of 0 2 M glycineamide and 0 15 M sodium chloride, (*c*) with 0 1 ml each of neutral 0 2 and 0 15 M sodium chloride solutions, and finally (*d*) with 0 1 ml of 0 2 M sodium chloride and 0 1 ml of the urokinase solution containing 90 units of activity

In all samples (each carried out in triplicate), clotting occurred between 6 and 7 min after blood was drawn They were then incubated at 37° Lysis was judged by visual disappearance of the clots, which were inspected at 15-min intervals during the first hour and hourly thereafter for 6 h of incubation Further observations were made at 18 and 24 h

Remarkably, all samples of set (*a*), containing both urokinase and glycineamide, showed complete lysis after only 1 h incubation In contrast, set (*d*), which contained urokinase but no glycineamide, retained undigested clot residues up to 18 h Two tubes of set (*b*), comprising glycineamide but no urokinase, lysed by 24 h, a third tube of this set contained a small clot residue at this time All samples belonging to set (*c*) showed firm, retracted clots at 24 h incubation

Comparable results were obtained with rabbit and rat bloods These preliminary experiments clearly suggest that glycineamide is an adjuvant of clot lysis Pharmacological potentialities of the cross-linking inhibitors[1], in general, are being further explored for possible use in thrombolytic therapy

This work was supported by grant *H*-2212 of the National Heart Institute, U S National Institutes of Health

L LORAND
A JACOBSEN

Department of Chemistry,
Northwestern University,
Evanston,
Illinois

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A Simple Comparative Method of measuring Red Cell Viability

A VARIETY of methods, including use of immunological or isotopic markers or the excretion-rate of products of hæmoglobin catabolism, have been used to estimate *in vivo* viability of red blood cells[1] In measuring the viability of cells subjected to storage or other *in vitro* treatment, it is necessary to calculate survival using an independent measurement of dilution since the experimental cells may in part be rapidly lost[2,3] The dilution volume, or red cell mass, of the recipient has been estimated indirectly from plasma volume and hæmatocrit determinations[3] or directly by use of simultaneous or sequential injections of control red cells marked with a labelling system different from that used for the experimental cells[3,4]

In principle it appeared to us possible to estimate survival of experimentally treated red cells most easily and reliably by direct comparison of test cell survival to that of control cells by use of consecutive injections of the two cell populations each labelled with the same isotope Effects due to differences in isotope elution, biological disposition, or methods of analysis would thus be avoided Chromium-51 has been utilized as the isotope inasmuch as several investigators[5] have demonstrated that red cell volume could be determined repeatedly and reliably in the same recipient using sequential injections of red cells labelled with chromium-51

New Zealand rabbits weighing about 3 kg were used in groups of 4–6 to evaluate the proposed method Autologous control cells, obtained by heart puncture, and homologous test cells were labelled as whole blood with chromium-51 ('sodium rachromate,' Abbott Laboratories), using respectively 10 μc and 100 μc per 15 ml of blood-ACD anticoagulant (3 1) Labelling was allowed to proceed for 30 min at room temperature and was stopped by addition of ascorbic acid (0 4 ml 10 per cent solution) Uptake of chromium-51 was 90–95 per cent of theory Labelled red cells were injected as whole blood without washing

An injection of 5 0 ml of autologous blood labelled with chromium-51 was made into each test animal, followed by duplicate sampling of the peripheral blood between 30 and 60 min for specific red cell radioactivity A second injection of 5 0 ml of homologous test blood, untreated or subjected to storage at 4° C or in the frozen state and labelled with chromium-51 at a 10-fold higher level, was made into the same animal, followed again by duplicate sampling of the peripheral blood for specific red cell radioactivity between 30 and 60 min and at later intervals of time (for example, 24 h)

Total amounts of chromium-51 infused as labelled red cells in each injection were determined on 1 0-ml aliquots of dilutions of the total red cell content of a volume of labelled blood equal to that injected Circulating specific red cell activities were determined for each peripheral blood sample on 1 0 ml volumes of packed red cells measured in syringes graduated to 0 01 ml All blood samples were centrifuged under constant conditions of time and speed Radioassays were carried out with a Nuclear Chicago Corporation model *DS* 5-5 scintillation well counter and model 186 decade scaler

The percentage survival of test red cells was calculated by use of the formula

$$\% \text{ Survival} = \frac{\bar{T}_r - \bar{S}_r}{T} \left(\frac{S}{\bar{S}_r} - V_x \right) 100$$

T and S refer, respectively, to the total net counts per minute infused as labelled red cells in the test and control injections $\bar{T}_r$ and $\bar{S}_r$ refer, respectively, to the average net counts per minute observed in 1 0 ml volumes of red cells following the test and control injections V_x is the difference in volumes (in ml) of test cells injected and of those removed in sampling after the control injection Survivals at 30–60 min and at 24 h are calculated in an equivalent manner using $\bar{T}_r$ determined at the respective time-intervals The higher level of chromium-51 in the test injection reduces to about 0 5–1 per cent the error which would be introduced into the calculation of 24-h viability by a 5–10 per cent loss of control cells in this period of time

Precision of pipetting and counting packed red cells has been ± 3 per cent or better , precision of measuring the injected dose of chromium-51 cells has been ± 1 per cent Mixing of the infused red cells into the circulation occurs rapidly Following injection of normal cells, the specific red cell radioactivity is stable within ± 2 per cent over a period of 6–69 min or more

Table 1 summarizes results obtained in assaying the early and 24-h post-infusion survival of normal untreated rabbit red cells and rabbit red cells subjected to 21-day storage at 4° C or to freezing and thawing in glycerol medium

Table 1 *In vivo* VIABILITY OF CONTROL AND PRESERVED RABBIT RED CELLS

Treatment	No of groups	No animals per group	% Red cell survival (average ± *S D*) 30–60 min	24 h
Control*	11	4–6	99±5	94±5
4° C storage 21 days (with ACD)	2	4–5	77±5	69±1
Frozen in glycerol†	2	6	86±8	80±1

* Untreated, freshly collected homologous cells in ACD (acid-citrate-dextrose)

† Frozen, thawed, and processed according to Jones *et al* (ref 6)

Variation within groups was about ± 10 per cent This has appeared to be largely accounted for in terms of analytical errors Biological sources of variation did not seem important Control cells, of course, must be subjected to a minimum of trauma in order to avoid erroneously high comparative survival figures of the test cells

The advantages of the proposed method lie in the simplicity of sample preparation and radioassay, in the use of chemically and radioactively identical markers for control and test cells, and in the minimum amount of handling required keeping the control cells as nearly normal as is experimentally attainable While our experience has been with rabbit red cells generally, the proposed method is readily adaptable to use with human beings In work to be reported elsewhere, we have in human recipients obtained precision of ± 5 per cent or better when using a ratio between control and test-levels of injected isotope of 2–3 An overall dose of chromium-51 of less than 100 μc or about 1/8 the recommended body burden was used Other cellular components of blood can be labelled with chromium-51, and a similar comparative isotope dilution technique should be applicable to the estimation of their relative viability

This work was supported in part by contract *NONR* 3003(00)(*NR* 105,208) between the Office of Naval Research and the Linde Company

G F DOEBBLER
R F DWYER
A P RINFRET

Research Laboratory,
Linde Co ,
Division of Union Carbide Corporation,
Tonawanda, New York

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RADIOBIOLOGY

Binding of Strontium in Blood

DURING an investigation of the relative availability to man of strontium in bread and cow's milk[1], three normal subjects ingested approximately 500 mμc of strontium-85 daily for 4 weeks Bulked samples of plasma from blood taken thrice weekly showed a lower specific activity (^{85}Sr/Sr) than urine for the same period These results are shown in Table 1 as mean values for the first three subjects Two further normal subjects followed a similar regimen, but less-frequent and larger samples of blood were taken This allowed the specific activity to be determined on each blood sample for comparison with the specific activity of urine over a contemporary 2-h period It will be seen that these results were substantially the same as those in the earlier case , and a similar finding was obtained in another subject given a single oral dose of strontium-87*m*

Table 1

Subject	Date	Specific activity of strontium Urine mμc/μg Sr	 Plasma mμc/μg Sr	Ratio urine/ plasma
T E F C		0 30	0 14	2 1
G E H	mean value	0 23	0 072	3 2
J F L		0 28	0 071	3 9
G R H	12/2/62	0 30	0 069	4 3
"	19/2/62	0 35	0 072	4 9
"	26/2/62	0 20	0 126	1 6
"	1/3/62	0 13	0 077	1 7
F S W	12/2/62	0 34	0 10	3 4
"	19/2/62	0 28	0 097	2 9
"	26/2/62	0 34	0 098	3 5
"	1/3/62	0 21	0 061	3 4
A J P *	3/5/62	0 27	0 11	2 4
"	30/5/62	0 23	0 090	2 6

*Single oral dose

The ratio of the specific activity of strontium urine/plasma was always appreciably greater than unity This implies either that the kidney is able to differentiate between different isotopic forms of strontium, which is most unlikely, or that strontium exists in two different chemical forms in blood and that the kidney is able to differentiate between these Further experiments to investigate this phenomenon are in progress

T E F CARR
G E HARRISON
J F LOUTIT
ALICE SUTTON

Medical Research Council,
Radiobiological Research Unit,
Harwell, Berkshire

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X-Irradiation of *Escherichia coli*

Escherichia coli is the bacterium probably most commonly used in radiobiological work It is generally agreed that the growth conditions before, as well as after, irradiation affect the number of cells which survive a given X-ray dose Nevertheless, contradictory results have been reported from experiments in which almost identical techniques have been utilized Thus Stapleton *et al* [1] report that irradiated cells recover to a certain extent from lethal effects of X-rays if, prior to incubation at 37°, they are held in presence of available nutrient at temperatures sub-normal for optimal growth, say, 24 hr at

19° Non-irradiated control cells showed no variation in viability with temperature

Opposite results were obtained by Alper[2] In her experiments, holding irradiated cells on nutrient agar for 6 hr at 19° led to an increase in sensitivity, that is, fewer cells survived than in cultures which had either been incubated at 37° immediately after irradiation, or had been kept for 2 hr at 45° and thereafter at 37° Moreover, only 60 per cent of non-irradiated control cells were stated to survive 6 hr at 19° These results are difficult to reconcile with previous reports[3] in which the general principle was evolved that irradiation damage in bacteria is the greater the faster the post-irradiation growth-rate This relation between irradiation effect and growth-rate, however, has not been found to hold good generally[4] and further instances have been described[5] in which bacteria do not conform to this 'principle'.

In view of these contradictory reports it was decided further to examine the part which changes in temperature and growth-rate play in influencing post-irradiation viability in *E coli*

Cells derived from a synthetic medium-agar slant were grown in liquid synthetic medium[6] to reach stationary-state concentration They were irradiated in the same medium in equilibrium with air or with oxygen-free nitrogen

Irradiation was carried out with 200 kVp X-rays, 10 m amp , no added filtration The dose-rate was 8 kr /min Two doses only were given, 4 and 8 kr Suitably diluted samples (0 1 ml) were spread on (Oxoid) nutrient agar and incubated at 37° or 15°, colony counts being made after 1 or 5–6 days, respectively

As in the experiments of Stapleton *et al* [1], temperature did not significantly affect the number of non-irradiated controls There was, furthermore, only a very small difference between the total viable counts of irradiated cells which had been incubated at 37° or 15°, respectively The oxygen enhancement ratio was also very similar at the two temperatures (Fig 1) In appearance, however, the colonies differed greatly, 90 per cent of the controls grown at 15° being petite colonies, the remainder being of normal size, as were all colonies grown at 37° Microscopic examination revealed the petite colonies to be composed entirely of filamentous forms while the large colonies consisted of single cells The ratio petite/large colonies in the surviving fraction of the irradiated cells was different from that in the controls On plotting the survivors of the two types of cells against X-ray dose the filamentous forms were revealed to be more radiation sensitive than single cells and to have a smaller oxygen enhancement ratio (Fig 2) Thus, in order to bring about the same reduction of the viable count in both single and filamentous cells the latter require only about half the dose on irradiation in air and a third of the dose on irradiation in nitrogen

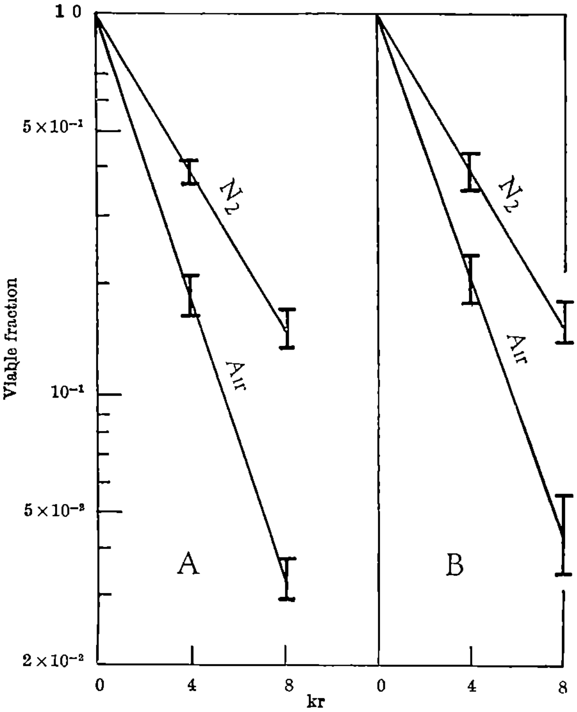


Fig 1 Survival of *E coli B*, post-irradiation incubation at different temperatures, *A*, 37°, *B*, 15° The oxygen enhancement ratio in both cases is approximately 1 6

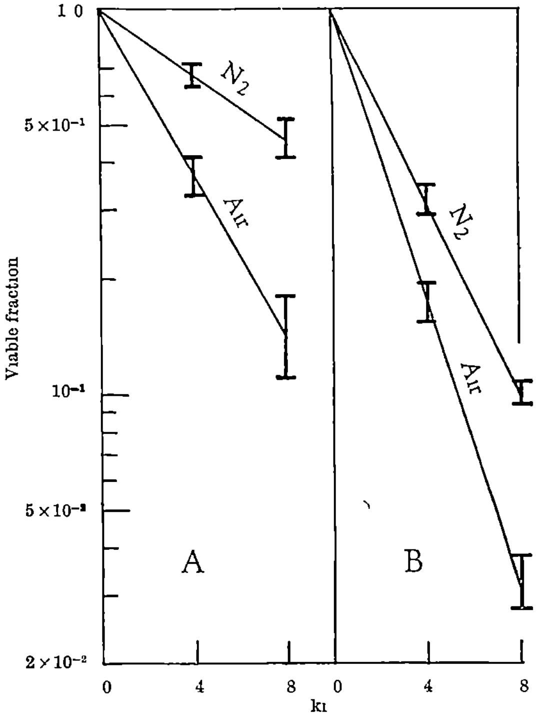
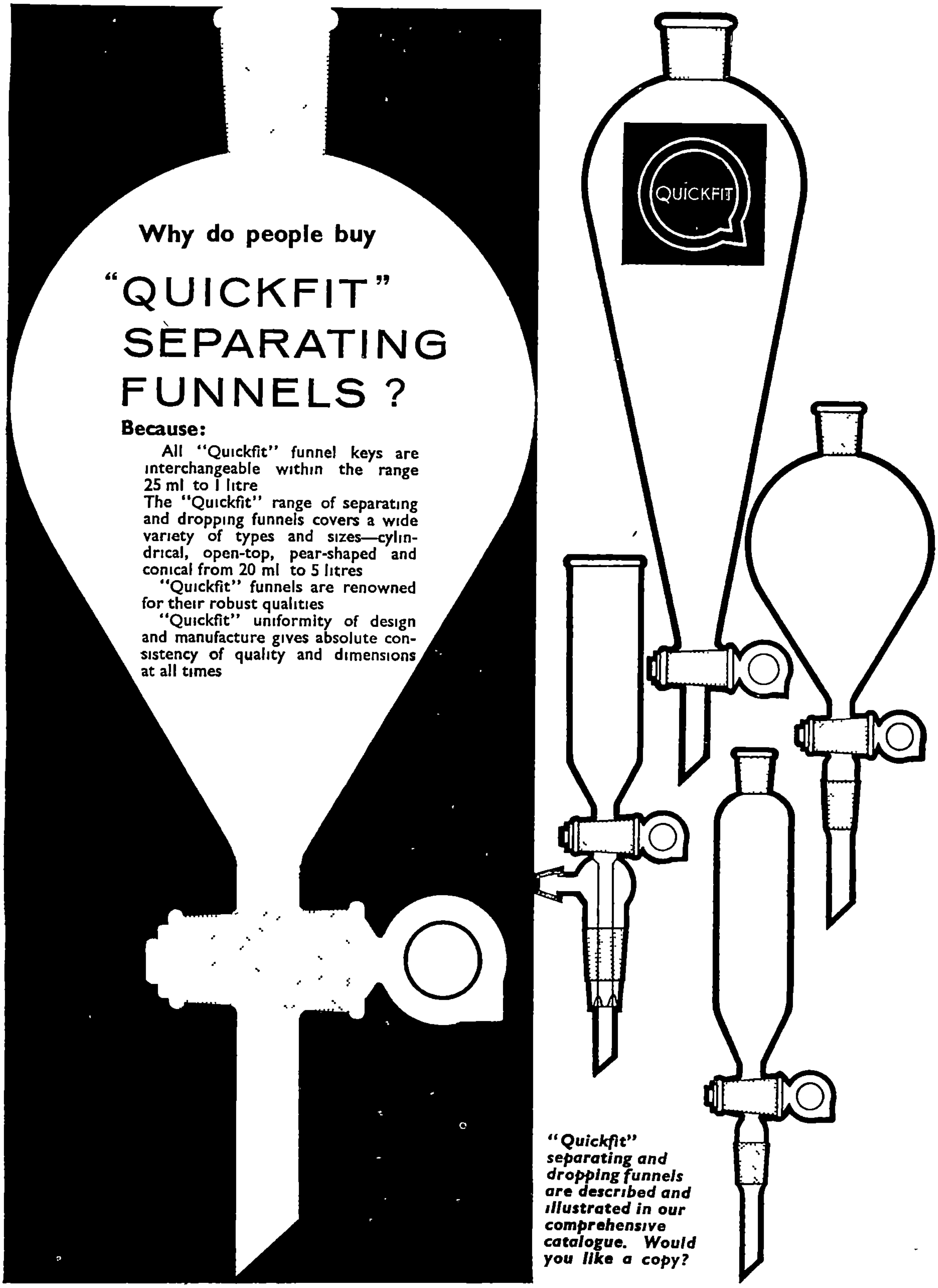


Fig 2 Survival of *E coli B*, post-irradiation incubation at 15° *A* Large colonies, single cells, *B*, petite colonies, filamentous forms Oxygen enhancement ratio in *A* = 2 5, in *B* = 1 5

It follows that the culture of *E coli* used was not uniform but contained two types of cells indistinguishable under conditions of optimal growth, except possibly in their metabolism[7,8], but potentially different in their reaction to unfavourable conditions, as low temperature and irradiation.

Cultures were, therefore, grown from single colonies of both types in order to see whether the characteristics of the cells were maintained on growth at different temperatures

The procedure was briefly as follows growth on solid synthetic medium at 24°, followed by growth in liquid synthetic medium at 30°, when all cells are single forms On plating on nutrient agar and incubation at 15° a clear division between large (single cells) and small (filamentous forms) colonies resulted, about 95 per cent of the colonies reverting to the type from which they derived Fresh cultures, grown



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again from single colonies through two passages at 30° and 37°, were next irradiated and viable counts obtained after incubation at 15°. A clear separation according to cell type resulted once more and viable counts and oxygen enhancement ratios closely resembled those shown in Fig. 2 for single cells and filamentous forms.

The fact that *E. coli* can form filaments in a certain percentage of cells growing under unfavourable conditions complicates interpretation of irradiation effects. Thus cultures which contain an unknown proportion of potentially filamentous, highly radiation-sensitive forms will show correspondingly varying effects of irradiation, especially when this is followed by incubation at a suboptimal temperature during part of the post-irradiation growth period. The conclusion that certain metabolic reactions occurring in the cold cause either attenuation[1] or accentuation of radiation damage in *E. coli*[2] may need reconsideration in view of the experiments reported here. These show that types of *E. coli* can be isolated which, when grown in the cold, are distinct in their form, that is, single or filamentous, and that these differ in their inherent radiation sensitivity.

H. LASER *

Molteno Institute,
University of Cambridge.

* Member of the Scientific Staff of the Medical Research Council.

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Deoxyribonucleic Acid Synthesis in Oocytes of Mouse Embryos

IN order to investigate the synthesis of DNA in mammalian oocytes an attempt was made to label oocytes of mice with a radioactive DNA precursor. If DNA is synthesized during the last premeiotic interphase, as it has been reported for the mouse spermatocyte[1], it should be possible to label oocytes by injecting a radioactive precursor during the last third of pregnancy. Meiotic prophase in the mouse oocyte begins about the 13th day of embryonic life[2–4]. Tritium-labelled thymidine was therefore injected into mouse embryos on different days during the latter part of embryonic life to see : (1) whether oocytes can be labelled in this way ; (2) what percentage of the oocyte population is labelled after injection on different days of embryonic life ; (3) whether labelled oocytes in progressive stages of meiosis can be obtained if autoradiographs are prepared at different intervals after injection of the DNA label.

Street mice 13–18 days pregnant were used in this work. On the day of injection of thymidine the pregnant mouse was anæsthetized, her abdomen opened and the uterine horns exposed. 10 μc of tritium-labelled thymidine (Radiochemical Centre, Amersham, England) was injected into the amniotic sac of each embryo. The abdomen of the mother mouse was then closed and the animal returned to her cage, where she usually recovered within a short time. The embryos were killed at different intervals after injection, fixed in Bouin's solution and serially sectioned at 5μ. Autoradiographs of Feulgen-stained sections were prepared with K_2 liquid emulsion (Ilford) and exposed between 7 and 28 days.

Examination of the autoradiographs shows that the nuclei of oocytes can be labelled by this method (Fig. 1). However, the number of cells which incorporate the label varies greatly and depends on the age of the embryo at the time of tritiated thymidine injection. The distribution of labelled oocytes in animals killed 24 h after injection of tritiated thymidine is shown in Fig. 2. The largest number is found when the precursor is injected on the 13th, 14th or 15th day of pregnancy : 27, 35 and 28 per cent of the cells take up the label on these days. Only a small fraction of the oocytes can be labelled if tritiated thymidine is injected after the 15th day of pregnancy. Injection on the 16th day results in 2 per cent of labelled oocytes whereas on the 17th and 18th day only 0·5 per cent of them incorporate the label. The fact that on each of the three days between the 13th and 15th day about one-third of the oocytes incorporate the label, as well as the sudden drop in the number between the 15th and 16th day, suggests that synthesis of DNA in the oocyte is a fairly synchronized process which in most cells is completed by the 16th day of embryonic life. The small number of oocytes incorporating the label after the 15th day could indicate either that synthesis of DNA in these cells extends over a much longer period of time than in the other cells, for this there is no other evidence, or it may suggest that DNA synthesis in these few cells started later in embryonic life. This assumption is

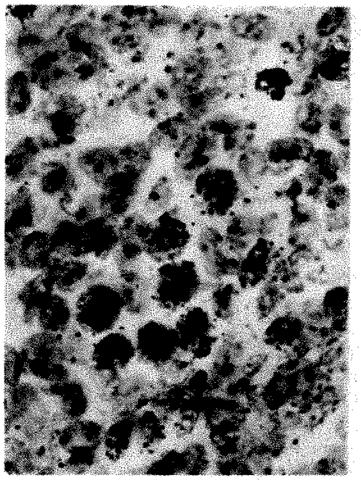

Fig. 1. Labelled oocytes in leptotene. Autoradiograph prepared 24 h after injecting tritiated thymidine into the amniotic sac of a 15-day-old mouse embryo (× 700)

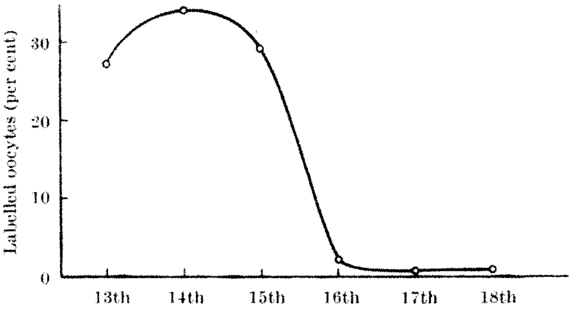

Fig. 2. Distribution of labelled oocytes 24 h after tritiated thymidine injection on different days of embryonic life

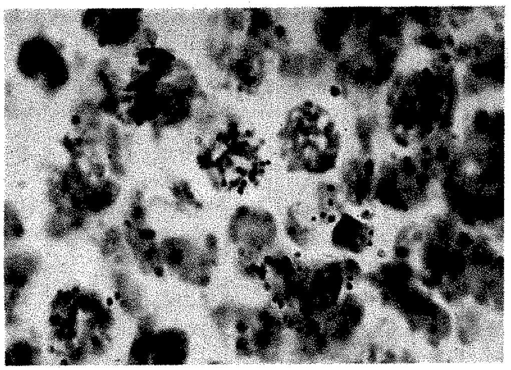

Fig. 3. Oocytes in pachytene, the one in the centre is labelled. Autoradiograph prepared 4 days after injection of tritiated thymidine on the 14th day of pregnancy (× 1000).

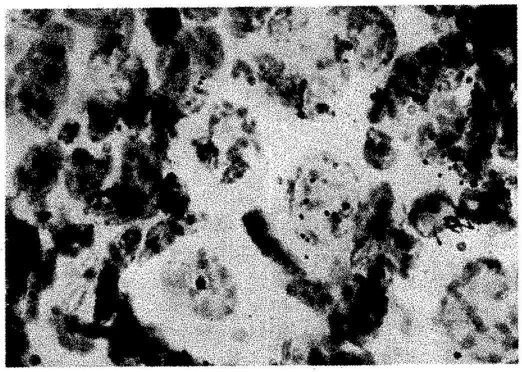

Fig. 4. A labelled oocyte in early diplotene next to an unlabelled one. Tritiated thymidine injected on the 13th day of embryonic life, autoradiograph prepared 8 days later, that is, 2 days after birth of the mouse (× 1000)

supported by the fact that some oocytes in leptotene can usually be found in histological sections of ovaries after the 15th day, indicating that they entered meiotic prophase recently.

The mouse oocyte in the embryo progressively develops and passes through the stages of leptotene, zygotene and pachytene. Just after birth the ovary contains oocytes in pachytene and early diplotene in about equal numbers[5]. Having determined the time of DNA synthesis to be between the 13th and 15th day of embryonic life, one should then be able to find oocytes in the progressive stages if autoradiographs are prepared on animals injected with the labelled precursor on one of these three days and killed later in embryonic life or after birth. This in fact has been done. All progressive stages of labelled oocytes have been observed. If autoradiographs are prepared within 24 h after the injection, the oocytes are in leptotene or early zygotene. If 4 days intervene between the injection and death of the animal, the oocytes have developed to the stage of pachytene, and many in this stage are labelled (Fig. 3). If autoradiographs of ovaries are prepared only after the birth of the animal, many of the labelled oocytes are in early diplotene (Fig. 4), indicating that synthesis of DNA in these cells occurred at the time of injection (13–15th day), and that they kept the label through the progressive development.

It has thus been possible to show that synthesis of DNA in the mouse oocyte occurs mainly on the 13th, 14th and 15th day of embryonic life and that the label incorporated into the oocyte nucleus during DNA synthesis can be followed through the progressive stages of the meiotic prophase until after birth.

Hannah Peters
Emilia Levy*
Monna Crone

Finsen Laboratory,
Copenhagen.

* Fellow of the International Atomic Energy Agency.

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BIOLOGY

Terminology of Some Biologically Active Substances and Validity of the Term 'Pheromones'

In 1957 I proposed the term 'telergones' (from the Greek *tele*—afar and *ergon*—action) for the designation of all biologically active substances secreted by animals into their environment and influencing other organisms[1]. These substances are products of external secretion and differ principally by their importance for the animals from hormones, parahormones and other substances which display their physiological action within the organism producing them. The site of production of telergones are special unicellular or multicellular glands, the ducts of which open singly or in groups on to different parts of the body surface or into various cavities opening to the outside. In many instances there are accessory adjustments for pushing out, sprinkling and exhalation of telergones or for introducing them into the body of other animals.

At present, the chemical nature of only few telergones is known. They can belong to various groups of organic substances. Since in various systematic groups of animals the substances with similar biological action can differ greatly in their chemical properties the classification of telergones is now based not on their chemical properties but on their biological importance to the animals producing them.

Telergones are subdivided into 'homotelergones' acting on other individuals of the same species and 'heterotelergones' acting on animals belonging to other species[1,2].

Karlson and Lüscher[3] have proposed the term 'pheromones' for the designation of those substances which I called 'homotelergones'. They derived this new term from the Greek *pherein*—to transfer and *hormān*—to excite. But Micklem[4] justly pointed out that in the word 'pheromone' there is no structural clue to its derivation from *hormān* and corrected it into 'pherormone'. Then Karlson and Lüscher, in a note to Micklem's article, gave a new etymological explanation for their term. They stated that the ending 'mone' was regarded as a proper suffix used in such scientific terms as hormones, gamones and termones[4].

However, the use of the term 'homotelergones' is to be preferred since it was proposed earlier and is more correct etymologically than the term 'pheromones'. Moreover it indicates exactly the relation of these substances to the telergones and their action on other individuals of the same species.

The substances belonging to the homotelergones are subdivided into the following groups:

(1) 'Gonophyones' (from the Greek *gonos*—sex and *phyein*—to create) acting on the development of the

sex glands and the formation or modification of the sex characteristics (for example, the substances produced by the females of the echiurid worm *Bonellia viridis*, by marine snails of the genus *Crepidula* and the royal jelly of bees).

(2) 'Gamophyones' (from the Greek *gamos*—marriage and *phyein*—to create) stimulate the maturation of the sex glands and the onset of the reproductive processes in the individuals of the other sex (such substances have been established in polychætes *Nereis* and *Grubea* and in some fishes).

(3) 'Epagones' (from the Greek *epagein*—to attract) are attractants of other individuals of the same species, most frequently of the opposite sex (for example, the sexual odoriferous substances of mammals, insects and other animals).

(4) 'Odmichniones' (from the Greek *odme*—odour and *ichnion*—trace) are deposited on the ground and other surrounding objects and serve for marking the locality and facilitating the orientation, such substances are produced by ants, termites and some mammals).

(5) 'Thorybones' (from the Greek *thorybein*—to provoke alarm) excite alarm reactions in other individuals of the same flock or shoal (such substances have been detected in fishes by von Frisch[5] and called *Schreckstoffe*; they are produced also in the mandibular glands of some ants).

The heterotelergones are subdivided into the following groups:

(6) 'Amynones' (from the Greek *amynein*—to drive away)—protective or repulsive substances protecting animals against the attacks of their enemies. Some of them have a repulsive odour, others irritate the skin or mucous membranes, and still others possess strongly poisonous properties.

(7) 'Prohaptones' (from the Greek *pro*—before and *haptein*—to snatch or to bind fast) are poisonous substances paralysing or killing the prey.

(8) 'Lichneumones' (from the Greek *lichneuma*—dainties) are produced by some myrmecophiles and termitophiles, greedily licked off by the ants and termites and show excitant or narcotic actions in them.

(9) 'Xenoblaptones' (from the Greek *xenos*—host and *blaptein*—to injure) are produced by parasites and have specific influences on the structure and physiology of their hosts.

The physiological mechanism of action is recognized only in few telergones. Some of them act on specialized chemical receptors (olfactory, gustatory and other) and provoke characteristic reflex actions by their irritation. Evidently in marine and fresh-water animals some telergones from the surrounding water percolate through the intact integument, the surface of the respiratory organs or the alimentary canal into the internal environment of the organism. Dispersed by blood or other circulating fluids through the whole body, they act on organs and tissues sensitive to them. The influence of the telergones on enzyme systems and the participation of hormones in the mechanism of their action has not been studied very much.

J. KIRSCHENBLATT

Department of Physiology,
Tchernovitsy Medical Institute,
U.S.S.R.

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Synthesis of Organic Acids in the Fruit of the Grape

TARTARIC and malic acids are the quantitatively important acids in the fruit of the grape (*Vitis vinifera* L.). These acids are believed to be synthesized in the leaves and translocated to the fruit[1,2]. However, there is little experimental evidence to support this theory. The work reported here was undertaken to determine whether organic acids are also synthesized in the berry.

In one typical experiment, performed on July 28, 1961, carbon dioxide labelled with carbon-14 was presented to the leaf immediately above the cluster on a girdled shoot of a mature Sultanina (syn. Thompson Seedless) vine. The fruit was hard, green, and immature. Eleven hours after the start of the $^{14}CO_2$ treatment, the phloem transport from the vine to the fruit was eliminated by girdling the shoot just above and below the cluster. At the same time, the treated leaf was removed. At intervals thereafter, the alcohol-soluble constituents of samples of the fruit were extracted. The cations were removed from the extract with 'Amberlite *IR*-120 (H^+)'. The organic acids were separated on a 'Dowex 1 × 8' column ($CO_3^{=}$)[3]. Malic and tartaric acids were then separated on a 'Dowex 1 × 8' column (formate)[4]. The radioactivity of the various fractions was measured at infinite thinness under a thin window, gas-flow counter.

The results (Table 1) showed that the amount of radioactivity in the organic acids increased as the time after isolation of the fruit from the carbon-14 source was lengthened. Similar results were obtained when berries were isolated by detaching them from the cluster. 62–73 per cent of the radioactivity found in the organic acid fraction was incorporated into malic acid and about 15 per cent into tartaric acid. The relative specific activity of both acids increased three-fold between the two sampling times shown. Stafford and Loewus[5] exposed detached grape leaves to $^{14}CO_2$ and found that 76–90 per cent of the label in the organic acid fraction was in malic acid and only 0·08 per cent in tartaric acid.

Table 1. RADIOACTIVITY IN THE ORGANIC ACIDS OF GRAPE BERRIES AFTER ISOLATION OF THE FRUIT FROM THE SOURCE OF CARBON-14

	Time (h) after isolation of berries		
	1	13	37
Activity in organic acids			
c.p.m. × 10^{-3}	210	272·7	510·6
% of activity in original extract	11·2	14·5	28
Activity in malic acid			
% of activity in organic acid fraction	62	—	73
specific activity (c.p.m. per μequiv.)	60	—	175
Activity in tartaric acid			
% of activity in organic acid fraction	13	—	15·5
specific activity (c.p.m. per μequiv.)	12	—	40

In another experiment, $^{14}CO_2$ was presented to the cluster on July 30, 1961. Samples of fruit were collected at 1 and 23 h after the start of the treatment with $^{14}CO_2$. The proportion of radioactivity found in the organic acid fraction increased from 30 per cent in the first sample to 43 per cent in the second sample.

The results indicate that the berry is an important site of synthesis of organic acids. It has been shown that acid content of the berry increases until cell division ceases[6]. The high level of incorporation of label into tartaric acid by the immature berry relative to that shown by mature leaves[5], suggests tartaric acid synthesis in the grape may be related to some aspect of growth metabolism. This is not inconsistent with the hypothesis that a sugar is the precursor to tartaric acid[5]. The high level of activity found in the organic acid fraction when $^{14}CO_2$ was presented to the cluster indicates that fixation of carbon dioxide into malic acid may make important contributions to the acid content of the berry.

C. R. HALE*

Department of Viticulture and Enology,
University of California,
Davis.

* Present address : Australian Wine Research Institute, Private Mail Bag, No. 1, G.P.O., Adelaide.

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Floral Initiation of *Xanthium* in Response to Application of an Extract from a Day-Neutral Plant

A PROCEDURE for the preparation of a floral initiating extract from the leaf tissues of *Xanthium strumarium* has been described in an earlier report[1]. In general, the procedure involves the rapid freezing in liquid nitrogen of fresh leaf material obtained from flowering *Xanthium* plants. The frozen tissue is lyophilized at temperatures below 0° C. In recent work the continuous extraction procedure has been modified and extraction of the dry leaf material is now accomplished by several washings with cold (0–10° C.) absolute methanol. The methanol is removed at a low temperature by evaporation at reduced pressure in a Rinco apparatus. The residue is diluted with 1·5–5 times its weight of lanolin and the mixture applied as a coating to the under surface of the leaves of vegetative *Xanthium* test plants. All treated plants and their corresponding controls are maintained until dissection on strict long-day conditions. Three weeks after treatment the plants are dissected and the extent and degree of flowering is recorded according to a standard procedure[2].

In the intervening year since the original work was reported we have repeatedly demonstrated the activity of the extract in causing initiation of flowers in *Xanthium*. Recently we have found the extract capable of causing floral initiation in *Xanthium* under conditions of continuous illumination of approximately 2,000 foot-candles intensity from a fluorescent light source. In a typical experiment 40 per cent of the treated plants flowered with all the untreated control plants remaining vegetative.

Extracts prepared from the indigenous sunflower *Helianthus annuus*, a day-neutral plant, also have been found capable of initiating flowering in *Xanthium* test plants. Leaves from flowering *Helianthus* were picked in the late summer of 1961 and were processed and extracted in accordance with the previously described method for obtaining *Xanthium* extract. In one of several successful experiments, 200 g of lyophilized *Helianthus* leaf material yielded 34 g of extract residue. The residue was blended with 50 g of anhydrous lanolin and applied to the leaves of 40 vegetative *Xanthium* plants. A group of 40 untreated plants was used as controls. All plants were then maintained on long-day conditions of 18 h of light and 6 h darkness at 25° C. for the subsequent three weeks. The plants were then dissected. All the control plants were vegetative. Twenty per cent of the treated plants flowered; of those flowering the average floral stage[2] at the time of dissection was 1·2.

The initiation of floral development in *Xanthium* in response to application of *Helianthus* extract strengthens the belief that there is a common chemical substance or closely related series of chemical substances governing the transition to flowering in many plants. Although the evidence is as yet incomplete, the active entity in the extracts is presumed to be the flowering hormone, florigen.

This investigation was supported by National Science Foundation grants Nos. 10854, 10855 and 14380.

RICHARD G. LINCOLN
DARWIN L. MAYFIELD
ROBERT O. HUTCHINS
ALAN CUNNINGHAM

Departments of Botany and of Chemistry,
Long Beach State College,
Long Beach 4, California.

KARL C. HAMNER
BRUCE H. CARPENTER

Department of Botany,
University of California,
Los Angeles, 24.

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Bud Rot Disease of the Oil Palm

FOR many years, a pathological condition of the oil palm, *Elaeis guineensis*, in which the spear and bud tissue becomes rotten, has been known, and has been reported from many countries where the palm is in cultivation. In general, the disease is occasional, but in some areas the incidence is high, and it has caused the death of a large number of palms in the plantations in South Congo.

Numerous possible agents have been suggested as causal, but in spite of many attempts at diagnosis, no one has succeeded in reproducing the condition.

The disease has now been shown to be due to a bacterium of the genus *Erwinia* and similar to *E. lathyri* (Manns and Taubenhaus) Holland, but only on palms which have become, or have been made, susceptible.

Continuous growth studies made on a group of palms in the geneological block at Branbanta (South Congo), show that before an attack of bud rot takes place, the growth-rate of the palm slows considerably. Natural rotting commences some three weeks after this in the region below the point of visible emergence of the spears through the funnel formed by the older leaf bases. If, however, the palm is inoculated immediately a slowing of growth is apparent, an attack can be started within two or three days. Rotting has also been induced by inoculation on palms which have remained for long periods on, or just above, the apparent threshold of susceptibility without succumbing to natural attack. Palms maintaining a normal growth-rate, that is, vigorous palms, have been inoculated, but invariably without effect.

Healthy palms can be made susceptible, and attacks of bud rot have been produced by both root and leaf cutting. A tourniquet applied low on a young spear renders that spear susceptible, but only above the tourniquet.

Inoculation experiments on detached spears show that the most susceptible tissue is that lying 15–25 cm. below the point of emergence of the spear. This is the area where growth is changing from elongation to maturation. Mature tissue is not susceptible, and the very young tissue near the bud is also comparatively resistant. Thus it appears that the bacterium is only able to attack palms which are unable to carry the spear tissue through this susceptible stage, sufficiently vigorously. Different genetical lines show different susceptibilities, and the lines which exhibit the greatest vegetative vigour are also the lines which are least attacked.

Growing conditions in South Congo are marginal. The soil is a poor sand and there is a prolonged dry season. However, wild palms, in which, presumably, natural selection has been operating, are rarely seen affected, and the occurrence of highly productive palms in devastated areas, together with the differences to be found in the geneological blocks, indicate that, while a bacterium is finally responsible, the root cause of the trouble is genetical, in that the material planted is not adapted to the rigorous growing conditions.

A full paper on this work will be published later.

My thanks are due to the Commonwealth Mycological Institute for the identification of the bacterium, and to Unilever, Ltd., for permission to publish this communication.

A. D. S. DUFF

Plantations Lever au Congo,
Departement de Recherches,
Yaligimba, Via Bumba,
Republic of Congo.

Compensatory Growth of Cattle on the Natural Grasslands of Northern Rhodesia

ON the natural grasslands of Northern Rhodesia cattle gain weight during the rainy season, but during the subsequent dry season they frequently lose up to 15 per cent, and in some cases as much as 25 per cent, of their previous maximum weight. It is well known that the greater the restriction in feed supply the greater the gain in weight on subsequent realimentation[1-4]. The degree of compensatory growth was measured for the indigenous (*Bos indicus*) Sanga and Zebu cattle (oxen). The level of nutrition was varied during the dry season to produce a range of changes in weight from slightly above maintenance to large losses, and the gains in the following rainy season were observed when all the cattle were grazed as one herd on the natural veld. A summary of the results is given in Table 1. Each age-group trial began at the end of a rainy season during which all the cattle had been under the same conditions. About one-fifth of the cattle were used in more than one year. The stocking-rate was such that ample forage was available during the rains, and the nutrition of the cattle was restricted only by the seasonal decline in the nutritive quality of the grasslands.

Table 1. AGE-GROUPS AND SUMMARY OF CATTLE WEIGHTS

	Cattle ages (yr.)	Mean initial weight (lb.)	No. of cattle	Weight gain (lb.) Dry season Mean	Weight gain (lb.) Dry season Max.	Weight gain (lb.) Dry season Min.	Weight gain (lb.) Rains Mean
1958/59	2	390	18	20	59	– 34	178
	3	669	38	– 8	86	– 80	213
1959/60	2	371	19	– 1	61	– 53	231
	3	580	17	– 28	58	– 157	226
	4	872	39	– 119	12	– 211	254
1960/61	2	372	16	15	82	– 80	190
	3	671	25	– 69	20	– 144	247
	4	778	16	– 60	24	– 176	197
	5	927	28	– 105	26	– 262	192

The response of the cattle on re-alimentation to the different levels of nutrition during the dry season was estimated by the linear regressions of the gains in weight during the rains on the gains during the preceding dry season. It has been suggested[5] that there may be a fundamental difference in response according as the degree of restriction permits small gains, maintenance or losses in weight. There was no evidence of a change in the pattern of response with these conditions, as shown by curvature in the response relationship, even with the widest range of weight changes extending to severe losses. Similar work in North America[6] and elsewhere showed no significant and consistent departure from linearity over the ranges studied. The residual variation was also of the same order as that observed by others[7,8] with groups of similar size.

The regression coefficients (Table 2) show by how much the gain during the rains was expected to be reduced for each 1 lb. increase in dry season gain (obtained by extra feeding) or alternatively, the expected amount by which the rains gain was increased in compensation for each 1 lb. lost as a result of a lower plane of nutrition during the dry season. This regression coefficient thus provides a measure of compensatory growth. It is more general than the recovery index defined for two planes of nutrition[5].

Table 2. COEFFICIENTS OF REGRESSION OF WEIGHT GAIN DURING RAINY SEASON ON GAINS DURING THE PRECEDING DRY SEASON

	Age of cattle (yr.) 2	3	4	5	Average
1958/59	– 0·427 ± 0·259	– 0·710 ± 0·189			– 0·568
1959/60	– 0·519 ± 0·130	– 0·682 ± 0·107	– 0·799 ± 0·100		– 0·667
1960/61	– 0·271 ± 0·124	– 0·710 ± 0·194	– 0·883 ± 0·125	– 0·642 ± 0·099	– 0·626
Mean regression (eliminating years)	– 0·369 ± 0·088	– 0·697 ± 0·093	– 0·822 ± 0·080	(– 0·642) (± 0·099)	– 0·632

The compensatory growth was less with the 2-year-old oxen than with the older groups (Table 2), possibly because they were at a more critical stage of development[5]. Under the conditions of these trials, the regressions were consistent from year to year for each age group, except the 2-year-olds.

The seasons were separated by using the dates of minimum and maximum cattle weights as end-points. The rainy seasons during the three years of the trial were reasonably typical, and varied only from 188 days to 218 days. A more detailed study of the mean growth-curves of selected sub-groups of cattle indicated that the effect on the regression coefficients due to variation in the duration of the rainy season is unlikely to exceed 7 per cent. The growth-curves suggest that the compensatory growth effect persists throughout the rainy season.

The cattle were all offered identical herbage during the rains, and the quantity of fæces produced was used as a measure of relative herbage intake. The cattle which had large weight losses in the preceding dry season consumed more herbage than those on a higher (maintenance) plane of nutrition. This result agrees with those obtained by others[9].

A quantitative knowledge of the response relationship provides a basis for an economic assessment of alternative systems of management. The average costs of feeding to produce various weight changes during the dry seasons can be compared with the values of the corresponding live weights at the end of the growing life of the cattle.

C. A. SMITH

Central Research Station,
Mazabuka,
Northern Rhodesia.

G. E. HODNETT

Regional Research Centre,
University College of the West Indies,
Imperial College of Tropical Agriculture,
Trinidad, West Indies.

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In vivo Isolation of *Hymenolepis nana* and Antibody-binding Sites

A RECENT preliminary investigation[1] suggests that antibodies are directed against adult specimens of *Hymenolepis nana* while located in the ileum of the mouse. The present investigation was carried out to substantiate tentative conclusions regarding the specificity of antibody binding sites previously observed. It appeared, from the earlier experiments, that antibodies were readily available to adult tapeworms, *in situ*, in that binding sites were partially or completely occupied in the adult stage as ascertained by immunofluorescent staining. Specimens were not, in view of antibody access and possibly excess, available for negative controls. *In vivo* isolation of the parasites from direct cellular contact and source of antibody for a period of time longer than the half-life of the mouse immune globulin was therefore considered. Under these conditions, previously localized sites should not exhibit fluorescence following application of indirect immunofluorescent staining.

Under similar conditions the direct technique could be used, utilizing an anti-*Hymenolepis* rabbit globulin, for a comparison of binding sites from the point of view of availability of antigens to the host during infection.

In present experiments, adult worms, obtained from mice following a three-week egg-induced infection, were transferred through several rinses of sterile phosphate-buffered saline of *p*H 7·2 and then incubated at 37° C. for 15 min. in 'CulturStat Medium 199' containing Earle's base, penicillin and streptomycin (Cappel Laboratories, West Chester, Penn.). This medium was selected since in previous experiments adult tapeworms were maintained *in vitro* at 37° C. for more than 30 days (unpublished results). Single worms were placed in individual sterile (autoclaved) diffusion chambers which had been previously moistened with the culture medium. The chambers were of the 'Sellotape' design[2] utilizing type *GS* porosity 'Millipore' filters having a diameter of 8 mm. The filters were immediately implanted, intraperitoneally, in mice which had never been infected with hymenolepids, as determined by fæcal and serological examinations. The surgical procedure was carried out under 'Nembutal' anæsthesia and aseptic conditions. Two chambers were placed in a 23 gm. mouse and removed 3–7 days following implantation. Worms were then subjected to direct and indirect immunofluorescent staining. Conjugates for direct staining were prepared with 'Lissamine Rhodamine *B* 200' (ref. 3) and fluorescein iso-thiocyanate conjugates for indirect staining were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Control adsorptions were used as cited in ref. 1. Fluorescent microscopic observation of whole mounts, and frozen sections, was accomplished by utilizing a Reichert-Hacker assembly and microscope with appropriate filters.

Although worms survived in chambers implanted for seven days (a longer period was not attempted), partial encapsulation of several chambers with fatty host tissue was observed which undoubtedly affected diffusion-rates of oxygen, carbon dioxide, liquid nutrients, and metabolic products. Worms from encapsulated chambers, although viable, nevertheless appeared abnormal morphologically.

Fluorescence of previously localized binding sites, in worms obtained from the ileum, was not observed following indirect staining of isolated worms with rabbit anti-mouse globulin. Tapeworms obtained from chambers implanted for only three days did not fluoresce specifically, which is not inconsistent with the catabolic decay period for mouse globulin. Fluorescent sites in isolated worms treated with mouse antiserum and then subjected to indirect staining were not distinguishable from sites observed in worms obtained from the ileum (Fig. 1). This was considered of interest since antibodies which materialize in the intestinal lumen may be somewhat distinct from those appearing in the blood stream, as observed by Barksdale and Ghoda[4] in patients with dysentery caused by *Sh. flexneri* or *Sh. sonnei*.

Antibody binding sites fluoresced strongly in worms obtained from diffusion chambers following direct immunofluorescent staining (Fig. 2). A comparison of these sites with those observed in tapeworms from the ileum did not permit conclusions concerning the number of antigens available during some stage of the infection. Immunoelectrophoretic investigations[5], however, indicate that whereas crude worm extracts possess at least nine antigens (or determinant groups) which engender antibodies in the rabbit, only four elicit a response in the mouse host during infection.

Results indicate that antibodies combine with adult worm antigens *in vivo*. It is not presumed that such antigens are, necessarily, stage specific.

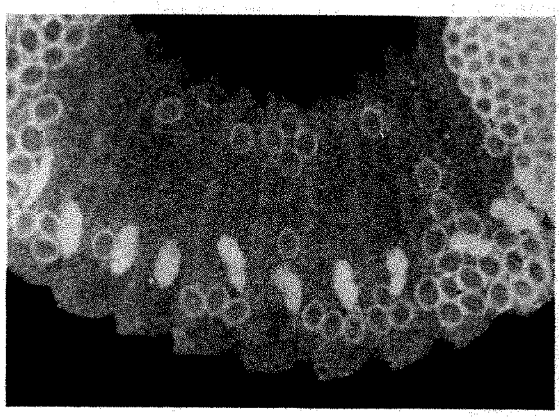

Fig. 1

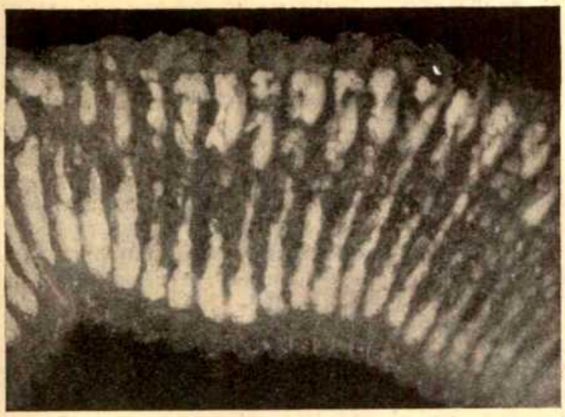

Fig. 2

Utilization of the diffusion chamber technique is suggested as a possible approach to immunological studies requiring *in vivo* isolation of animal parasites.

This investigation was supported by a research grant, *G* 14342, from the National Science Foundation.

R. M. COLEMAN*
N. M. FOTORNY

Department of Biology,
Russell Sage College,
Troy, New York.

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* Present address: Department of Biology, Boston College, Chestnut Hill, Mass.

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Transatlantic Journey of a Tagged Cod

ALTHOUGH cod (*Gadus morhua*) populations in the North Atlantic form more or less discrete stocks, many tagged cod have travelled long distances, for example, between Iceland and Greenland, from Iceland to the Faeroes, and the Faeroes to the North Sea[1], and from Bear Island to Iceland and Greenland[2]. Since 1956 more than 4,000 cod have been tagged in the central North Sea from English vessels, and until this year none had been returned from outside the North Sea. On January 4, 1962, the Lea-type tag[3] No. 1137 which had been attached by a monofilament nylon loop between the first and second dorsal fins to a cod caught on the North-west Rough (central North Sea, 55° 6′ N., 1° 24′ E.) on June 9, 1957, was handed to Mr. G. R. Williamson by a member of the crew of the Polish stern trawl factory ship *Kastor* at St. Johns, Newfoundland, as having been caught on the Grand Banks. At the time no special significance was attached to this fish, as it was presumed to have been tagged in the Newfoundland area. When the exceptional movement was known, further inquiries were made through Mgr. Jozef Wolek, director of the Polish Sea Fisheries Institute, Gdynia, who visited the ship and interviewed the fishermen. They confirmed that the fish was caught on December 7, 1961, on the north-eastern slope of the Grand Banks (47° 54′ N., 47° 54′ W.) in 300–320 m., as witnessed by several fishermen, including the second mate. The fish was still alive, with the tag attached normally, and was measured before the tag was removed and the fish sent for filleting and freezing. It had grown from 57 cm. total length when tagged to 75 cm. when recaptured. In the North Sea a 60-cm. cod might grow 15 cm. a year, but on the north-east slopes of the Grand Banks only about 3 cm. a year.

It is impossible to make more than a guess at the route by which the fish moved. The shortest route around the north of Scotland is a distance of more than 2,000 miles, which would be one of the longest distances travelled by a tagged cod, if not the longest, though it would be by no means impossible in the four and a half years since tagging. It is more probable that the fish travelled via the Faeroes, Iceland and Greenland, or even farther north.

J. A. GULLAND

Fisheries Laboratory,
Lowestoft.

G. R. WILLIAMSON

Fisheries Research Board of Canada,
St. Johns,
Newfoundland.

---

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Cultivation of *Eisenia foetida* (Sav.) Free from Infection with Acephaline Gregarines

A PLENTIFUL supply of earthworms known to be free from any infection by acephaline gregarines is required for the examination of the mode of transmission of these parasites. Vigorous cultures of *Eisenia foetida* (Sav.) free from any gregarine infection were successfully established by means of the following technique.

Cocoons gathered from laboratory cultures of the wild host worm were washed thoroughly for 15 min in a flour sieve with a strong jet of water, and then surface sterilized in Nance's solution (0·1 per cent mercuric chloride in 0·5 per cent 'Teepol') for 1 min. They were then washed again for 5 min and finally placed in non-infective soil. The latter was contained in metal biscuit tins holding some 10 lb. of moist soil and having a layer of activated animal charcoal at the base. Each container received 50 cocoons before being set aside, with the lid replaced, to allow the worms to develop. The main difficulty experienced was the preparation of soil which was non-infective, but which would yet support the normal growth of worms from the cocoons. This was finally achieved by first sterilizing the soil in the biscuit tins and then recolonizing it with as many of its living constituents as possible without re-admitting gregarine sporocysts.

The soil was autoclaved, already in the biscuit tins, for 3 h at 60 lb./in.^{2}. Sterility was judged to be complete when attempts to culture fungi on 'Dox' agar and bacteria on nutrient agar from soil inocula failed. (It appeared that the type of soil used was of some importance, the most successful being a light friable loam, the least, a mixture containing dung.) The sterilized soil was now inoculated with a crumb of normal soil in order to re-introduce living constituents. In order to prevent the re-entry of gregarine sporocysts this inoculum was packed into the middle of a glass cartridge (open at both ends) between two firm plugs of sterilized soil (Fig. 1). Soil organisms capable of growth or movement would gradually recolonize the sterilized soil, while any gregarine sporocysts, being non-motile, would remain inside the cartridge. The latter was removed intact 4–6 weeks after implanting, at which time profuse growth of common soil fungi and bacteria could be obtained by plating suspensions of the remaining soil on appropriate media. Even so, such soil would not support

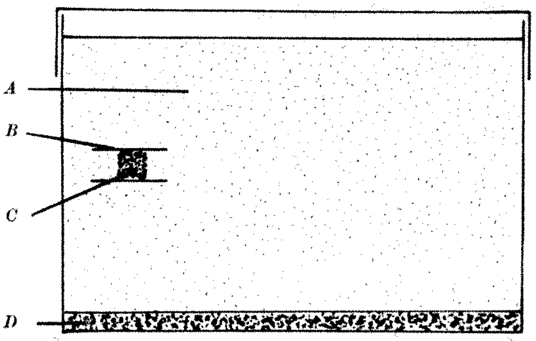

Fig. 1. Method of inoculating sterilized soil with normal soil so as to exclude gregarine sporocysts. *A*, sterilized soil; *B*, glass cartridge containing inoculum of normal soil (*C*) sandwiched between two plugs of sterilized soil, *D*, activated animal charcoal

the growth of worms to full size and sexual maturity until it had been further inoculated with soil protozoa. The method of doing this which was first adopted was to culture soil protozoa in soil extract or hay infusion[1]. A crumb of normal soil was placed at one side of a Petri dish of culture medium which was then incubated for seven days at 24° C. Then an aliquot of the medium was withdrawn from the side of the dish opposite to the point at which the crumb had been added and used to inoculate fresh culture medium.

The subculture after further incubation for 7 days, containing protozoa (mainly ciliates and flagellates) could safely be used to inoculate soil, as any gregarine sporocysts, being non-motile, would have been left behind in the first Petri dish. Later, a less-laborious method of obtaining soil protozoa free from gregarine sporocysts was used in which humus-rich soil was shaken in distilled water, then after standing for 1 h to settle the water was decanted off and filtered through butter muslin. This supernatant was spun at relative centrifugal force 400 for 5 min and then incubated at 24° C overnight. Centrifuging would take down any gregarine sporocysts (which are denser than water) while, as subsequent examination showed, motile micro-organisms recolonize the upper layers of fluid during the incubation overnight. The upper two-thirds of each vessel of soil water so treated was carefully pipetted off and used to inoculate soil which had previously been inoculated with a crumb of normal soil some four weeks earlier, in the manner already described.

The soil now proved an entirely successful culture medium for *E. foetida*. Successive generations have been grown in it continuously over a period of more than 3 years, the only regular further addition being food material in the form of oatmeal. At laboratory temperatures around a mean of 15° C worms develop from cocoon to maturity in 6–7 months. This compares with 3 months growth period reported by Herlant-Meewis[2] for this species when grown in untreated soil at 19° C. No worms cultured under the conditions described here have ever shown infection with acephaline gregarines apart from those infected experimentally.

I thank Prof. H. Stenton, Department of Botany, Hong Kong University, and Prof. J. N. R. Grainger, Department of Zoology, Trinity College, Dublin, for advice, and Prof. Paul G. 'Espinasse, Department of Zoology, University of Hull, for facilities.

H. B. MILES

University of Hull.

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MICROBIOLOGY

Trypsin Inhibitors produced by *Clostridium botulinum* Cultures

TRYPSIN inhibitors have been demonstrated in a large variety of biological material, involving, among others, pancreas, milk, egg-white, blood, placenta, urine, skin, gut parasites, beans, peas, potatoes[1] and bacteria[2]. In this communication the presence of trypsin-inhibiting material in the culture supernatants of *Clostridium botulinum*, types *A*, *B* and *E*, is reported.

The organisms utilized were *Cl. botulinum* type *A* strain Hall (obtained from Dr. C. Lamanna, Johns Hopkins University, Baltimore), *Cl. botulinum* type *B* strain Beans (obtained from Dr. J. Keppie, Ministry of Supply Experimental Station, Porton), and *Cl. botulinum* type *E* strain Fredriksberg (obtained from Dr. H. O. Pedersen, Copenhagen).

For the cultivation of the types *A* and *B* the media used by Bonventre and Kempe[3] were utilized. For type *E* the medium of Gordon *et al.*[4] was used. The incubation temperatures of the types *A* and *B* cultures were 35° C and for type *E* 28° C.

Samples were withdrawn from the cultures with 24-h intervals for 6 consecutive days. An aliquot was used for turbidimetrical determinations of bacterial growth at 600 mμ. The rest of the sample was centrifuged at 5,000 rev/min for 20 min in order to remove the bacteria, and the supernatant was used for the demonstration of trypsin-inhibiting activity.

A modification of the procedure described by Kunitz[5] was used for the demonstration of trypsin-inhibiting activity. A 3 per cent casein solution was adjusted to *p*H 7·6 with dilute sodium hydroxide. 5 ml. of the casein solution were placed in each of four test tubes (1·7 × 18 cm), 2 ml. of 0·1 M phosphate buffer (*p*H 7·6) were added to tubes 1 and 2, while 2 ml. of the culture supernatant were added to tubes 3 and 4. To tubes 1 and 3 1 ml. of 0·025 per cent solution of trypsin (Difco, 1 : 250) in phosphate buffer was added, while 1 ml. of boiled trypsin solution was added to tubes 2 and 4.

The mixtures were incubated at 37° C in a water bath. After 3 h, 3 ml. of each incubation mixture were added to a tube containing 3 ml. of 10 per cent trichloroacetic acid, mixed, and left at room temperature for 30 min. The precipitated material was then centrifuged at 5,000 rev/min for 15 min. The supernatants were diluted ten-fold with 5 per cent trichloroacetic acid and measured at 280 mμ in a Beckman spectrophotometer, model *DU*, using 5 per cent trichloroacetic acid as blank.

Fig. 1 shows the occurrence of trypsin inhibitors in typical experiments.

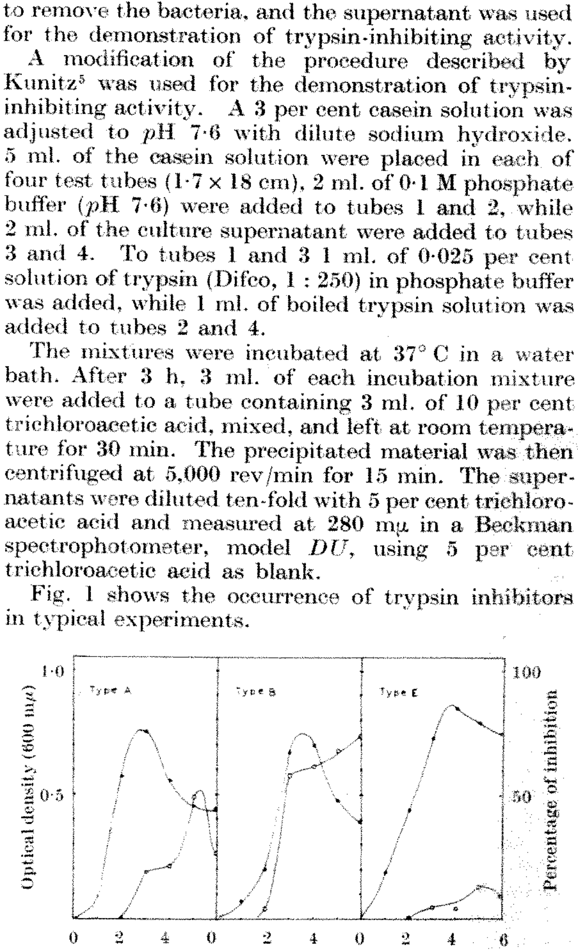

Fig. 1. Trypsin-inhibiting activity (open rings) compared with the bacterial growth (closed rings)

The trypsin-inhibiting activity varied greatly in different cultures of the same bacterial strain Under the conditions described the following ranges of maximum trypsin-inhibiting activities (per cent) were observed *Cl botulinum A* 13–51, *Cl botulinum B* 62–74, *Cl botulinum E* 5–35

Controls using sterile media incubated for the same period showed no trypsin-inhibiting activity

The trypsin inhibitor produced by the type *E* strain has been investigated to some extent The inhibitor was found to be easily dialysable, leaving the dialysis bag completely within 1 h when using a rotating technique The inhibitor also possesses great heat stability It may be kept at 100° C for 1–2 h without detectable loss in activity Later on the activity decreases slowly It has also been autoclaved at 120° C for 15 min without loss in activity

These results lead to the suggestion that the inhibitor is a small molecule, presumably organic in nature because ashing destroys the activity completely

TORE HOYEM
ANTON SKULBERG

Department of Microbiology and Hygiene
Veterinary College of Norway,
Oslo

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VIROLOGY

A Simple Method for Total Particle Counts of Trachoma and Inclusion Blennorrhœa Viruses

DARK-GROUND illumination has been used to examine the elementary bodies of large viruses in stained and unstained preparations[1,2] We find that unstained crude suspensions of chick embryo yolk sacs infected with the viruses of trachoma and inclusion blennorrhœa, examined under dark-ground illumination, contain particulate host material which obscures the virus particles and which can be confused with them However, when such suspensions are dried, fixed and stained with Giemsa, many bright yellow-green particles are seen by dark-ground illumination, resembling elementary bodies stained with acridine orange and viewed by ultra-violet microscopy Their colour and intensity clearly distinguish them from background material, even if they are enveloped by it They resemble virus particles in size and shape, and their distribution in yolk sac smears is the same as that of the purple elementary bodies in films stained by Giemsa viewed by ordinary illumination, they are not present in smears of normal yolk sacs Our conclusion that these are virus particles is also supported by dark ground microscopy of inclusions found in HeLa cells infected with trachoma or inclusion blennorrhœa viruses[3] The inclusions contain numerous yellow-green particles similar to those in yolk sac smears and suspensions Large forms of virus are also recognizable, but are much less bright, and reddish in colour

We estimated the number of elementary bodies in suspensions of yolk sacs infected with these viruses Suspensions were made by shaking membranes for 1 min at room temperature with 0 5 per cent (w/v) trypsin in phosphate-buffered saline[4], removing the membrane and incubating the remaining suspension at 37° C for 30 min After removal of gross debris by low-speed centrifugation virus was deposited at 8,000*g* and resuspended in buffer to make a suspension containing the equivalent of 1 g yolk sac per 10 ml buffer A small amount of suspension was taken up in a 0 1 ml glass capillary tube ('Microcaps', Drummond Scientific Co), the length of the liquid column measured, a drop expelled on to a clean glass slide, and the length of the remaining column measured Since the capillary tube contains 0 1 ml and the bore is uniform, the volume of the drop placed on the slide could be calculated The drop was spread with a fine glass rod to give a thin circular film which was dried in air, fixed in absolute methyl alcohol for 10 min and stained for 30 min in Giemsa (Hopkins and Williams Ltd) diluted 1/10 in phosphate buffer *p*H 6 8 The film was then washed in buffer and dried at room temperature Elementary bodies were more easily seen when preparations were soaked in a mixture of 5 ml glacial acetic acid and 95 ml of chloroform after fixation and before staining To facilitate counting under dark ground illumination, an eye-piece stop was used, only those particles within the standard central area were counted, and at least 600 were counted in each drop The number of particles per ml of original suspension is

$$\frac{\text{No of particles counted} \times \text{area of drop}}{\begin{matrix}\text{No of fields}\\ \text{counted}\end{matrix} \times \begin{matrix}\text{volume of}\\ \text{drop}\end{matrix} \times \begin{matrix}\text{area of central}\\ \text{counting square}\end{matrix}}$$

the area of the drop being obtained from measurement of its diameter with calipers

If the distribution of virus particles within the drops conforms to Poisson's law, the variance of the distribution of the number of particles per field should equal the mean number per field In fact, the variance was 1 7–6 0 times greater than the mean, indicating that the particles were not distributed at random among different fields of the same drop This would arise if particles accumulated at the edge of the drop, or if they were clumped To reduce errors from these causes, drops were carefully spread before drying and consecutive fields across the drop were always counted Notwithstanding their non-random distribution in the drop, the number of particles estimated per drop was evidently proportional to the dilution of the suspension, since a similar count per ml of undiluted suspension was obtained by counting diluted or undiluted suspension (Table 1) Furthermore, the maximum variation in estimates of particle counts for seven drops of different sizes of the same suspension was only ± 20 per cent, for example, $2 9 \times 10^9 \pm 0 6 \times 10^9$

This technique is simple, quick and reproducible, and requires no special apparatus An advantage

Table 1 PARTICLE COUNTS ON YOLK SAC SUSPENSIONS OF TRACHOMA AND INCLUSION BLENNORRHŒA VIRUSES

	Virus strain	Dilution counted	Estimated No of particles per ml
Trachoma	*BOUR*[5]	1/1	$1 2 \times 10^9$
		1/5	$1 1 \times 10^9$
	ASGH[5]	1/1	$6 5 \times 10^8$
		1/5	$6 0 \times 10^8$
	*SA*2[6]	1/1	$1 0 \times 10^9$
		1/5	$1 3 \times 10^9$
	*TE*55[7]	1/1	$2 7 \times 10^7$
		1/4	$2 9 \times 10^7$
Inclusion blennorrhœa	*LB*1[8]	1/1	$5 0 \times 10^8$
		1/5	$5 5 \times 10^8$

over electron microscopy is that particles can be counted in infected yolk-sac suspensions without purification or concentration The relationship between particle counts and infectivity for chick embryos and HeLa cells is being investigated, using several strains of virus

Peter Reeve
Janice Taverne

Medical Research Council Trachoma Research Unit,
Lister Institute of Preventive Medicine,
London, S W 1

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Correlation between the Surface Structure of Viruses and Latency

By the use of DEAE-cellulose chromatography, we have endeavoured to obtain in their pure form various factors developed by adenoviruses during their propagation in tissue culture cells[1–4] HeLa or human amnion cells were inoculated with adenoviruses of types 1, 2, 3, 5, 7*a*, 9, 10, 11 and 15 On the 3rd–5th day the culture fluids were collected and were dialysed against 0 005 *M* borate buffer solution, *p*H 8 10 The dialysed fluid was then introduced into a DEAE-cellulose column (1 cm diam , 10 cm high) which was previously balanced by 0 005 *M* borate buffer solution, *p*H 8 10 Fractionation was carried out by adding 0 005 *M* borate buffer solution, *p*H 8 10, containing sodium chloride, to the column in linearly increasing concentrations of 0–0 8 *M* 5-ml fractions were collected and analysed with the view of detecting hæmagglutinins (by the reaction of hæmagglutination with the erythrocytes of rats and monkeys), also an early cytopathogenic factor[5,6], the complement-fixing antigen and an infectious virus.

Certain results obtained by these experiments are illustrated in Fig 1 Further detailed results will be published elsewhere

Attention is directed to the fact that adenoviruses of types 3, 7*a*, 9, 10 and 15, which cause acute diseases in human beings, can be eluted from the DEAE-cellulose column with smaller concentrations of sodium chloride (0 2–0 3 *M*) than types, 1, 2 and 5, which act as the latent ones eluted at 0 45 *M*–0 55 *M* sodium chloride The intermediate position is occupied by adenovirus, type 11 The observed regularity testifies to the difference in the structure of proteins which form the surface of adenoviruses, causing latent and acute infections respectively One may imagine that the proteins forming the membrane of latent adenoviruses contain a greater number of groups which are negatively charged at *p*H 8·10 than the proteins forming the surfaces of 'acute' adenoviruses

The characteristics instrumental in developing the latent nature of some infections caused by definite viruses are not known What is known, however, is that the viruses which cause latent infections sometimes lead to the development of acute diseases The viruses which are characterized by causing acute infections alone, never behave as the latent viruses It can therefore be assumed that the cause of latency lies not in the properties of the cells affected in this or that case but in the virus itself

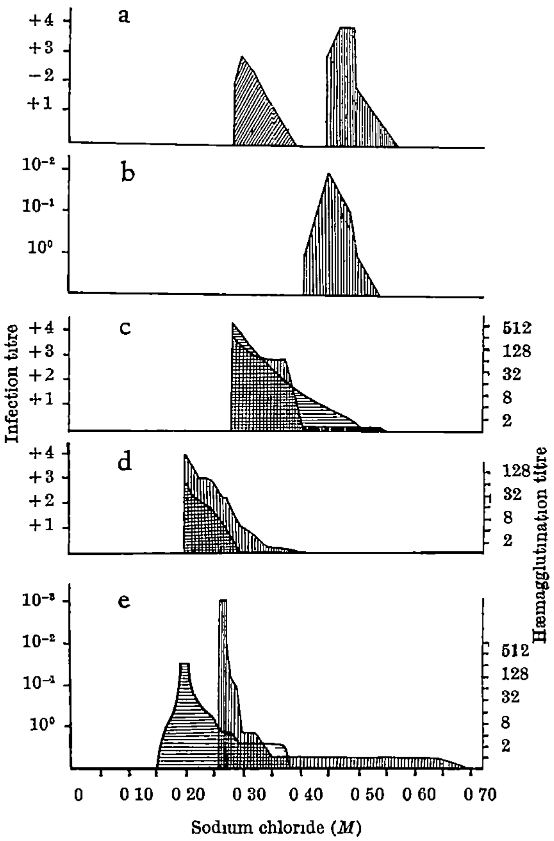

Fig 1 Fractionation of infected cultural fluids of adenoviruses of different serotypes on DEAE-cellulose Vertical shading, virus, horizontal shading, hæmagglutinin, oblique shading, early cylopathogenic factor *a*, Serotype 5 (similar to type 2), *b*, serotype 1, *c*, serotype 11, *d*, serotype 7*a* (similar to type 3), *e*, serotype 10 (similar to types 9 and 15)

In attempting to interpret the possible significance of this regularity we focused our attention on the ability of polyanions to hamper the course of the infectious process when tissue cultures were inoculated with polio virus[7] The question arises whether latent viruses do not contain similar polyanionic structures in their very elementary particles (unlike the 'acute' ones)—a phenomenon which is actually reflected in the greater number of anions on their surface Experiments in this direction are now in progress

V M Zhdanov
L B Mekler

Ivanovsky Institute of Virology,
Academy of Medical Sciences of the U S S R ,
Moscow

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[7] Salganik, G I , Scherbatich, V P , and Protas, L K , *Physiology and Genetics of Viruses* (in the press)

FORTHCOMING EVENTS

Sunday, September 2—Wednesday, September 5

British Association for the Advancement of Science (at Manchester)—Continuation of 124th Annual Meeting

Sunday, September 2

At 11 a m.—Service in Manchester Cathedral Preacher The Lord Bishop of Manchester, the Right Reverend Dr W D L Greer, D D

Monday, September 3

At 10 a m —Prof F W Shotton, F R S "The Physical Background of Britain in the Pleistocene Period" (Presidential Address, Section C)

At 10 a m —Mr L P Kirwan "Nubia—an African Frontier Region" (Presidential Address, Section E)

At 10 a m —Mr W B Reddaway "The Economics of Underdeveloped Countries" (Presidential Address, Section F)

At 10 a m —Prof Bruce Pattison "English as an International Language" (Presidential Address, Section X)

At 8 p m —Prof Sir Alister Hardy, F R S "Man and the Beneficent Sea" (Evening Discourse)

Tuesday, September 4

At 10 a m —Dr M R House "Bursts in Evolution" (Darwin Lecture)

Wednesday, September 5

At 10 a m —Mr R A French "The Making of the Russian Landscape" (Lister Lecture)

Monday, September 3—Friday, September 7

International Conference on Water Pollution Research (at Church House, Westminster, London, S W 1)

Wednesday, September 5

Institute of Petroleum (at 61 New Cavendish Street, London, W 1), at 5 30 p m —Mr F S Beardmore and Mr W C G Kosters "Application of Sulpholane in Udex Units"

Monday, September 10—Friday, September 14

Institution of Gas Engineers, and the Institute of Fuel (at the White Rock Pavilion Main Hall, Hastings)—Conference on "Gasification Processes"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Lecturer in Agricultural Economics—The Registrar, The University, Manchester 13 (September 8)

Experimental Officers or Senior Experimental Officers in the Polymer Science Group in the Chemistry Department of the Faculty of Science, to assist in the research work of the group under the general direction of Prof Gee—The Registrar, The University, Manchester 13 (September 8)

Lecturer or Assistant Lecturer (preferably with experience in teaching and research) in Zoology—Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S W 3 (September 10).

Research Assistant (with an honours degree in physics or mathematics or equivalent qualifications) to Prof N H March, to work on the density matrix theory of imperfection in metals—Prof N H March Department of Physics, The University, Sheffield 10 (September 10)

Scientific Officer (science graduate) to be the Senior Non-Medical Scientist dealing with the diagnostic work of a new Chromosome Laboratory which is to be opened early in 1963 by the United Sheffield Hospitals in conjunction with certain university departments—The Superintendent, The Children's Hospital, Sheffield 10 (September 10)

Assistant in Bacteriology—The Secretary, University of St Andrews, c/o Queen's College, Dundee (September 14)

Lecturer in Physics—The Registrar and Clerk to the Governors, College of Technology, Park Road, Portsmouth (September 14)

Assistant in the Department of Statistics—The Secretary, The University, Aberdeen (September 15)

Electronics Designer (with a degree in physics or electrical engineering, or a comparable qualification) in the Department of Chemistry, to collaborate with members of the teaching staff in designing and constructing electronic equipment to be used in research work in the Department—The Registrar, The University, Manchester 13 (September 15)

Research Assistant or Research Fellow in the Department of Livestock Husbandry within the Faculty of Rural Science, University of New England, Armidale, N S W , Australia, to study growth and type in beef cattle in relation to carcass attributes, undertake carcass appraisal studies, and to conduct research into aspects of meat quality—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W.1 (Australia and London, September 15).

Trainee Biochemist (with a degree or equivalent qualification in chemistry, with experience in, or wishing to take up, clinical biochemistry)—The Professor of Pathology, Cardiff Royal Infirmary, Cardiff (September 15)

Senior Lecturer and a Lecturer (preferably with qualifications in inorganic chemistry or biochemistry) in Chemistry, a Senior Lecturer and Lecturers in Physics, and Lecturers in Mathematics at Kwame Nkrumah University of Science and Technology—The Assistant Registrar, Kwame Nkrumah University of Science and Technology, 15 Gordon Square, London, W C 1 (September 17)

Lecturer (Grade II) in Veterinary Pathology in the Clinical Laboratory of the Veterinary Medicine Department at Langford (Bristol, 13 miles)—The Registrar and Secretary, The University, Bristol (September 21)

Lecturer in Pathology at the Western Infirmary—The Secretary of University Court, The University, Glasgow (September 22)

Senior Lecturer or Lecturer in Radiology at University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W C 1 (September 28)

Senior Lecturer (with an honours or higher degree in chemical engineering with research and teaching or industrial experience) in Chemical Engineering at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (September 28)

Cotton Agronomist (with a university honours degree in science or agricultural science or equivalent qualifications, experience in organizing, carrying through and reporting on agronomic studies, and preferably with experience in the agronomy of cotton) at Kimberley Research Station, Division of Land Research and Regional Survey, C S I R O , to take part in the cotton research programme of the Station and undertake studies of an agronomic and plant nutritional nature—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2, quoting Appointment No 620/28) (September 29)

Associate Professor in the School of Electrical Engineering, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales Australia (September 30)

Senior Lecturer in Logic and Metaphysics in St Salvator's College—The Secretary, The University, College Gate, St Andrews (September 30)

Postdoctoral Fellows (2) (with at least a Ph D degree or equivalent, backed by research experience in the field of organic chemistry) in the Division of Protein Chemistry, C S I R O , Melbourne, one Fellow to study methods of synthesizing peptides, including oligomers, and the other Fellow to study the action of oxidizing agents on amino-acids, peptides and proteins—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2, quoting Appointment No 462/161 (October 6)

Biochemistry Graduate (with experience in microbiology), for research work in chemotherapy—Dr G T Stewart, Department of Pathology, Queen Mary's Hospital for Children, Carshalton, Surrey

Entomologist (under 40) at the Royal Horticultural Society's Gardens, Wisley, Surrey, for duties principally advisory and instructional, but may include research work on insect pests affecting horticultural plants—The Secretary, Royal Horticultural Society, Vincent Square, London, S W 1, marking envelope "Confidential"

Geologist (Water Supply) (with an honours degree in geology or equivalent qualifications, and at least five years experience in water supply geology, including the electric logging of boreholes) in Northern Nigeria, to supervise the water supply exploration drilling projects and the training of Nigerian staff—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S W 1, quoting Ref RC 254/105/01.

Hospital Physicist (Basic grade) (honours physics graduate)—Dr B Levin, Pathology Department, Queen Elizabeth Hospital for Children, Hackney Road, London, E 2

Lecturer or Senior Lecturer (with good academic qualifications and preferably research experience to doctorate level or equivalent) in the Department of Electrical Engineering—Dr J H Calderwood, Royal College of Advanced Technology, Salford

Lecturer (with an honours degree in pharmacy or equivalent qualification, experience in research methods, and preferably industrial experience) in Pharmaceutics, and a Lecturer (with an honours degree in science or pharmacy, with special qualifications and research experience in pharmacology) in Pharmacology—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C 1

Mathematician (well qualified) in the Mathematical Department—The High Master, Manchester Grammar School, Manchester 13

Physical Chemists or Physicists (with a Ph D and preferably in the age group 24–28) in the London Research Station and in the Basic Research Group, both located at Fulham, for original research into gas/solid catalysis and experimental methods for the examination of catalysts—The Staff Controller, North Thames Gas Board, 30 Kensington Church Street, London, W 8, quoting Ref NE/1181

Professor (normally national of the United Kingdom or the Republic of Ireland, at least five years university teaching experience or five years relevant industrial experience, and preferably a Ph D) of Electrical Engineering (Power) at the Middle East Technical University, Ankara—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S W 1, quoting Ref RC 203/152/03

Research Assistant (with a degree in botany, diploma in food science or equivalent qualifications) in the Potato Branch, for work on quality in potatoes—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge

Scientific Information Officer (Abstractor) (with a degree in agriculture or natural science, and able to translate from German)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire

Technician (with biochemical experience), for research on enzymes and metabolites of neurological interest—The Secretary, Institute of Neurology, The National Hospital , Queen Square, London, W C 1

TEMPORARY RESEARCH ASSISTANT (with a degree in chemistry or biochemistry), for the staff of the Low Temperature Research Station, Cambridge, but seconded to the Department of Chemistry, Royal Holloway College (University of London), Englefield Green, Surrey, to work on phenolic metabolism in plants—The Secretary, Low Temperature Research Station, Downing Street, Cambridge, quoting Ref RHC

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Privy Council Medical Research Council Special Report Series No 303 Refraction and its Components in Twins By Arnold Sorsby Michael Sheridan and G A Leary Pp viii+43 (London H M Stationery Office, 1962) 7*s* net [97

Leicester Museums and Art Gallery Department of Geology A Catalogue of the Figures and Cited Specimens in the Department of Geology By C A Sizer Pp 46 (Leicester The Museums and Art Gallery, 1962) [127

Department of Scientific and Industrial Research Forest Products Research Bulletin No 48 Properties of 30–37 Year Old Sitka Spruce Timber By J A H Broughton Pp iv+24+4 plates (London H M Stationery Office, 1962) 3*s* net [127

General Register Office The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages, Infectious Diseases, Weather, Population Estimates, quarter ended 31st March 1962 (No 453, 1st Quarter 1962) Pp 28 (London H M Stationery Office, 1962) 2*s* 6*d* net [127

Library Association North Western Group Reference, Special and Information Section Occasional Papers, No 3 The Creed of a Librarian—No Politics—No Religion—No Morals By D J Foskett Pp 13 (London The Library Association, 1962) 3*s* 6*d* [127

Sir John Cass College Prospectus, Session 1962–63 Pp 56 (London Sir John Cass College, 1962) [137

A Postscript to Science and Government By Sir Charles Snow Pp ii+37 (Cambridge, Mass Harvard University Press London Oxford University Press, 1962) 3*s* 6*d* net [137

United Kingdom Atomic Energy Authority Eighth Annual Report for the period 1st April, 1961—31st March, 1962 Pp ix+87+4 plates (London H M Stationery Office, 1962) 6*s* 6*d* net [137

Federation of British Industries Pattern of Research in British Industry Report of a Conference at Eastbourne Pp v+84 (London Federation of British Industries, 1962) 5*s* [137

Second Annual Report of the Council of The Institute of Physics and The Physical Society, 1961 Pp 24 (London The Institute of Physics and the Physical Society, 1962) [137

The International Nickel Company (Mond), Ltd The Properties and Applications of the Nickel Silver Alloys Pp 24 (London The International Nickel Company (Mond), Ltd , 1962) [137

British Standards Institution Draft British Standard Recommendations for the Selection, Formation and Definition of Technical Terms Pp iii+16 (DC$_2$/4492) (London British Standards Institution 1962) [137

Meteorological Office Scientific Paper No 15 The Errors of the Meteorological Office Radiosonde, Mark 2B By Dr D N Harrison Pp ii+40 (M O 724) (London H M Stationery Office, 1962) 5*s* net [167

The Water Research Association Seventh Annual Report for the year ended April 30, 1962 Pp 44 (Medmenham, Marlow The Water Research Association, 1962) [167

Broadcasting Memorandum on the Report of the Committee on Broadcasting, 1960 Pp 12 (Cmnd 1770) (London H M Stationery Office, 1962) 1*s* 3*d* net [167

The British Journal for the History of Science, Vol 1, Part 1, No 1, (June 1962) Edited by Dr H D Anthony Published twice annually Pp 95 (Ravensmead Keston, Kent The British Society for the History of Science, 1962) 15*s* per part [187

Other Countries

United States Department of the Interior Fish and Wildlife Service Fishery Bulletin No 199 Tank Culture of Tilapia By Richard N Uchida and Joseph E King Pp iv+2–152 (Washington D C Government Printing Office, 1962) 30 cents [137

Occasional Papers of the Division of Systematic Biology of Stanford University No 10, (June 15 1962) The Hong Kong Newt described as a New Species By George S Myers and Alan E Leviton Pp 4 (Stanford, Calif The University 1962) [137

Annals of the South African Museum Vol 46, Part II The Hydrozoa of the South and West Coasts of South Africa Part 1 The Plumulariidae By N A H Millard Pp 261–320 (Cape Town South African Museum, 1962) 89 cents [137

Metropolitan Life Insurance Company Statistical Bulletin, Vol 43 (May 1962) Population Changes and Their Health Implications Rapid Population Growth in Small Cities Reduction in Perinatal Mortality Human Factors in Motor Vehicle Accidents Pp 12 (New York Metropolitan Life Insurance Company, 1962) [137

Republic of Cyprus Annual Report of the Geological Survey Department for the year 1961 Pp 43 (Nicosia Geological Survey Department, 1962) 100 mils [137

Educational Services Incorporated, Watertown Mass *E S I Newsletter*, Vol 1, No 1, (May, 1962) Pp 30 (Watertown, Mass Educational Services, Inc , 1962) [137

Ministère de l'Agriculture de la Province de Québec Noms Français des Maladies des Plantes au Canada (avec équivalents Anglais) (French Names of Plant Diseases in Canada, with English Equivalents) (Supplément du 41^{e} Rapport Annuel Société de Protection des Plantes du Québec) Pp 169 (Publication Nr 263) (Québec Ministère de l'Agriculture de la Province de Québec, 1962) [167

Research Institute of Oncopathology, Budapest Report on Research Work in the period 1958–1962 Pp 24 (Budapest Research Institute of Oncopathology, 1962) [167

Ontario Research Foundation Annual Report, 1961 Pp 64 (Toronto Ontario Research Foundation, 1962) [167

The Weizmann Institute of Science Report of Scientific Activities 1960–1961 Pp 151 (Rehovoth The Weizmann Institute of Science 1962)]167

Instituto de Investigacao Cientifica de Mocambique, Lourenco Marques Novos Taxa Entomológicos (Suplemento a Revista Entomologia de Mocambique) No 25, (Outubro 1961) New Baetidae (Ephem) from South Africa By J D Agnew Pp 18 No 26, (Novembro 1961) New Transvaal Leptophlebiid (Ephem) By J D Agnew Pp 27 No 27, (Janeiro 1962) Mais cinco novas espécies de Tabanideos (Diptera Tabanidae) de Mocambique Por J A Travassos Santos Dias Pp 16 No 28, (Fevereiro, 1962) The New Rhinoestrus Species from the Springbuck (*Antidorcas marsupialis* (Zimmermann)) in South Africa (Diptera Oestridae) By Fritz Zumpt and Evelyn Bauristhene Pp 23 (Lourenco Marques Instituto de Investigacao Cientifica de Mocambique, 1961 and 1962) [167

Arctic Institute of North America Technical Paper No 9 A Geobotanical Survey of Northern Manitoba By J C Ritchie Pp 48+2 maps (Montreal Arctic Institute of North America, 1962) 2 dollars [187

Canada Department of Northern Affairs and National Resources National Museum of Canada Bulletin Nº 178 Social Networks in Great Whale River Notes on an Eskimo, Montagnais-Naskapi, and Euro-Canadian Community By John J Hinigmann Pp vi+110 (9 plates) (Ottawa Queen's Printer, 1962) 1 dollar [187

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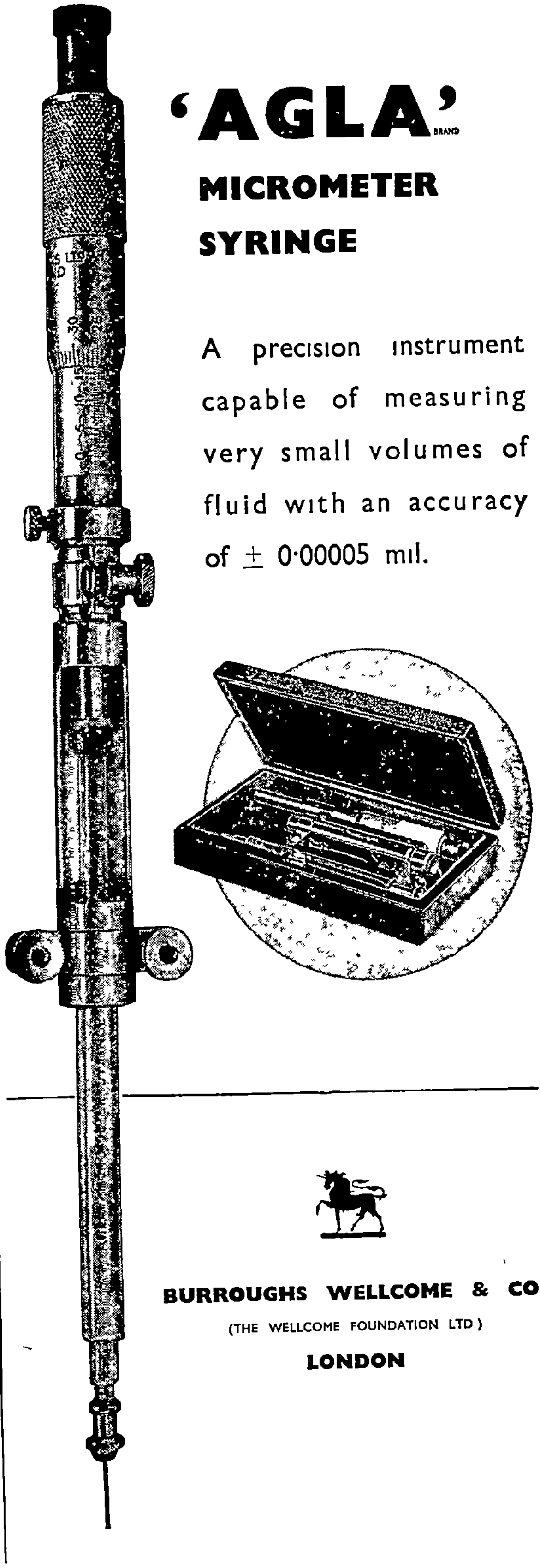

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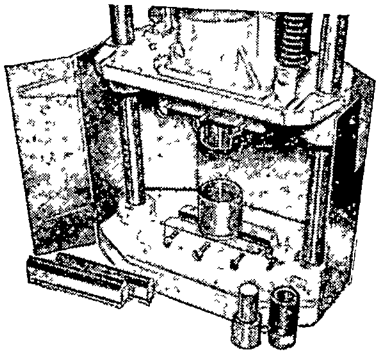

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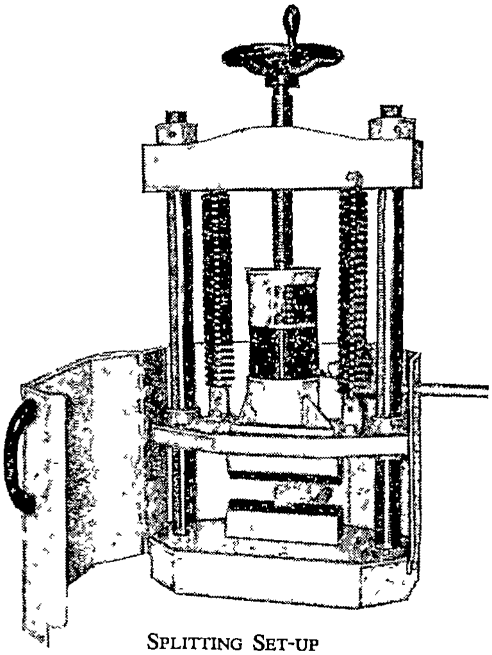

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THE FUTURE FOR BRITISH TELEVISION AND BROADCASTING

ALTHOUGH the Report of the Pilkington Committee on Broadcasting[*] deals with several matters of direct concern to the scientist, which are summarized elsewhere (see p 949 of this issue), the report is of primary importance as a social document As the Committee itself points out, broadcasting is by common consent of profound social significance, and the considerations which have shaped the exercise by successive Governments of their control of broadcasting have been, above all, social considerations Moreover, a control which derives from the need to ensure the orderly use of scarce frequencies, and is thus technical in its purpose, is applied in the United Kingdom for reasons which are essentially social

This point can scarcely be overstressed, for it has determined fundamentally the outlook of the Pilkington Committee itself and the extent to which the Government in its Memorandum[†] on the Report accepted the Committee's recommendation on one hand and on the other deferred some for further consideration In fact, so far as television is concerned, since the Television Advisory Committee was concerned only with technical factors affecting a change in line-definition standards, it fell to the Pilkington Committee to examine the social and economic factors It may be regarded as having given insufficient weight to the effect of competition on the British Broadcasting Corporation, and it has been suggested that it might have sought more convincing or scientific evidence on certain matters Despite the presence of two scientists among its members, the Committee was not, however, a scientific or a technical body It was chosen to provide a representative sector of intelligent lay opinion, and it may be surmised that its advice would have been regarded as suspect could it have been described as in any sense expert or highbrow Furthermore, the Committee emphasizes at the start of the Report that there is so far very little conclusive evidence on the effects of television on values and moral attitudes There is no attempt in the Report to overstate a case or to suggest that trends and possible dangers to which a wide range of witnesses directed attention was proved or were as yet more than contingencies to which regard should be paid by responsible authorities

This has been so ignored in much of the early criticism and uproar over the Report that its significance and true character can scarcely be appreciated without some summary of the Committee's views on the purposes of broadcasting This chapter, which follows on a general survey of the present position, is basic to the Committee's appraisal of the services now provided and its proposals for the future In the light of this chapter it can best be seen how far the Government proposals outlined in the White Paper are in harmony with the spirit of the Report It should be remembered, moreover, that not only did the Committee make its examination at a time when important developments are impending, and when the limited frequency space available makes it essential, that what is available should be used to the best advantage it was also conducting the first examination of a broadcasting authority other than the B B C , of broadcasting services operating under conditions of competition, and of broadcasting when television has become the major force

This chapter, then, is the real core of the Report, and at the start the Committee notes that neither the British Broadcasting Corporation nor the Independent Television Authority attaches any significance to the difference in wording or to the difference in order in which the three elements of information, education and entertainment are placed in the Corporation's Charter and in the Television Act The unanimous opinion was expressed to the Committee that no written formula for good broadcasting is possible it is a practice not a prescription, and the Committee, while setting its standards high, took the view that a service of broadcasting should be judged not by the stated aims of the broadcasters but by their achievements, and that the structure of their organizations should be examined in the light of those achievements Broadcasting is more nearly an art than an exact science, dealing in tastes and values, without being precisely definable, and the test of success is the programmes

In the main, submissions made to the Committee about sound broadcasting expressed satisfaction with the service Comment, critical or otherwise, was relatively lacking, and there seems to be no significant failure to realize the purposes of broadcasting For television, on the other hand, there was a large volume of sharply critical submissions, and those from people and organizations as viewers showed much disquiet and dissatisfaction The disquiet derived from the view that the power of the medium to influence and persuade is universal, and from a strong feeling, amounting often to a conviction, that the power was often being so used as to suggest a lack of awareness of, or concern about, the consequences Those who represented this view saw television as one of the major long-term factors that would shape the moral and mental attitudes, the values of our society Dissatisfaction derived from the view that there was something lacking in the programmes as such, possibly of items of one kind or another, or a more general lack of quality

It was not suggested that the picture was uniformly black or grey, but the submissions left no impression that there was little wrong with television

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[*] *Report of the Committee on Broadcasting,* 1960 Pp xii+342 (Cmnd 1753) (London H M Stationery Office, 1962) 18s net

[†] *Broadcasting Memorandum on the Report of the Committee on Broadcasting* 1960 Pp 12 (Cmnd 1770) (London H M Stationery Office, 1962 1s 3d net

Admittedly this is not evidence, and the Committee does not suggest that this assessment of the power of the medium is proved Nevertheless, those who work professionally in this sphere held that what evidence there was showed that there was an effect, perhaps persistent, apparently imperceptible but in the end prevailing It may be that in this sphere cause and effect can never be absolutely demonstrable, but the Committee is clearly right to hold that there must be a presumption one way or the other on which to found policies for the conduct of the services, and against which to measure the responsibilities of those who provide them Whatever difficulty may arise in giving it practical expression, the Committee would have been socially irresponsible had it reached any other conclusion in the face of the available evidence than that television is and will be a main factor in influencing the values and moral standards of our society

By its nature, broadcasting must be in a constant and sensitive relation with the moral condition of society Broadcasters cannot evade the consequent responsibility, either because the effect has not been conclusively proved or because of the difficulties that arise in offering a wide range of choice to the public Here again the Pilkington Committee puts the issue fairly and sensibly "To give the public what it wants" is a misleading phrase and should only be used in the sense that what the public wants and what it has the right to get is the freedom to choose from the widest possible range of programme matter Anything less is deprivation

Here particularly, the Report has been widely misrepresented The Committee reiterates that the broadcasting authorities have a duty in so far that they should keep themselves aware of the public's tastes and attitudes as they now are and in all their variety Furthermore, they should respect the public's right to choose from the widest possible range of subject-matter and/or to enlarge worth-while experiences In actual fact the Committee found that the antithesis "broadcasting should give the public what it wants, and not what someone thinks is good for the public" was regarded as a gross over-simplification of a complex and continuing problem No broadcasting authority accepted either of the two extremes, all saw their policies as lying between them There were differences of emphasis and there is obviously a very wide range of possibilities between the two extremes No one, however, argued against any departure from "giving the public what it wants" on the grounds that it implied a measure, however small, of 'paternalism' and was, for reasons of democratic doctrine, inadmissible On the contrary, all accepted that there was a responsibility to help towards a broadening and deepening of public taste

Nevertheless, the Pilkington Committee found reason for supposing that in television the purposes of broadcasting were to a material extent not being realized, and specifically it urges the responsibility of broadcasting, and especially television, to focus attention on the growing points of experience These are usually most significant, and at these points the challenges to existing assumptions and beliefs are made, the claims to new knowledge and new awareness stated If our society is to respond to the challenges and to judge the claims, they must be put before it All broadcasting, and television especially, must be ready and anxious to experiment, to show the new and unusual, to give a hearing to dissent Here, broadcasting must be most willing to make mistakes, for if it does not, it will make no discoveries"

For that passage alone the Pilkington Committee has earned the respect of the scientist, and its whole spirit makes nonsense of much of the attack that was immediately launched on the Report It provided a firm basis for Lord Hailsham's appeal in opening the House of Lords debate on July 18 to discard the generalizing about paternalism, commercialism, monopoly and free-enterprise which has so long dominated discussion of this subject and to seek an agreed broadcasting policy based on technical necessity and practical politics Lord Hailsham agreed that the unease emphasized by the Committee could not be dismissed as contemptible or ridiculous Moreover, apart from his firm warning to the programme companies about profits, he fully supported the thesis that the state of public morals and the standards of taste in public entertainment could never be a matter of indifference to patriotic men and women and are not necessarily adequately catered for by purely commercial considerations

It is unnecessary to pursue further for the present purpose the Pilkington Committee's appraisal of either the broadcasting or the television services in order to appreciate the significance of the recommendations of its Report It will suffice to reiterate the major elements which in its judgment, good television broadcasting should comprise First, programme planning and content must respect the right of the public to choose from among the widest possible range of subject-matter Secondly, in every part of this wide range, approach and presentation must be of high quality Lastly, and of overriding importance those who handle this powerful medium must be animated by a sense of its power to influence values and moral standards and of its capacity for enriching the lives of all of us The broadcasting authorities must care about public tastes and attitudes, and must keep aware of them, and of their capacity to change and develop

The Committee's recommendations for British television number 120 and are summarized under organization, existing services, new services and technical It proposes no change in the arrangements for the discharge of the Government's ultimate responsibility for the social consequences of broadcasting, and the Postmaster-General should continue to be responsible for the exercise of the Government's powers under the general authority and direction of the Prime Minister In accepting this recommendation in the White Paper, the Government states that there will be no separate Minister for Broadcasting, and no major change is proposed in the powers of the Government, which will remain largely as at present. Furthermore, in effect, the Committee's recommendation that there should be unremitting observance of the fundamental independence of the broadcasting authorities from Government intervention in the day-to-day management of their affairs, including programme content, is also accepted, though changes may be proposed in the new Charter and Act The Committee also suggests that the presentation to Parliament of the annual reports of the broadcasting authorities should be the occasion for an explanation of policy and for a revealing examination of criticisms

The Committee also recommends continuance of the power of the Postmaster-General to veto any particular broadcast or class of broadcast, and that the Independent Television Authority should be empowered to announce the use of this veto. It suggests that discussion between the broadcasting authorities and senior officials of the Post Office on the appointment or reappointment by the Government of governors of the British Broadcasting Corporation or the Independent Television Authority, should be conducted on behalf of the broadcasting authority by its Chairman, and that the Postmaster-General should continue to prescribe the hours of broadcasting It emphasizes that there should be no marked disparity between the number of hours of television for which each of the two broadcasting organizations is allowed to broadcast on any one service

On these further recommendations regarding the powers and duties of the Government the White Paper makes no comment. Those relating to the British Broadcasting Corporation are largely accepted. The Corporation will continue to be constituted by Royal Charter, and the view that the Charter (and also the Television Act) should be renewed for 12 years from July 30, 1964, is accepted The Government also agrees that where it is possible at Royal and State occasions of national interest for only one camera team to be present the Corporation should have the right and duty of undertaking the broadcast The Government also accepts the Committee's proposals on the Chairman and Governors of the Corporation, but does not propose to make ability to speak Welsh an absolute qualification for the National Governor for Wales It also agrees that the appointment of the Corporation's General Advisory Council, but not its size, which should vary between eight and twelve members, should continue to be prescribed by the Charter It accepts the recommendation that the Corporation's National Broadcasting Councils for Scotland and Wales (and for Northern Ireland, if established) should exercise the same powers in regard to the content of television services as they already do for sound It commends to the Corporation the Committee's comments about an advisory committee on scientific programmes, but the recommendation regarding the Corporation's Central Religious Advisory Committee is for discussion with religious bodies and leaders.

On finance, the Pilkington Committee recommends that the British Broadcasting Corporation should continue to be financed out of licence revenue, and only out of licence revenue, and while no material change is recommended in the principles governing the licence fee system, or in the arrangement whereby the proportion of net licence revenue payable to the Corporation is negotiated with the Paymaster-General and the Treasury, the Charter should be amended to empower the Corporation to borrow money up to a limit of £10 million This recommendation is accepted by the Government, but beyond accepting its responsibility to see that the Corporation can secure sufficient revenue to finance adequate services, the Government in the White Paper makes no comment on finance, particularly on the Committee's view that a fee of £6 annually for a combined sound and television licence would be reasonable

On independent television the Report recommends major changes in its constitution and organization The Independent Television Authority should plan programming and sell advertising time, the programme companies producing and selling to the Authority programme items for inclusion in the planned programme The Authority, after making provision for reserves, should pay any surplus revenue to the Exchequer On these proposals the Government has reserved its decision, and wishes first to be satisfied that any new structure will remedy the defects it is intended to overcome, without throwing up equally serious difficulties of its own or depriving the existing system of those features for which it can fairly claim credit This is a reasonable view, and the White Paper makes it the key point for public (including Parliamentary) discussion of which the Government promises to take full account

Meanwhile, the Government has accepted some minor recommendations relating to independent television It agrees that the Authority should set up a Committee for Wales to advise on the general conduct of independent television in Wales as it has done for Scotland and Northern Ireland, but that this does not require statutory force Decision is reserved on the recommendation that the Authority should be relieved of its statutory obligation to appoint an Advertising Advisory Committee and a Children's Advisory Committee as too closely connected with the question of the future structure of independent television The Government accepts the recommendations that advertising magazines should be prohibited, and that the maximum and average amounts of advertising time should be prescribed by the Authority and not by legislation It proposes to discuss with the Authority the question of a possible change in the minimum interval of time between breaks for the insertion of advertisements, as well as the permissible content of advertisements and the formulation of the Authority's code of principles for television advertising There is no comment on the recommendations that the British Broadcasting Corporation should continue to engage in research into the scientific and technical aspects of broadcasting, and in continuous and perceptive audience research, giving both areas of research the resources they reasonably need, or that the Independent Television Authority, on the other hand, should engage in, or commission, research and development work, including continuing and perceptive audience research, the cost of which should be an admissible charge on revenue In the House of Lords debate it was suggested that the Government should establish a central fund for research and development in television, possibly from the Authority's surplus revenue from the sale of advertising time, and the idea of centralized or co-operative research in this field at least calls for consideration

The Pilkington Committee takes the view that no additional national sound broadcasting services are needed, although it recommends that the two broadcasting organizations should give special attention to the needs of the deaf With this view the Government agrees The Committee recommends rejection of the proposals for separate, independent Scottish and Welsh Broadcasting Corporations, but recommends the planning of one service only of local sound broadcasting, not financed from advertising revenue but from licence revenue, provided by the British Broadcasting Corporation and planned to serve the largest possible number of distinctive communities that technical considerations will permit Within these local sound services it suggests that the Corporation and the motoring organizations should conduct an experiment of broadcasting information to motorists on road and traffic conditions While the Government argues that the justification for local sound broadcasting would be the provision of a service genuinely 'local' in character, it questions the existence as yet of any such demand and proposes to take note of public reaction before deciding The Pilkington Committee itself recommends that the completion of the transmission coverage, on very-high-frequencies, of the Corporation's three national sound services should take precedence

Otherwise, on new services the Committee recommends rejection of the proposals to create an additional public corporation to provide an additional television service, or that an additional television programme should be provided jointly by the British Broadcasting Corporation and independent television Instead, it recommends that the Corporation should provide the next additional television programme and should be authorized to do so as soon as possible, and this the Government proposes to do, but it does not commit itself to the further recommendation that no additional television service should be provided by independent television before the authority is reconstituted and reorganized Meanwhile, the new programme will be on 625 lines in U H F , starting in London by mid-1964 and extending to the rest of Britain as rapidly as possible thereafter The Pilkington Committee recommends rejection of the proposals for a service of sponsored television, and no service of subscription television should be authorized

Technically, as noted elsewhere, it recommends that the line definition standard in use in the United Kingdom should be changed from 405 to 625, and that a compatible service of colour television should be introduced on the 625-line definition standard The Government appears to accept the Committee's view that an ultimate pattern of six programmes is required and that two more programmes should be provided in the next four years The new programmes in U H F will be on 625 lines from the start, although the present services will continue on 405 lines for some considerable time The Government accepts the Committee's view that the first call on the unallotted frequencies in Band III should be to enable the Corporation to separate its service to Wales from that to the English Regions It proposes to authorize the Corporation to proceed as early as possible with the provision of a new transmitter to give a distinctively Welsh service to South Wales Stations to serve North-East Wales and South-West Scotland may also be required, and it is also proposed to authorize the Corporation to start transmitting some programmes in colour as part of their second programme, and similarly for any future independent television programme although the system of transmission is not yet finally decided Decision on pay-television is reserved and the Government does not accept the Committee's rejection of television for public showing, suggesting that for the time being applications to provide such a service should be considered on their merits Party political broadcasting is to be discussed, in the light of the Committee's comments, with the political parties and the broadcasting authorities

The Government has thus manifestly adopted a considerable number of the conclusions of the Pilkington Committee, some of which are far from being minor ones, and is taking the appropriate action On others, as on local broadcasting, its own assessment of the situation is reasonable enough Beyond this, as the Bishop of Leicester remarked in the House of Lords on July 19, the Report has made it more difficult for any Government to ignore or repudiate its responsibility in those realms of human life where moral attitudes are formed and developed Government and Opposition alike are clearly hesitant in facing the financial issues and reluctant to adopt the recommendation for an increase in the licence fee to £6, though this seems both the most salutary course and one that compares well with what is charged in most West and Northern European countries. There are difficulties, though not necessarily insuperable, in other methods of finance, such as subsidy, if political pressure or the suspicion of political pressure is to be avoided, and even if this risk could be avoided by some system of grants over a term of years, while even the proposed increase would leave the fee lower than that of so many other countries, the straightforward increase seems the more desirable method

The real question remains the future organization of independent television Here, the White Paper is scarcely convincing that the Government has accepted the essence of the Committee's conclusion. Although the Government has decided so promptly to give the third channel at once to the British Broadcasting Corporation, to give independent television also a new channel in advance of re-organization or reconstitution might defeat the whole purpose of the Pilkington proposals On this the Postmaster-General was perhaps a little more reassuring in the debate in the House of Commons on July 31 than is the White Paper itself Meanwhile, the change-over itself from 405 to 625 lines involves problems enough, and although the White Paper gives no details it seems that the Government's policy is reasonable. The creation of a national network of U.H.F. stations is a major undertaking and while existing V.H.F. sites and masts will be used to the fullest possible extent, many new U.H.F. stations must be built. Further, as is pointed out in the *BBC Record* for June, much more care has to be taken in locating the receiving aerial, which by and large will itself be more costly. In most locations it will be necessary, first, to have an outside aerial and secondly to have an aerial of high-gain costing appreciably more than the indoor and outdoor aerials which are often adequate for Bands I and III. The method by which the change-over is best affected is a technical problem which has still to be examined fully, and if the adoption by the Government of the Pilkington Committee's recommendation in this field has made this a matter of some urgency it is as well that the user should appreciate that the cost of technical advance is not something which should be borne entirely by the State. It is right and salutary that some of the charge should fall on those who immediately benefit by it, and that in itself should help to keep the further discussion of the organization of television and sound broadcasting on sensible and realistic lines attuned to technical and social needs rather than political prejudices.

METAPHYSICS FOR EMPIRICISTS

Foundations of Empiricism

By James K. Feibleman. Pp. xi+389. (The Hague: Martinus Nijhoff, 1962.) 27 guilders.

THIS book is very ably written. The author has a long list of philosophical works to his credit, dealing with almost every aspect of the theory of knowledge, including the social sciences. There are seven parts: categorematics, axiomatics, systemics, ethics, practices, historics and epistemics. Such a formal framework keeps the argument neat and tidy. The aim throughout is to regenerate metaphysics in a way calculated to make the subject amenable to empiricism. In other words, the metaphysician should be constrained within the boundaries of logic and fact. It is true that there have been several varieties of this discipline in the past, on the whole unsuitable, or at least unhelpful, for an age of advancing scientific enterprise. A writer of Thomist sympathies, Fr. F. C. Copleston, has remarked that metaphysics must come to terms with empiricism. The changeless view of being, as we should say, "independent of a mechanism", is too uncritical, and it is no wonder that scientists have found it unacceptable. By a deliberate search for a finite metaphysics we are invited to overcome this defect.

So far so good. Perhaps subject and object have become out of step in the course of time, with the former predominant. It is maintained that subjectivism, Greek as it was, represented at least a partial decline from the summit of Hellenic philosophy. There is no reasonable doubt that such is a true reading of history. What is odd, is that the author should belabour the theologians for their handling of these matters. Nobody to-day would try to justify the unhappy events of a century ago, for they are over and done with: so much so, in fact, that no reputable scholar is likely to make the mistake of asserting that theoretical physics can be used as a prop for religion. (Symbolic logic may possibly capture ethics and æsthetics, but that is something quite different.)

All this, however, is not to deny a place for a further plane of discourse whereon epistemic conditions are "wholly other", and to which its own metaphysics may apply. They should indeed be humble and searching. As regards the Metaphysical Society of 1869, the author has presumably read the penetrating record by his fellow-countryman, Prof. A. W. Brown (1947), of this phase of thought, remembering that the original title was "Theological" and not "Metaphysical". The whole purpose of the change was to make for obvious impartiality.

The association of metaphysics with some kind of transcendentalism is largely a matter of language, and one can sympathize with the writer in his desire to reduce the number of assumptions supporting an objective ontology.

The interplay of belief and reason goes on. The West has inherited the Augustinian "*Credo ut intelligam*", and passed it on through Anselm and Duns Scotus. Without rejecting it, there is an honoured place awaiting the empirical metaphysics for which this volume so clearly strives. F. I. G. RAWLINS

THE M.K.S. SYSTEM AND ELECTRICAL THEORY

Electrical Theory on the Giorgi System

By P. Cornelius. Translated from the third Dutch edition by Dr. L. J. Jolley. Pp. x+187. (London: Cleaver-Hume Press, Ltd., 1961.) 32*s*. net.

PUBLICATIONS emanating from the Philips Research Laboratories have a high reputation, and this English translation of a book which has reached a third edition on the Continent will be read with great interest throughout Great Britain.

The battle for the M.K.S. system advocated by Giorgi is almost won. There can be few electrical engineers remaining who are not convinced of its merits and the objections of physicists to this system do not appear to be insuperable. Advocacy of the M.K.S. system is, however, only a minor purpose of the author, whose main objective appears to be the expounding of electrical theory up to quite advanced levels with a minimum use of mathematics.

His starting point is Ohm's law, and one suspects that he would have preferred both the volt and the ampere to be regarded as fundamental units, dispensing with one of the mechanical units. From this familiar law he develops the ideas of electric field strength and current density as vectors, passing easily from there to the concepts of electric and magnetic flux. In the space of 45 pages electrical theory is developed up to Maxwell's laws and divergence and curl are introduced so naturally that the least mathematical of his readers should have no difficulty in following his argument.

Mechanical forces are not introduced until the second chapter, but this inversion of the normal order of developing electrical theory is not without its difficulties. In establishing Ohm's law the author uses mechanical forces to detect and measure both voltage and current. The power consumed in a resistance is stated as an axiom, since the real nature and dimensions of voltage and current are not revealed until much later. The book is indeed more valuable for the technologist wishing to tie up loose ends of his electrical knowledge than it would be for the young student aiming to grasp the fundamentals of his subject. For him, Coulomb's law of forces between electric charges remains the natural starting point of his subject. The best method of introducing the concept of the magnetic field is perhaps more controversial; but it is doubtful if many teachers of electrical engineering will be prepared to relegate mechanical forces to the secondary position which they occupy in this book.

About half the book is devoted to an exposition of selected problems. The author's self-denying ordinance with regard to the use of mathematics prevents the study of these problems to any great depth, but the reader's interest is maintained throughout.

Electrical engineers in Britain should be grateful to Dr. Jolley for the most attractive translation of this work and to the Cleaver-Hume Press for the publication of it. No misprints were found, the contrast of print and paper are excellent and the diagrams clear.

A. H. M. ARNOLD

AN ATLAS FOR SPECTROCHEMICAL ANALYSIS

Atlas of Analysis Lines

Prepared by Halina Jaffe, with the technical assistance of N. Turkeltaub. Pp. 13 + 50 plates. (London: Hilger and Watts, Ltd., 1962.) £26 5s.

THE publication by Halina Jaffe presents the enlarged iron spectrum in three ranges, 2478 Å – 3499 Å, 3324 Å – 8943 Å and 3981 Å – 5350 Å, the last range being taken with glass optics. The spectra are presented on 50 cards, the iron arc spectrum being in the centre, the persistent lines of 69 elements having been added manually on either side of it.

In assessing the value of this *Atlas*, comparison will naturally be made with its well-known predecessor by Gatterer and Junkes, and also with others that have appeared from time to time in text-books on the subject, of which that by Brode (*Chemical Spectroscopy*) is probably the most widely used. Jaffe's *Atlas* has advantages over either of these but at the same time is not devoid of its own limitations.

The charts are of handy size and are particularly suitable for use with Hilger instruments. The author emphasizes this, and specifies type and number of spectrograph and projector used in the production of the charts; using other projectors by the same maker, however, they are somewhat less convenient. The arc lines appear as black lines on a white ground as in a normal spectrogram, at a degree of enlargement well chosen for the purpose. Two tables are included which list the most persistent lines of the 69 elements which have been included in the charts, in order of element and in order of wave-length, respectively. These are not intended to be comprehensive wave-length tables; in fact only two lines are included for copper and silver, for example, up to eleven for dysprosium. A wave-length scale is lacking, which seems a rather serious omission; if one could have been included in Ångstrom units for the shorter wave-length range and in five Ångstrom units for the higher ranges, it would have been a considerable advantage. The inclusion of a range using glass optics is to be commended for those who have instruments with this additional equipment; hitherto workers in this medium have been rather ignored when aids of this type have been offered.

On general grounds, the spectra suffer from the inherent 'graininess' of the photographic emulsion, a defect that could scarcely be entirely avoided. The wisdom of glossy photographs on thin card for use in a laboratory is questionable, though users can overcome the flimsiness by mounting each card on a thicker backing; a matt finish would probably have been more serviceable.

The price of £26 may seem high, but this is probably justified when one considers the man-hours necessary to make a similar series of charts, even if it be confined to the range more commonly used in any particular laboratory. The ready comparison of the charts in this *Atlas* with spectrograms prepared with Hilger equipment should make it worthy of a place in the spectrographic laboratories where such equipment is to be found, and by no means out of place where other equipment is in use.

J. A. C. MCCLELLAND

π-ELECTRON CHEMISTRY

Molecular Orbital Theory for Organic Chemists

By Prof. Andrew Streitwieser, Jr. Pp. xvi + 489. (New York and London: John Wiley and Sons, Inc., 1961.) 109s.

THIS is a book for which there has long been a need. For although the greatest successes of the theoretical chemists since the advent of the wave equation in 1926 have been in the field of organic chemistry, there has hitherto been no simple account of the whole topic.

Prof. Streitwieser is well qualified to write such an account, for he combines both the experience of the experimental chemist, who knows how hard it is to sort out a genuine measure of the charge around each atom of a molecule, and also the ability of the theoretical chemist, since he has made some notable contributions to our store of calculated quantities. It is this balance between theory and experiment which must count as one of the strongest points in favour of this book. The literature abounds in 'bastard mathematics'—by which I mean poor-quality mathematics which serves practically no purpose because it solves no problems. There is none of that here.

The author has concentrated on π-electron chemistry—which seems for him to be almost synonymous with organic chemistry—and he deals only with the molecular-orbital approximation. This is wise, because by far the greatest successes and the widest applications are within this approximation. He also concentrates almost entirely on the simplest Hückel theory, with no overlap integrals and no allowance for electron exchange (except in the development of the ω-technique, largely developed by the author himself). As a result, the account is excellent for ground states, but becomes much less convincing in those few places where excited states are involved. But it is quite amazing to see what can be done, even with so gross an approximation.

The book is a veritable mine of information and is almost encyclopædic. The author says that in this field, during the 1930-decade, there were published some 20 papers, in the 1940-decade 70 and in the 1950-decade 600. He seems to have included them all. Certainly I could think of scarcely any significant ones that he had left out. Moreover, the applications are multitudinous—to charge distributions, bond orders, polarizabilities, electrode potentials, and particularly chemical reactivity, where he has provided easily the best and most comprehensive account yet published. The text is liberally scattered with excellent diagrams and graphs to show the measure of agreement with experiment.

This is a book which should be accessible to every theoretical organic chemist, and to most experimentalists also. For the story is not too difficult, and the whole pattern of π-electron chemistry is changed because of the work here described. There will be those who shudder occasionally at what will seem hard treatment for their beloved English language ("the hydrogen molecule cannot be solved exactly", "this function peaks at the nuclei", "The reader will recall from his algebra days"). But there seem to be very few actual mistakes, despite the inevitable over-simplifications. If there are still any organic chemists who wonder what good has come out of wave mechanics let them read this volume, they will do well if they avoid being converted by the range of the ground covered, and the author's enthusiasm as a guide. C. A. COULSON

THE MIOCENE TRANSGRESSION

Fundamentals of Mid-Tertiary Stratigraphical Correlation

By Dr. F. E. Eames, Dr. F. T. Banner, Dr. W. H. Blow and Dr. W. J. Clarke. With a contribution by Dr. L. R. Cox. Pp. viii + 163 + 17 plates. (Cambridge: At the University Press, 1962.) 50s. net; 9.50 dollars.

THE correlation of the Mid-Tertiary marine sediments has frequently been based on uncritical, even dogmatic statements in the past, so that some peculiar conclusions have been reached, notably those relating to the fauna ascribed to the Oligocene in America. The authors of the present work have briefly made known in the past decade some of their reasons for rejecting some of the old correlations. Using their wide knowledge of the stratigraphical palæontology of both the Old and New Worlds, they now give their correlation of the Mid-Tertiary marine successions throughout the world, and produce their arguments for lowering the base of the Miocene to include much formerly referred to the Oligocene. They lay considerable emphasis on the Foraminifera, both the larger forms and the assemblages of planktonic forms, especially the Globigerinaceae, but (a basic principle) they also take into account the evidence of the other phyla. The authors point out that assessments so made are liable to error if certain factors are disregarded, such as the influence of facies on the local ranges of a species in different localities, the changed time-values of some fossils owing to modern, more refined evaluation of their characters, and the recognition of widespread disconformities and unconformities. The last, in particular, have sometimes been overlooked in the past, so that re-worked Eocene fossils, often beautifully preserved and incorporated into post-Eocene sediments, have led to false conclusions, especially in America.

Another fundamental principle of the authors is that "the type region for a stage or series of stages must be regarded as constituting the fundamental basic standard". Any discussion of the Miocene in its relation to the Oligocene should, therefore, be based on a clear understanding of the faunas of the European Oligocene and Aquitanian, and the contribution of Dr. L. R. Cox in Part 1 on their marine molluscan faunas is thus of much importance.

Part 1 consists of a review of the criteria for correlating the Mid-Tertiary deposits and a critical revision of the correlations made. The Aquitanian is taken as the base of the Miocene, and naturally much of the evidence for the authors' conclusions concerns the recognition of the Lower Miocene (Aquitanian and Burdigalian) and its relationship to the Oligocene. As their revision moves on from Europe, through the Tethyan area, to Indonesia and beyond, the authors argue cogently for several new interpretations, for example, in Indonesia. It is in the Central American area, however, where they make their most profound changes, lowering the base of the marine Miocene everywhere in the United States, Caribbean region and northern South America, to include nearly all that was formerly designated as Oligocene. The Antigua Limestone is of key importance, for in addition to a fauna of *Lepidocyclina* species of Lower Miocene age, it yields corals (and other macrofossils) which enable correlations to be effected with other Central American areas. Thus the coral-bearing formations previously referred to the Oligocene are shown to be Miocene. The biggest changes affect the Vicksburgian, which had come to be regarded as typical of the American Oligocene, but which the authors argue belongs to the Miocene, and the successions in Venezuela and Trinidad, where anomalies can be explained by the re-working and derivation of Eocene fossils.

Part 2 deals with the Globigerinaceae. A series of biozones, based on the planktonic Foraminifera, and founded on the succession in Tanganyika and on a revision of the species occurring there, is proposed for the Upper Eocene, Oligocene and Lower Aquitanian. The distribution of the assemblages so recognized confirms the results reached in Part 1, and particularly the absence of the Upper Bartonian and the whole of the Oligocene from the Central American region.

A reconsideration of Mid-Tertiary palæogeography follows from the establishment of such a major unconformity (or disconformity), and the authors suggest that, while the Oligocene orogeny was occurring in the archipelagic Caribbean area, "the whole of the southern portion of the United States was uplifted *without* orogeny as a gigantic coastal plain". The widespread Aquitanian transgression that succeeded was in accord with the geological history of the rest of the American region and of the Tethys as a whole.

The arguments put forward by the authors are so logical and well thought out that they carry conviction, and it seems that, in general, only slight adjustments will be necessary to the stratigraphical conclusions at which they arrive. Their correlation-tables are clear and easy to interpret, and the text-figures and plates are excellent. The whole volume is well produced and typographical errors are very few.

H. DIGHTON THOMAS

Annual Review of Microbiology

Vol 15 Edited by Charles E Clifton, in association with Sidney Raffel and Mortimer P. Starr Pp viii+349 (Palo Alto, Calif Annual Reviews, Inc , 1961) 7 dollars

THE fifteenth *Annual Review of Microbiology* provides concise surveys of literature covering a wide range of topics, in this respect it lives up to the standard set by its predecessors The volume contains thirteen reviews, cites more than two thousand references and includes a cumulative index of authors and chapters for Volumes 11–15

Microbiological literature has, during the past decade, become liberally sprinkled with jargon Most microbiologists are now familiar with the terms 'chemostat' and 'bactogen', but 'auxanometer' and 'symbiocon' may leave many puzzled The principles of these and other devices now used for the continuous culture of micro-organisms are admirably presented by James

Studies of microbial metabolism continue to reveal new synthetic capabilities in micro-organisms the biosynthesis of C_2, C_3 and C_4 compounds from C_1 units is discussed in a comprehensive review by Quale All who study microbial metabolism will, ultimately, be faced with problems of cell permeability and membrane transport Cirillo points out that, until recently, it has been common practice either to ignore these problems or eliminate them by the mechanical disruption of cells This is no longer the case Much has been learnt in recent years about active transport mechanisms and 'permeases', these studies have been well summarized by Cirillo in an article entitled "Sugar Transport in Micro-organisms"

Two articles in the present volume will be of the greatest interest to virologists Questions of virus structure are discussed by Colter and Ellem, Zhdanov reviews recent experience with anti-viral vaccines This latter contribution contains some interesting information about the relative merits of different poliomyelitis vaccines

Other topics reviewed include physiology of actinomycetes, fine structure of protozoa, interactions between pesticides and soil micro-organisms, the family Brucellaceae in veterinary research, phage-host relationships in some genera of medical significance, plant-nematode inter-relationships, gaseous sterilization and antigenic variation in unicellular micro-organisms B A Newton

The Ovary

Vol 1 Pp xiii+619 157*s* 6*d* Vol 2 Pp xiii+600 157*s* Edited by Sir Solly Zuckerman Assisted by Anita M Mandl and Peter Eckstein (New York Academic Press, Inc , London Academic Press Inc (London), Ltd , 1962)

THIS magnificent treatise will certainly long remain a standard reference work for the literature on the morphology and function of the ovary

Volume 1 consists of eleven chapters and covers the development and structure of the ovary, including its histochemistry, the ovarian cycle, ovarian pituitary relationship, the mechanism of ovulation, ovarian physiology in the non-pregnant and pregnant female, the menopause, and derangements in ovarian function

The twenty-three chapters of Volume 2 deal with such varied problems as the chemistry and biochemistry of ovarian hormones, the experimental embryology of the ovary, the actions of ovarian hormones on the body, the influence of nutrition and external factors on the ovary, relationship between ovarian hormone secretion and behaviour, transplantation of the ovary, and the relationship between tumorogenesis and the ovary

Each chapter is written by specialists, well recognized in their respective fields, and, thanks to the excellent editorial work of Sir Solly Zuckerman, the whole treatise has a character of harmonious unity despite the multiplicity of necessarily overlapping subjects

It would be impossible to review each chapter of an extensive treatise of this kind in detail, but there can be no doubt about the value of these two volumes not only to endocrinologists but also to comparative anatomists, zoologists and experimental embryologists The treatise is illustrated by numerous photographs, graphs, and tables, its usefulness is greatly enhanced by carefully prepared author- and subject-indexes Hans Selye

Science and World Affairs

History of the Pugwash Conferences By Prof J Rotblat Pp 92+4 plates (London. Dawsons of Pall Mall, 1962) 8*s*. 6*d* paper bound, 14*s* cloth bound

THE most recent Pugwash Conferences have met in the United Kingdom the first at Cambridge on August 25 was attended by 60 specialists on disarmament and arms control, the second in London on September 3, when some 200 scientists from many nations sought solutions to some of the problems created or made tractable by the modern growth of science Prof. Rotblat's readable account of the origin and development of these Conferences on science and world affairs as an important and effective channel of communication between scientists for the study and discussion of the most complex problems now facing mankind thus appears at a most opportune moment

It gives a concise, purely factual account of the first eight conferences, and although Prof Rotblat does not attempt to discuss in general terms the organization of international discussion among scientists, its effectiveness, or the relation of such discussion to Government action, he provides all the material required for an independent and impartial assessment of what has already been achieved by these conferences. He appends to his account the original Russell–Einstein manifesto of December 1954 from which the movement may be said to have stemmed and the several statements issued by the successive Conferences, including the Vienna Declaration of 1958, and the detailed proposals for international co-operation in science which form the substance of the statement from the seventh Conference last September, and there are lists of the participants in all these Conferences He is content to let the facts speak for themselves, and the success which the movement has already achieved is manifestly such as makes the book one which has strong claims on the attention of scientists generally

Prof Rotblat has earned the gratitude of all scientists who believe that science has in its power to make a serious and constructive contribution to world peace and order His book should make a definite contribution to the understanding of what the two Conferences in the United Kingdom represent and their implications for human welfare and scientific advancement R Brightman

THE CHALLENGE OF SPACE RESEARCH

By Sir BERNARD LOVELL, O.B.E., F.R.S.*

Director of the Nuffield Radio Astronomy Laboratories, Jodrell Bank, and
Professor of Radioastronomy in the University of Manchester

OUR present knowledge of the universe is largely based on the work of the optical telescopes, particularly of the 100-in. on Mount Wilson and the 200-in. on Mount Palomar. More recently the radio telescopes have achieved great successes in astronomical research. It is important to appreciate the scale of cosmic distances with which these telescopes are concerned so that we can understand the limitations of the experiments which it is possible to do in space probes.

About 25 years ago, we believed that all the stars of the universe were concentrated in the Milky Way system which we see with the naked eye in the sky at night. We believed that the totality of the cosmos was represented by an almost spherical enclosure with the Sun and its planets conveniently situated in the centre of the system. But observations made with the Mount Wilson telescope just after the First World War showed that the real situation was quite different. Now we know that the Milky Way is an assembly of about 100,000 million stars arranged in a flattened disk so large that a ray of light, travelling at 186,000 miles per second, would take 100,000 years to traverse the system. Moreover, the Sun, instead of being situated in the centre of this stellar system, is in a very unprivileged position right out in one of the spiral arms of the galaxy. The distance of the Sun from the nucleus of the galaxy is about 30,000 light years, and we are so unfortunately situated that with the optical telescopes it is not possible to study these central regions because of the obscuration by the dust which exists between the stars. This entire system is rotating like a cartwheel and the stars on the perimeter take about 200 million years for a whole rotation. It was soon realized that this enormous assemblage was only a very small part of the cosmos. Herschel speculated that the nebulosities which could be seen in the Milky Way might be systems of stars far removed from it. This remained a speculation until Hubble succeeded in measuring the distances of some of the stars in these nebulosities. Then it was appreciated that we were dealing with other entire galaxies of stars situated so far away from our own Milky Way system that the light from the nearest of them in Andromeda was taking about 2 million years on its journey towards us. This great spiral galaxy in Andromeda is the only extragalactic nebula which can be seen with the naked eye. We know now that there are vast numbers of similar galaxies in the universe each of which contains, perhaps 10,000 or 100,000 million stars.

The large telescopes reveal the existence of these galaxies of stars out to distances such that the light takes thousands of millions of years on its journey through space. The 200-in. telescope on Mount Palomar, a few years after it came into operation, succeeded in photographing a cluster of galaxies in Hydra the distance of which was determined as 2,000 million light years. More recently, with the co-operation of the radio telescopes, the range of identification has been increased to something like 5,000 million light years. Within this region of time and space there must be thousands of millions and probably trillions of these galaxies which appear to be distributed uniformly, at least on the large scale.

One of the most remarkable features of these observations is that the galaxies are receding from one another at high speed. At the limit of our present penetration the speed of recession is about 46 per cent the velocity of light, that is about 86,000 miles a second. These distances are quite vast compared with the kind of distances with which we are concerned on Earth. An imaginary journey at the speed of light, 186,000 miles a second, would enable us to encircle the Earth 7 times in the first sec, after $1\frac{1}{2}$ sec we could pass the Moon, in 8 min we would pass the Sun, and in about $6\frac{1}{2}$ h we would be removed entirely from the Solar System. After $4\frac{1}{2}$ years we would reach the nearest star, after about 50 or 100 thousand years we would pass out of our own Milky Way system, after 2 million years we would reach the nearest nebula in Andromeda and so we could go on for many thousands of millions of years traversing the regions of time and space which are accessible to our present-day instruments.

These results with the big telescopes have been obtained because man has the ability to look out into space through rather a small transparency or gap which exists in the Earth's atmosphere. This extends over the colours of the rainbow, and it is through that region of the spectrum that it is possible to get a clear view of the heavens. If human beings had evolved with eyes which were sensitive in a slightly different part of the spectrum towards the infra-red or ultra-violet we would have had very little knowledge of outer space. This restriction of man's view of the universe was not thought to be of any great consequence because it was known that a hot body, like a star or the Sun, generates most of its energy in the visible part of the electromagnetic spectrum. Even after Jansky discovered that radio waves were incident on the Earth, in the radio part of the spectrum, astronomers were not particularly excited about it. Now we realize that this other transparency in the atmosphere is extremely important, because the radio telescope which picks up the radio waves from space has enabled us to penetrate very far indeed into the depths of space. Indeed, it seems likely at the present time that the big radio telescopes are penetrating much farther than the known 5,000 million light years limit of the most distant identified cluster of galaxies, and probably so far as man ever will be able to penetrate into space. We believe that this new-found ability to investigate the remote epochs of space and time is of great consequence to the problem of the origin of the universe, but the present occasion is not appropriate for further discussion of this exciting work.

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* Substance of an address delivered before the Institute of Strategic Studies at Brighton on May 14.

Scientific Significance of the Earth Satellite and Space Probe

Our earthbound instruments look out into space through the transparencies in the optical and radio parts of the spectrum where there is no significant absorption in the atmosphere or ionosphere It was realized that there were a number of problems awaiting solution when it became possible to fly scientific instruments outside the atmosphere and ionosphere but it was not anticipated that our ability to launch instruments in space probes or Earth satellites would give us entirely unexpected results about the conditions in the Solar System or in the universe In the event, the early experiments with scientific instruments in the Earth satellites produced some remarkable results

For the scientist the importance of these space activities is the ability to get instruments outside the obscuring and absorbing layers of the Earth's atmosphere in order to investigate those parts of the spectrum which are wholly or partially obscured from instruments on the Earth It is important to emphasize this point because on the cosmic scale the possible penetration of these vehicles into space is minute Indeed, so far as it is reasonable to look ahead, the useful penetration of the space-vehicles will be confined to the nearer parts of the Solar System The Earth is 93 million miles distant from the Sun or 8 light min The orbits of the inner planets Mercury and Venus lie between the Earth's orbit and the Sun At closest approach Venus is about 25 million miles from Earth, and Mercury about 50 million miles The orbit of Mars lies outside that of the Earth and at closest approach its distance from the Earth is about 34 million miles These distances of a few tens of millions of miles represents the immediate ambition of space technologists so far as the possibility of getting scientific instruments into space is concerned It seems unlikely that in the foreseeable future there will be much hope of placing scientific instruments into the region of space of the outer planets Jupiter, Saturn, Uranus, Neptune and Pluto In the case of Jupiter a journey of about 368 million miles would be involved and ten times that distance for the outermost planet Pluto

If success is ultimately achieved in sending space-vehicles to the extremes of the Solar System the distances involved so far as penetration into cosmic space is concerned are insignificant Even with the outer planet of the Solar System, Pluto, we are concerned with a planet which is only a few thousand million miles distant from Earth, whereas the nearest star is 20 million million miles away The light from the Sun takes 6½ h on its journey to the orbit of Pluto, but 4½ years to the nearest star Thus, for any space experiments in which proximity of instruments to the object of investigation is concerned, only the inner reaches of the Solar System are at present open to study and in decades to come perhaps the region of Jupiter and the nearer of the outer planets

Scientific Dividends of Space Research

Until 1958 we believed that the immediate environment of the Earth was defined by the zones of electrified regions, known as the ionosphere, extending to 400 km or so above the Earth There seemed no reason to doubt that the upper part of this ionosphere represented the extent of the Earth's geophysical control It was a reasonable assumption that if scientific instruments were despatched to a height of more than 500 km above the surface of the Earth they would be in a truly interplanetary environment, and would be influenced only by the particles and radiation under the influence of the Sun, largely unaffected by the presence of the Earth

It is probably correct to say that most of the original concepts for work with Earth satellites were concerned either with information about the Earth itself or with the more detailed studies of the pressure, temperature and depth of the atmosphere, and with a fuller understanding of the incident radiations which controlled the ionosphere and atmosphere In actual fact the discovery made by Prof J A Van Allen in the first American satellite, *Explorer I*, demonstrated that this concept of the environment of the Earth was far too restricted *Explorer I* carried instruments which were specifically designed to study the radiation impinging on the atmosphere from the Sun and outer space The instruments ceased to function because they became blocked with intense radiation in certain parts of the satellite orbit Van Allen had discovered that the Earth is surrounded by radiation belts in which fundamental particles—mostly protons and electrons—are trapped in the Earth's magnetic field

These radiation zones are now known to extend from about 4,000 miles above the Earth to 50,000 miles, where there is a gradual merging into the interplanetary environment At the moment there is uncertainty about the precise arrangement and origin of the particles in these zones For some time after the initial discovery it was believed that there were two main zones of trapped particles, the one nearer the Earth consisting of protons, and an outer zone consisting of electrons However, it seems from the recent analysis of the American results that it may be wrong to think of these zones as two separate entities, and that they may be manifestations of a continuum of trapped particles extending out to 50,000 miles from the Earth

The origin of these particles is a question of dispute It is believed that the protons in the inner zone may be the result of processes which have taken place in the atmosphere of the Earth There, neutrons are produced by cosmic ray bombardment The neutrons decay into protons and electrons as they move out into space and the protons are trapped in the magnetic field The trapped particles spiral around the Earth's field making the journey between the two hemispheres in a second They are reflected at the mirror points and continue in this trapped condition with the whole shell moving around the Earth once a day The origin of the electrons in the outer zone is also uncertain One significant observational fact is that the density of the trapped electrons is very closely under solar control in the sense that when there are magnetic storms or solar eruptions the particles tend to drain from the zone and it takes some time for it to be repopulated This suggests that this zone is composed of particles which are actually streaming from the Sun—the solar wind This interpretation is not altogether satisfactory because it is not certain how such particles could actually be injected into these orbits around the Earth

The further details of this new work need not concern us here, but for our present purpose it is important to remember that in the few years since Van Allen made his first observations in *Explorer I* our entire conception of the immediate environment of the Earth has been transformed in a way which no one could have anticipated. It is true that many years ago the Norwegian astrophysicist Stormer described how particles could be trapped in the Earth's magnetic field in this way, but his theory concerned the formation of auroræ and I do not think that any scientist really anticipated that the Earth was permanently enclosed in such a shell of particles In 1957 we believed that we would move into outer space a few hundred miles above the Earth's surface Now we know that we would have to travel to distances of about 50,000 miles above the Earth's surface before we would find ourselves in a truly interplanetary environment

These new results are well correlated with the development of our general concepts about the solar system For a long time the Earth and the planets have been regarded as bodies under the gravitational control of the Sun but otherwise physically isolated Now it appears that the whole system is much more of a unity and that we must regard ourselves as being enveloped in the very tenuous corona of the Sun itself It seems probable, both from this work with the space probes and from some recent experiments with the radio telescopes, that we are, indeed, enveloped in the corona of the Sun and closely under the control of the solar forces This probably applies also to the other planets in the Solar System Here we have one example demonstrating in a remarkable manner the unexpected dividends which have come from the investments in space research

A subject which has been reserved for experiments in Earth satellites and space probes is the problem of the dust which exists in the Solar System These dust particles are manifested on Earth as shooting stars in the atmosphere, and occasionally by meteorites which plunge to Earth These particles move under the gravitational attraction of the Sun, and are often associated with comets Contrary to older beliefs we are now certain that these meteoric particles are confined to the Solar System and are not transient visitors from interstellar space Occasionally, the Earth in its journey around the Sun passes through a particular concentration of débris which may itself be in a cometary orbit Then we see a display of shooting stars which may last for several nights until the Earth emerges from the débris The Earth sweeps up vast quantities of material in this way. With the unaided eye it is possible to see meteors down to about the 5th or 6th magnitude in a clear dark sky Down to this limit there are something like a hundred million of these particles burning up in the atmosphere of the Earth every day

The techniques of radio astronomy have greatly facilitated and extended these observations, and we now have observational data which take us down to the extremely small infra-visual particles Something like 10,000 million of these particles bombard the Earth's atmosphere every day, and the smallest of them which give a detectable trail observable with a radio telescope weighs only a ten-thousandth of a gram Particles of even smaller size exist in much greater quantities but these are so small that they are stopped by the atmosphere before they burn up, and they descend slowly to Earth as small particles of dust known as micrometeorites Our knowledge of the existence of these actually came from the inspection of the sediments at the bottom of the ocean The investigation of these micrometeorites in space as solid bodies could only be made by instruments in satellites moving outside the Earth's atmosphere In the past few years our knowledge of these particles has been greatly increased, since many of the satellites and space probes have measured the impact of dust grains weighing only a thousand-millionth of a gram It is believed that the Earth possibly collects something of the order of a few million tons of this material every year

As a third and last illustration of the discoveries which have already been made in space-vehicles we refer to another unexpected result This work concerned the investigation of cosmic rays and was carried out by Prof J A Simpson of Chicago in the space probe *Pioneer V* It has been known for a long time that the recordings on Earth of the cosmic rays which originate somewhere in the universe were effected by magnetic disturbances When the Earth's magnetic field is disturbed, for example on the occasion of a solar flare, there is a decrease in the intensity of the cosmic rays recorded on Earth It had been commonly assumed that the effect was local and that it was the change in the Earth's magnetic field which was altering this influx of cosmic ray particles When the space probe *Pioneer V* was about 10,000 km from the Earth there was a magnetic storm and Simpson's apparatus in the probe recorded a decrease in the cosmic ray intensity simultaneously with the decrease observed on Earth This straightforward observation is of great importance, since it demonstrates without ambiguity that the original interpretation that these decreases are confined locally to the Earth was erroneous Clearly we are dealing with a modulation of the cosmic ray particles from space in the Solar System itself Apart from the importance of Simpson's observations to our understanding of the cosmic ray phenomena the experiment has significant repercussions on our beliefs about the magnetic fields in the Solar System The effect demonstrates that there are variable magnetic fields in the interplanetary space, presumably carried by the plasmas ejected from the Sun during the upheavals caused by the solar flares

Tasks for the Future

These experiments which have been made in space so far are merely a beginning to the work which scientists hope and expect to carry out with the space probes of the future From the astronomer's point of view it seems possible that the future space probes can make great contributions to our understanding of the origin of the Solar System I think that this question of the origin and evolution of the Solar System, coupled with the study of the problem of the existence of organisms elsewhere in the Solar System, are issues which will become vital when space probes with working instruments can land on the Moon and reach the vicinity of Mars and Venus I would assess this general problem as the most significant task to which space science should adapt itself on a large scale

The problem of the evolution of the Solar System is one of extreme difficulty, and I sometimes think that it is even more difficult than the problem of the evolution of the cosmos itself After all, if one is concerned with the study of how the universe came into existence, then our modern radio telescopes penetrate so far into the cosmos that it is now possible to study the conditions in the universe as they existed more than 5,000 million years ago It is not possible to study the past history of the Solar System in this way Our knowledge of the Solar System is effectively of time present and we have no possibility of observing similar events happening elsewhere at the present time Therefore we must try to devise some observational test of a multitude of theories as to how the Earth and the planets came into existence.

The old idea was that the primeval material of the Earth and the planets was torn out of the Sun by a passing star and that this material afterwards cooled down and formed the Solar System as we know it to-day. This theory had two important consequences. One was that the process which gave rise to the birth of the Solar System must have been an extremely rare one, because, although there are such a great number of stars in the Milky Way system, a close approach of two stars in this way is an unlikely event. The second corollary was that the Earth was formed in a hot state and therefore the evolution of any life forms on it must have been a subsequent event after it had cooled down.

Our present knowledge leads to the belief that such a sequence of events is quite out of the question. Although many details remain to be completed it seems probable that the planets and the Earth came into existence by quite a different process. About 5 thousand million years ago the Sun was surrounded by a nebula of gas and dust. The Sun attained this nebula either in the process of its birth in a great star cloud, or it collected the nebula as it passed through one of the dust clouds in the Milky Way system. During the course of the next few thousand million years this nebula of gas and dust aggregated into successively larger bodies which eventually formed the Earth and the planets as we see them today. The evidence for this comes from the assessment of the relative abundances of the different kinds of elements in the solar atmosphere, in the meteorites, which are probably bodies of lunar size which were formed in the beginning and then broke up in collisions and from the studies of the abundances of the elements in the planetary atmospheres and on Earth. Analysis of the meteorites indicates that they lost their uranium about 4½ thousand million years ago, and this would appear to be the last occasion on which any really violent processes took place during the evolution of the Solar System.

In order to fill in the details of this problem it is important to obtain access to material which lies in the Solar System other than that which is brought to us occasionally by the meteorites which come to Earth, because the trouble there is that we do not really know how the meteorites themselves originated. This I think is the most significant part of the lunar space programmes now in preparation—namely the possibility of studying the details of the composition of the lunar dust and the lunar substrate in order to assess the relative abundances of the elements, compared with those which we have on Earth, on meteorites, and those which we find in the solar atmosphere.

The second problem concerns exobiology. That is the great issue as to whether life as we know it is unique. On the old theory of the origin of the Solar System it seemed inevitable that life was unique because the processes of formation of the Solar System appeared to be unique. Now the position has changed and the discovery of the probable existence of organisms elsewhere in the universe is important. As an astronomer I do not think there is an astronomical problem remaining, because the processes which we believe led to the formation of the Solar System must have been of frequent occurrence in the universe. It is not difficult to estimate how many stars in the universe are likely to have planetary systems. Even on the most pessimistic assumption this comes to about 5 per cent of the stars in the galaxy and in all the extragalactic nebulæ. How many of these stars with planetary systems have at least one planet which is in a sufficiently stable condition for a few thousand million years to support long-term evolution? The answer is that about half of one per cent of the stars in the cosmos have planets of which at least one member may be in a sufficiently stable condition to support long-term evolution. This means that there are vast numbers of possible abodes of life in the universe. In the Milky Way alone the one-half per cent means a thousand million. Then in the entire cosmos there must be many trillions of possible abodes for organic evolution.

The question is, therefore, not astronomical, but biological. This is where the future space probes could make their greatest impact on man's knowledge of the universe with the utmost repercussions on our life and thought. The question which the space probe has the possibility of answering is whether the primeval organisms exist elsewhere in the Solar System. This evidence must be carried by the planets Mars and Venus, and there is reason for believing from the recent discoveries of Calvin and Vaughn and of Prof. Nagy and others of life-forms in the meteorites that the answer will be in the affirmative [*Nature*, **193**, 1119, 1962]. Therefore, I would class the investigation of the lunar structure, from the point of view of the evolution of the Solar System, and the exobiological investigations of the atmospheres and surfaces of Mars and Venus, as the great and unique tasks of space science in the future.

A grave warning must be given, however. In the various excitements of carrying out these probings neither of the great nations who are engaged on this and have the only opportunity of carrying it out at the moment—the U.S.S.R. and the U.S.A.—must ruin our chances of making the discoveries because of national anxiety to be first. It is quite clear that the ejection of a rocket such as *Lunik II* on to the surface of Mars and Venus would be a very great scientific and moral disaster because by its contamination it would prejudice for ever the possibility of reaching a valid conclusion about the existence of organisms developing elsewhere. It is absolutely essential that these biological investigations be carried out carefully in a considered way with an assessment of the situation by probes moving remotely from the planets before actual impacts are attempted.

Value of the Investment

It is against this background of the present and future scientific activities in space that one must inquire if the investment is worthwhile. May I say straight away that I am a great believer in space research; but I have been asked to attempt to separate the worthwhileness of the investments in space for purely scientific research from all the other associated activities particularly the military, industrial and commercial enterprises such as, for example, communications, weather forecasting and navigation. Even with all these associated benefits removed my answer is a simple one, that no community can continue to thrive unless it invests in space research to the utmost. On the other hand this must be accompanied by a warning. In an extreme case, suppose a decision was reached that Great Britain had to engage in these activities, then her entire scientific budget of about £400 million would be only sufficient to make a start with the kind of space activities which are now being carried out by the National Aeronautics and Space Administration of the United States. Therefore to do that, that is to shift all the science of Britain on to these space activities, would be nonsensical, because the quantitative yield of results per investment per million pounds per thousand scientists in the space programmes is rather small compared with the results which can be obtained with the same kind of investment in earthbound instruments, such as optical telescopes and radio telescopes. Hence, although the case for investment to the utmost in space research is so strong it must be done at the sacrifice of something other than a country's current scientific budget. It is useless to attempt to fit these new activities into an existing scientific budget. There must be a new outlook and a new budget on the biggest possible scale. Unfortunately in Great Britain we have not realized this. In the United States the situation has been realized admirably and in time, because it is evident that the battle between East and West is seen by the U.S.S.R. as a conflict in the field of science and technology. This issue has been joined by the Americans, but in Great Britain there is little appreciation of the fundamental issues at stake.

May I conclude by saying that in spite of this enthusiasm which I display and this optimism for the future of scientific research I must confess that my belief in the inevitability of progress has been very considerably undermined during this past year by the realization that some of the American and probably some of the Russian space activities are not being guided by the purest of scientific motives. I refer of course to the military programme by the U.S. Air Force for the orbiting of 'Project Needles (West Ford)' around the Earth and more recently the proposed explosion of a megaton nuclear weapon in the region of the Van Allen belts [see following article]. These subjects are unusually controversial, and the only point I would make now is to emphasize the extreme importance of carrying out such projects only by agreement of the International Scientific Unions. If I might end on this rather sad note I do beg the Americans to use their influence to the utmost to make quite sure that such projects are carried out only by international agreement and particularly within the framework of the resolution of the International Astronomical Union which was phrased only a few months ago in California. If this is not done then the United States may bear the awful responsibility of having started a chain of events leading to the militarization of space and the destruction of astronomy on Earth. [See leading article in *Nature* of August 25, p. 739.]

Note added in proof by the author. This address was delivered to the Institute of Strategic Studies nearly four months ago. In this time there have been further space activities which underline the anxiety expressed in the last paragraph of my address [see also p. 945 of this issue]. The United States exploded the megaton bomb outside the atmosphere and have thereby enormously confused the study of the natural radiation belts by setting up a new long-duration zone of trapped particles. A few more explosions of this type for military purposes by either of the Powers will obviously add so much artificially trapped material to the radiation zones that the investigation of the natural effects will have to be abandoned before we know their true nature or origin. The U.S.S.R. have launched *Vostok* 4 into an orbit so accurately controlled that it came within a few miles of *Vostok* 3 launched 24 h earlier, and recovered both space ships on Earth within 6 min of each other in the assigned landing zone. Whether or not this exploit had military backing, it demonstrates further the military potential of space in terms of rendezvous, and orbital bombs. Finally, a highly successful communication satellite, *Telstar*, has been placed in orbit and thereby encouraged the commercial as well as the military communication interests in space. The anxiety expressed about 'Project Needles' must be paralleled by the anticipation that many *Telstar* or *Echo* balloon satellites will have a similar detrimental effect on earthbound astronomy and radioastronomy. The need for international agreement about the use of space and the control of launchings, either of rockets or space-vehicles into it, has become a matter of the utmost urgency.

GEOMAGNETICALLY TRAPPED RADIATION PRODUCED BY A HIGH-ALTITUDE NUCLEAR EXPLOSION ON JULY 9, 1962

By Dr. B. J. O'BRIEN, Dr. C. D. LAUGHLIN and Prof. J. A. VAN ALLEN
Department of Physics and Astronomy, State University of Iowa, Iowa City

THIS is a preliminary report of investigations made with the satellite *Injun I* of the artificial radiation belt produced by a nuclear device recently exploded at a high altitude. The officially available information on this burst is contained in the international *AGIWARN* message of July 9, 1962, "... a megaton yield range device was detonated in the ionosphere at an altitude of hundreds of kilometers in the vicinity of Johnston Island in the Pacific at 0900 U.T. on July 9, 1962". The geographic position of Johnston Island is 16.7° N., 190.5° E.

For every kiloton fission explosion in the device, there were produced about 10^{23} fission nuclei the radioactive decay of which yielded some 5×10^{23} electrons[1] having, presumably, a differential number energy spectrum approximated by $3.88 \exp(-0.575 E - 0.055 E^2)$, for the range $1 \lesssim E \lesssim 7$ MeV, where E is the β-ray energy in MeV, and the spectral expression is in units of β-rays per fission per MeV (ref. 2). Some of these electrons were injected at such pitch angles to the geomagnetic field vector that they were temporarily trapped, executing oscillatory motion in latitude along magnetic field lines and drifting eastward in longitude to form an artificial radiation belt encircling the Earth. This is the first reported case of a significant, artificial injection of durably trapped particles into the geomagnetic field since the *Argus* tests of 1958[3,4].

Experimental Details

The present article is based on observations made with the State University of Iowa satellite *Injun I*

(1961 omicron 2) which was designed for study of the naturally occurring radiation belts It was launched at 0423 U T on July 29, 1961, into an orbit with apogee altitude 1,010 km, perigee altitude 890 km, inclination 67° and period 104 min The satellite transmits real-time data from its array of radiation detectors on command from several ground stations, distributed around the world[5]. For the present purposes the telemetry data from the following stations are of significance Quito, Lima, Salisbury (Southern Rhodesia), Iowa City and several Pacific Island stations

There are three particle detectors on *Injun I* the outputs of which are used in this preliminary investigation, but in a more detailed analysis now being undertaken another seven detectors will give useful information

Detector 1 An end-window Geiger tube (Anton type 213) with shielding of 1 2 mg cm^{-2} over a conical field of angular diameter 30°, and 4 5 g cm^{-2} of lead elsewhere (L Frank, private communication)

Detector 2 A similar Geiger tube completely encased in a shield of 3 5 g cm^{-2} of lead and a further 1 g cm^{-2} of stainless steel

Detector 3 A *p-n* junction detector with an electronic bias of 1 MeV and a conical field of view of angular diameter 30° shielded by 2 mg cm^{-2} Shielding elsewhere is $\geqslant 3$ g cm^{-2} (G F Pieper, private communication)

An abridged summary of properties of the detectors is given in Table 1

Table 1 PARTICLES DETECTABLE BY DIRECT PENETRATION

	Electrons	Protons
Detector 1	Energy $E \gtrsim 40$ keV	$E \gtrsim 500$ keV
Detector 2	$E \gtrsim 6$ MeV	$E \gtrsim 45$ MeV
Detector 3	($E \gtrsim 1$ MeV order of 1 per cent efficiency)	$1\ 5 \leqslant E \leqslant 15$ MeV

It is tentatively estimated that the response of *Detector* 2 to the fission electron spectrum is due dominantly to direct penetrations of the shield by energetic electrons ($E \gtrsim 6$ MeV) and not to the bremsstrahlung of the more abundant non-penetrating ones This estimate is being re-examined by further laboratory examination The following investigations of the spatial extent and the time-decay of the artificial radiation zone are essentially independent of the validity of the foregoing working hypothesis

Detectors 1 and 3 are directional, and their response depends on the angle at which they point with respect to the magnetic field vector Both have associated background detectors which measure any penetrating contaminating radiation *Detector* 2 is an omnidirectional detector and provides the simplest means of mapping out the natural and artificial radiation zones, since its response is independent of the orientation of the satellite

A fuller description of the *Injun I* system is given in the previously cited paper of O'Brien *et al* [5]

Some General Considerations on Geomagnetic Trapping

The dynamics of geomagnetically trapped particles as recently reviewed by Van Allen[6] are applicable to the particles injected by the Johnston Island burst In particular, it is necessary to use the fully detailed properties of the real geomagnetic field in order to make a systematic study of spatial distribution and time-decay The co-ordinate system of McIlwain[7] is used here to replace the three geographic co-ordinates longitude, latitude and altitude with the two 'natural' geomagnetic co-ordinates B and L

The value of L is constant for a given line of magnetic force along which a charged particle oscillates in latitude It also labels a unique magnetic shell on which the particle drifts in longitude Numerically, L is such that if the geomagnetic field were that due to a simple dipole, then the equatorial radial distance from the centre of the Earth to a given magnetic shell would be L Earth radii The strength of the magnetic field at a point in space is B gauss

In a time-stationary state, at any two points in space with the same values of B and L the intensity of trapped particles is the same In the equatorial regions at *Injun* altitudes of $\sim 1,000$ km there are trapped the particles of the inner radiation zone which is relatively stable with time During the first year of observations by *Injun I*, some ten million measurements were made in the inner zone Hence the counting-rates of all detectors due to naturally occurring trapped particles are well known and are tabulated in B, L co-ordinates The intensities of the natural inner radiation zone as it was before the burst are used in this article as reference-levels As will be seen in the following, the intensities due to the artificially injected particles are much greater than normal intensities in the inner zone, especially at the lower altitudes This fact must be clearly recognized by those conducting flights in this region during 1962 Furthermore, because of it, we do not treat in this article any possible effects of the burst on the former inner zone particles themselves

The dependence on B and L of particle intensity in the inner zone is shown in Figs 5 and 6 of ref 7 using data from detectors on the satellite *Explorer IV*, which also observed the artificial belts of electrons resulting from the three *Argus* bursts[4] The shielded Geiger tube on *Explorer IV* had shielding significantly less than that of *Detector* 2 above and had an effective area about five times larger than that of *Detector* 2

Before the Johnston Island burst, the counting-rates of *Detectors* 1, 2 and 3 in the equatorial region were dominantly due to inner zone electrons, protons and protons, respectively After the event, there are three possible contributions (*a*) from penetrating electrons from fission decay, (*b*) from decay products of neutrons produced in the explosion, (*c*) from a redistribution in space of naturally occurring trapped particles The observed spectrum in equatorial regions is in crude accordance with the assumption that the dominant contribution is from penetrating fission-decay electrons

As to contribution (*b*), according to Latter, Herbst and Watson[1] about one neutron per fission escapes from a nuclear burst The subsequent neutron-decay injection process yields a more-or-less uniform 'source function' of electrons with $E \lesssim 780$ keV and energetic protons mostly with $E \sim 1$ MeV over an immense region of space By contrast, there is a comparable number of fission-decay electrons, which come from a localized 'source function' and are much more energetic and penetrating In this article we consider only the equatorial regions where the localized 'source function' of fission-decay electrons is large In this region it can be shown that neutron-decay products would have had less effect on *Detectors* 1, 2 and 3 than would the naturally occurring particles of the inner radiation zone

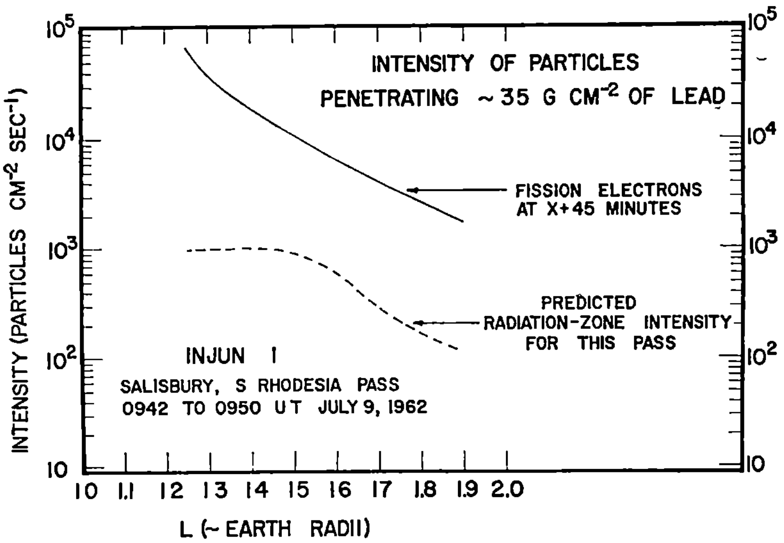

Fig. 1. Comparison of the intensity of the new artificial radiation belt and the natural inner radiation belt measured with *Detector* 2 in the same region over Southern Rhodesia. In calculating the intensity, the assumption is made that the detector is efficiently counting only fission electrons with $E \gtrsim 6$ MeV (see text)

As to contribution (*c*), the pre-burst peak counting-rate of *Detector* 2 at *Injun* altitude was only about 700 counts sec^{-1}, and its extrapolated rate in the heart of the inner zone was only about 1,200 counts sec^{-1}. Yet it reached rates of more than 11,000 counts sec^{-1} after the burst, and it seems clear that such rates could not have resulted from any reasonable redistribution of naturally occurring trapped particles.

Therefore, it is tentatively assumed that the dominant contribution to *Detectors* 1, 2 and 3 in equatorial regions after the burst was from penetrating electrons from fission decay. The validity of this assumption and its applicability at higher latitudes are being further examined.

Early Effects of the Explosion

Injun I was at longitude 224° E., latitude 36° N. and altitude 886 km and was moving north-east at the nominal explosion time of 0900 U.T. on July 9, 1962. We call this time $t = X$ h. *Injun* was not transmitting at the time.

Injun was transmitting over Salisbury, Southern Rhodesia, at $X + 45$ min. Thus it raced the slow electrons eastward around the world, and only electrons with energy $E \gtrsim 500$ keV would have drifted around from the explosion to Salisbury by the time of the pass (Figs. 1.2 and 1.3 of ref. 6 for longitudinal drift period as a function of energy).

Table 2. EARLY MEASUREMENTS OF PARTICLE INTENSITIES (ASSUMING THAT ONLY ELECTRONS ARE PRESENT)

	Detector 1 Intensity electrons $E \geqslant 40$ keV particles (cm^2 sec sterad)$^{-1}$	*Detector* 2 Intensity electrons $E \geqslant 6$ MeV particles (cm^2 sec)$^{-1}$	*Detector* 3 Intensity electrons $E \geqslant 1$ MeV particles (cm^2 sec sterad)$^{-1}$
$X + 45$ min			
(*a*) Normal to $\vec{B}$ at $L = 1.32$	3×10^7	3.5×10^4	$\sim 10^7$ (order of magnitude estimate)
(*b*) Peak intensity at $L = 1.26$	—	6.5×10^4 (see Fig. 1)	—
$X + 100$ min			
(*a*) Normal to $\vec{B}$ at $L = 1.35$	2×10^6	2×10^2	—
(*b*) Peak intensity at $L = 1.13$	—	2.5×10^3	—

By $X + 45$ min there was a shell of trapped electrons extending out to at least $L \sim 1.9$ over South Africa (Fig. 1).

The satellite was also transmitting over Johnston Island at $X + 100$ min and so it arrived there ahead of any electrons with energy $E \lesssim 500$ keV which were drifting around the world, but it would have started to overtake lower-energy electrons after passing Johnston Island.

In these two passes, the directional *Detectors* 1 and 3 were not pointing at right angles to the magnetic field at the times when the omnidirectional *Detector* 2 indicated that the particle intensity was at its maximum. In the intensity measurements listed in Table 2 we quote:

(*a*) Intensities measured by *Detectors* 1 and 3 when they were pointed perpendicular to the magnetic field, that is, when they were measuring the peak directional intensity of particles at that place and time. The intensity measured by the omnidirectional *Detector* 2 at the same place and time is also given. From such data the electron energy spectra may be obtained. Then

(*b*) The peak intensity observed by *Detector* 2 during the same pass is listed. The spectral data of (*a*) may then be normalized to the peak intensity of (*b*) if it be assumed that the electron spectrum is the same at the two locations. This matter is being investigated in detail.

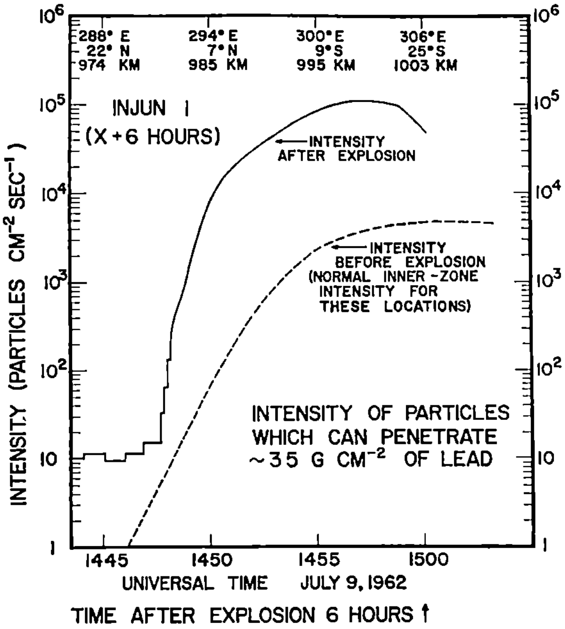

Fig. 2. Comparison of the observed particle intensity after the explosion with the normal zone intensity for a pass over South America. The locations shown are in geographic co-ordinates

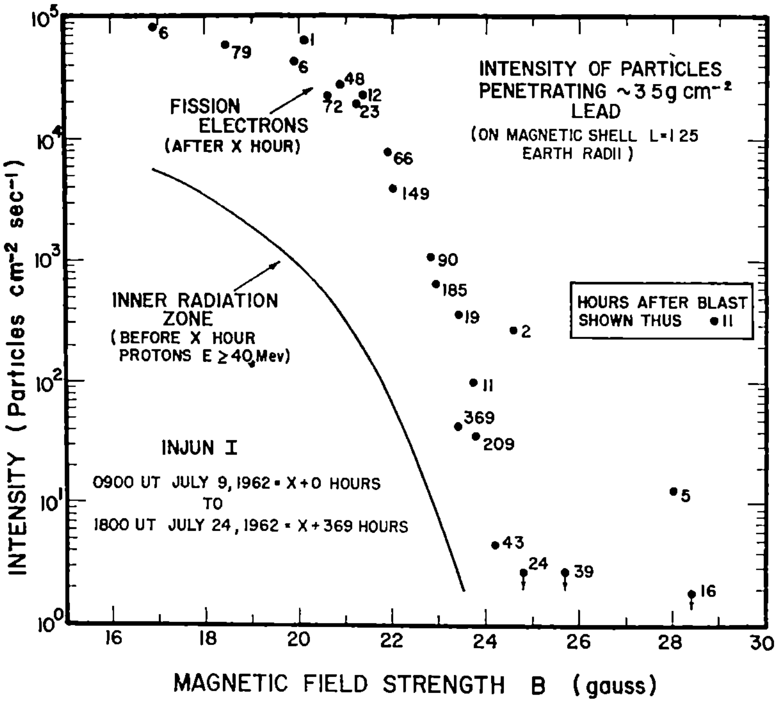

Fig. 3. Variation of particle intensity on the magnetic shell $L = 1·25$ Earth radii as a function of magnetic field strength (B) and the elapsed time since the explosion. The intensity decreases most rapidly at large values of B, that is, at low altitudes. A measurement obtained very recently and not plotted was an intensity of ($4·3 \times 10^4$) particles cm^{-2} sec^{-1} at $B = 0·192$ gauss at ($X + 459$) h. Note that this was after the moderate magnetic storm of July 26

The lower intensity over Johnston Island than over Salisbury is due presumably to the fact that a given geographical altitude over Johnston is equivalent, geomagnetically, to one several hundred kilometres lower over Salisbury. The decay of intensity in both the short term and the long term is dominated by the atmospheric density at the longitude at which the mirror points of particles are deepest in the atmosphere (see later). The dependence of particle intensity at a given value of L and at low altitudes is an extremely strong function of B for both the natural and artificial radiation zones for $L \lesssim 1·5$. For example, in the normal inner radiation zone the particle intensity changes by an order of magnitude over an altitude change of less than 100 km.

In Fig. 1, the enhancement after the explosion of the intensity of particles which could penetrate more than 3·5 g cm^{-2} of lead is clearly shown for Salisbury at $X + 45$ min. The enhancement is about one hundred-fold around $L \sim 1·2$. Over Johnston Island the enhancement near the peak of the artificial zone around $L \sim 1·13$ was more than one thousand-fold.

From data such as those shown in Figs. 1, 2, 3 and 4, it is estimated that there were of the order of 10^{24} freshly injected and trapped electrons of energy $E \geq 1,500$ keV in the geomagnetic field at $X + 1$ h. The total mass of these particles which have created this intense radiation belt is therefore only of order 1 mg.

Long-Term Observations

There is considerable interest in a detailed observational study of the time decay of the artificial radiation belt over a period spanning both magnetically quiet and magnetically disturbed conditions. Such a study is in progress. The moderate magnetic storm of July 26, 1962, did not have any pronounced effect, but further studies with other storms must be made. The results may be expected to be of value in determining the loss-rates of naturally injected particles and hence the source strength for such particles. Hopefully, some of the uncertainty concerning the origin of the inner zone can be dispelled.

In this preliminary report, the rate of loss of electrons of $E \gtrsim 6$ MeV only is considered, and this briefly. Data from a single pass are shown in Fig. 2. From many such passes, the particle intensity on a given magnetic shell, say $L = 1·25$ as in Fig. 3, is plotted as a function of B. The time-dependence of intensity at chosen sets of values of B and L can be

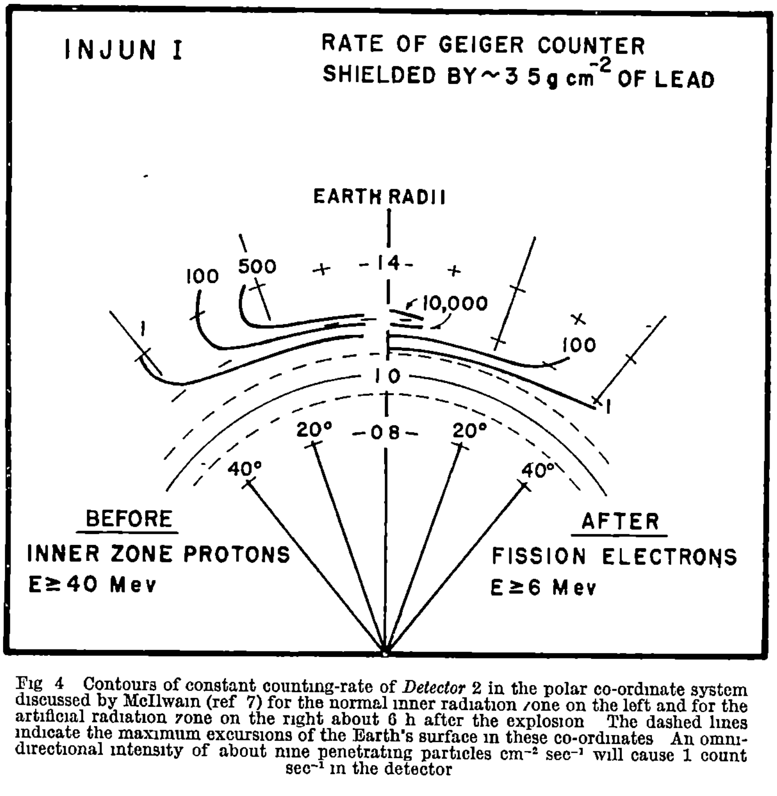
INJUN I
RATE OF GEIGER COUNTER SHIELDED BY ~3·5 g cm^{-2} OF LEAD
EARTH RADII
100
500
10,000
100
1
1
1·4
1·0
0·8
20°
20°
40°
40°
BEFORE
INNER ZONE PROTONS E≥40 Mev
AFTER
FISSION ELECTRONS E≥6 Mev

Fig. 4. Contours of constant counting-rate of *Detector* 2 in the polar co-ordinate system discussed by McIlwain (ref. 7) for the normal inner radiation zone on the left and for the artificial radiation zone on the right about 6 h after the explosion. The dashed lines indicate the maximum excursions of the Earth's surface in these co-ordinates. An omnidirectional intensity of about nine penetrating particles cm^{-2} sec^{-1} will cause 1 count sec^{-1} in the detector

examined with the help of such graphs. For the magnetic shell $L=1·25$, the minimum altitudes at which $B=0·20$, 0·22 and 0·25 gauss are respectively about 540, 350 and 140 km. Hence from Fig. 3 it appears that, for this magnetic shell and this phenomenon, the intensity of electrons with $E\gtrsim 6$ MeV which mirrored as low or lower than 140-km altitude became negligible (that is, became undetectable with this instrument) within a few hours. The intensity of those mirroring at or below 350 km was greatly reduced within a few weeks, while high intensities of those mirroring above 500 kilometers will persist for months. A proper treatment of the problem using transport theory and the combined results from all detectors is being undertaken.

Comparison with *Argus* Series

Three fission devices of about 1 kiloton yield were detonated by the United States at high altitudes in August–September 1958. The resulting artificial radiation belts were less than 0·1 Earth radius thick at the equator and had peak intensities at about $L=1·7$, 2·1 and 2·2 Earth radii respectively[7].

A preliminary comparison of the shielded Geiger tube data (*Explorer IV*) on the *Argus* shells with the present data from *Detector* 2 in *Injun* shows that the initial intensity in the Johnston Island shell was of the order of one thousand times as great as that observed by *Explorer IV* on the *Argus* shells. Because *Explorer IV* sampled the *Argus* shells only at altitudes less than about 2,000 km and so considerably below their equatorial altitudes, it did not measure the peak *Argus* intensities. However, *Injun* samples the artificial radiation belt near the equator and hence near the peak intensities. A detailed comparison of the phenomena is being made.

Discussion

It appears likely that the electrons artificially injected by the Johnston Island burst will continue to be present in measurable numbers for many months on the higher-altitude shells.

As a consequence, the electron spectrum and intensity at high altitudes for magnetic shells with $L\lesssim 2$ will differ from those of the previously studied inner radiation zone for some months. A short tabulation in terms of B and L of the electron intensities now in this region has been prepared and circulated privately.

Perhaps the best overall view of the artificial radiation belt as available at present is obtained by examination of Fig. 4. One should note in particular the low altitudes to which the belt extends, and the counting-rates very much higher than those observed in the natural inner belt with the same detector. For comparison also, a similar detector has a peak counting-rate of only about 100 counts sec^{-1} in the heart of the outer radiation zone.

Invaluable telemetry reception at equatorial stations was obtained through the co-operation of Roger Tetrick of the Goddard Space Flight Centre, Rolf Dyce and George Johnson of the Stanford Research Institute and W. S. Carey of Salisbury, Southern Rhodesia.

This work was supported in part by the Office of Naval Research under contract *N9onr*93803 and by the National Aeronautics and Space Administration under grant Nos. 9233–62.

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RADIOCARBON DATING

FIFTH INTERNATIONAL CONFERENCE

By Prof. H. GODWIN, F.R.S.
University of Cambridge

IN November 1954 the first representative international symposium on Radiocarbon Dating was held in Copenhagen and reported in *Nature*[1]. It was followed by others in Cambridge, Andover, Mass., and Groningen. The series has been continued by the organization of a fifth conference in Cambridge during July 23–28, under the ægis of the University Sub-department of Quaternary Research. By the courtesy of the respective governing bodies, the members were largely housed in Clare College and St. John's College, Cambridge, and meetings were held in the University Departments of Botany and Geography.

More than thirty laboratories were represented, and a large number of communications were made to the meeting by research workers from very widely separated parts of the world. We at Cambridge were very happy to welcome for the first time colleagues from the newly established Carbon Dating Laboratories in Poland and Yugoslavia.

Although there is a strong tendency to the general use of proportional gas counters, usually with a filling of carbon dioxide, it is apparent that alternative methods have by no means been totally neglected. Prof. H. R. Crane (Ann Arbor) described his considerable experience on the successful use of carbon bisulphide counters operating in the Geiger region. Representatives from the Centre d'Etudes Nucleares de Saclay (France) described their very successful development of a scintillation-counting technique. As one might have anticipated at this stage in the exploitation of the technique several contributors described their own experiences of factors leading to error in the carbon-dioxide proportional counting system, and the devices which could be used for obtaining maximum efficiency and stability, including particularly devices concerned to reduce the level of the background. Consideration was given to the theoretical consequences of counting at higher pressures than are usual, the possibility of the use of epoxy resins in counter construction and the modern improvements in the techniques of conversion of carbon dioxide to methane for use as a counting gas. The Conference listened to an account of the development at Zagreb of a method of radiocarbon assay by means of a carbon-dioxide filled ionization chamber.

The conference heard with great interest the reports of three different laboratories—Uppsala, the U.S. National Bureau of Standards, and Aldermaston, on their redeterminations of the half-life of radiocarbon. In each instance the technique used was based on mass spectrometry in combination with activity measurements in a gas proportional counter (supplemented, in one instance, by Geiger counter measurements). The three laboratories produced very similar results, which will be separately published. For the purposes of the conference it was agreed that they could conveniently be taken to indicate a best new working value of 5,730 ± 40 years. The effect of these redeterminations on the the best method of calculating and presenting radiocarbon ages was fully discussed by the Conference, with the results that are conveyed in a separate communication (see p. 984 of this issue).

Uncertainty as to the correct value for the half-life of radiocarbon is, of course, not the only unevaluated source of error in carbon dating. The method depends on the assumption that the initial radiocarbon activity of the atmosphere has been constant in the past and so far as this may be proved incorrect the method will need refinement in its application. Several laboratories have in consequence concerned themselves with activity measurements of material of known age. One important source of such material is the annual rings of trees of great age, such as redwoods and bristle-cone pines of North America and the kauri of New Zealand. The laboratories at Tucson, Pennsylvania, La Jolla, Seattle and Lower Hutt all reported measurements of this kind. Their results seem to confirm the reality of past deviations from the average atmospheric activity such as have been earlier indicated by the work of de Vries[2] and of Willis, Tauber and Munnich[3]. There seems to be some convergence of evidence of the reality of a maximum of atmospheric activity round about A.D. 1500, but there was a considerable lack of agreement between the results obtained on kauri from the southern hemisphere and those obtained from the northern hemisphere. In the discussions which followed the presentation of these results, emphasis was placed on the necessity for adequate dendrochronological assessment of the tree ring samples, and this was most evident in respect of the extremely eccentric growth of the high altitude bristle-cone pine *P. aristata*. A further source of material of known age lies in dated archæological material, particularly from Egyptian sources, the most systematic investigation of which is that being pursued in the University of Pennsylvania. There is still some discrepancy between the archæological dates and the radiocarbon determinations but the significance of the difference cannot yet be determined.

Underlying the question of the constancy of radiocarbon activity in the atmosphere, are the problems of transfer between stratosphere, troposphere and the ocean reservoirs, and several laboratories have been making use of the high concentrations of radiocarbon injected into the stratosphere by recent atom bomb tests to measure the attributes of this system. Laboratories at Los Angeles and Cambridge reported the results of preliminary sampling by aircraft from the lower stratosphere and upper troposphere, and other laboratories reported on the seasonal variations of bomb radiocarbon in the atmosphere. The Laboratories at Lamont and La Jolla reported similarly on radioactivity determinations of surface and deep water in the oceans of the world, and the inferences that these permitted regarding the turnover and exchange problems of the ocean.

The attention of the Conference was naturally directed also to a series of geochemical problems which are associated with the circumstances of deposition and preservation of material afterwards sampled for radiocarbon activity. The radiocarbon activity in certain types of meteorites was reported on as yielding no evidence of terrestrial origin. The results were also described of attempts to determine the suitability for radiocarbon age determination of snail shells from American loess deposits and of laboratory experiments based on feeding active carbon to laboratory snails. Prof. E. S. Deevey reported an extremely interesting examination of limnological problems by studying the isotopic fractionation in lakes and produced a tentative balance sheet for the carbon circulation of the ecosystem of the much-investigated Lindsey Pond. An account was given of the attempt to use determinations of the radiocarbon activity of the organic (non-carbonate) content of streams in North America to provide preliminary information on the sources of domestic, industrial and agricultural pollution of rivers. There were also reported the results of experiments to discover the extent to which woods and charcoals might take up in natural conditions contaminant carbon which could not be removed afterwards by laboratory treatment.

The concluding day of the Conference was occupied by consideration of two of the major fields of application of radiocarbon dating, namely, geological climatic phenomena and the archæological problems associated with the Neolithic culture and the spread of agriculture. From very high altitudes (5,000 m) in the Sierra Nevada of Colombia, Prof. R. F. Flint reported the early stages of inquiries comprising glacial geology, palynology and radiocarbon dating that promised ultimate correlation with European and North American events. Dr. M. Stuiver described the dating of the alternating salt and mud deposits of Searles Lake in the south-western United States, indicating an alternation of dry–warm as against cool–wet climatic episodes extending some 50,000 years back into the last (Wisconsin) glaciation. Dr. W. S. Broecker reported a close re-examination and re-dating of the famous Two Creeks site, where a forest bed beneath till has been generally thought to correspond with the mild Allerød oscillation in Europe. He produced evidence to suggest the possibility that it might alternatively be referred to the preceding Bølling oscillation. Two further contributions from Dr. M. Stuiver and Dr. I. Olsson concerned themselves with the dating of isostatic uplight in regions recently relieved from the burden of ice, namely, north-east Greenland and Spitsbergen. Dr. S. Jelgersma described the close and effective co-operation between the Geological Survey of the Netherlands and the Carbon Dating Laboratory at

Groningen in the resolution of the complex history of the progressive submergence of the Netherlands, an inquiry made particularly urgent by the need to comprehend the causes and nature of the marine incursions still being suffered in Holland The investigation of submerged peats and raised beaches in the British Isles by the Cambridge Sub-department of Quaternary Research was shown to lead to the conclusion that whereas the south-west was relatively stable, there had been isostatic uplift in the north and, in all probability, continuing tectonic subsidence in regions adjacent to the southern North Sea

Prof J G D Clark introduced a discussion on the spread of Neolithic civilization across Europe from the Middle East, stressing our changed conceptions of the absolute dating and of the routes of migration of the culture Dr H Barker indicated the nature of those British Museum dates bearing on the Neolithic period, and Dr A G Smith described the results of pollen analyses at very close intervals through two forest clearance phases at a site in Northern Ireland which had been closely carbon-dated by Dr E H Willis, the first clearances occurred before 3,500 B C and the whole episode of clearance, occupation and regeneration was shown to have occupied about 400 years, a period consistent with the ecological explanation Dr J D van der Waals described the elegant and thorough analysis of dates from the Neolithic of the Netherlands, showing among other things that within so short a time as 300 years there had been a remarkable diversification of cultural types of the Bandkeramic culture in that country Dr F E Zeuner described the significance of the British Mesolithic dates at Oakhanger, Surrey, and Torbryan in Devonshire, and commented on the Neolithic land surface of the Essex coast

A working conference of this kind has a particularly important co-ordinating function to discharge in securing the adoption of standards, furtherance of international co-operation in research, and adequate and proper publication of results The earlier conferences had already led to the annual publication of *Radiocarbon* and the constitution of a scheme for punched-card indexes of all published radiocarbon dates

It was now agreed further to support publication of a *Cumulative Index* to all radiocarbon dates published prior to the first volume of *Radiocarbon*, to seek every means to secure that all radiocarbon dating laboratories shall publish definitive date lists in *Radiocarbon*, and to secure as wide a circulation for that journal as possible The conference further urged on all authors the practice of quoting station index numbers alongside all radiocarbon dates mentioned in scholarly articles and texts

The decision not for the time being to adopt a new half-life value for radiocarbon age calculation, and to maintain statement of ages in years B P , was not reached without much discussion and some dissension, but the majority view is expressed in the communication on p 984 of this issue of *Nature*

The formal business of the conference was broken pleasantly and very instructively by a visit to the Research Laboratory for Archæology and the History of Art at Oxford, where Dr E T Hall, Dr M J Aitken and their colleagues demonstrated the variety of physical and chemical techniques now used in archæometry The apparatus in the Department of Geology for potassium–argon age determination was also demonstrated to us by courtesy of Prof L Wager Members of the Conference naturally made occasion to visit the Cambridge Radiocarbon Dating Laboratory under the guidance of Dr E H Willis, and several informal discussions on active topics were organized outside the conference programme

We owe very warm thanks for financial support to the Royal Society, the University of Cambridge, and for accommodation and hospitality to the University and the Departments of Botany and Geography We also thank our scientific colleagues and laboratory staffs in Oxford and Cambridge for all their assistance, and most particularly Dr E H Willis and Mr R Burleigh, who bore the main burden of organizing the meeting

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NEWS and VIEWS

The High-Altitude Nuclear Explosion of July 9

THE U S Atomic Energy Commission and the U S Defence Department have announced that the new radiation belt created by the American high-altitude nuclear explosion on July 9 is stronger than was originally thought, and may persist for several years (see also articles by Prof J A Van Allen *et al* and Sir Bernard Lovell on pp 939 and 935 respectively of this issue of *Nature*) It was already known that the new radiation had stopped transmissions from the British satellite *Ariel* and now, on September 1, it was announced that transmissions ceased from two American satellites

It is to be hoped that those having political and/or military authority in the United States and elsewhere will take note of these serious events Although the high-altitude explosion was, for the military authorities, a legitimate test of a military situation in that they were interested in the ionospheric effects which would have been short-lived and not particularly damaging to fundamental scientific progress, it must now be recognized that a consequential and quite unintentional effect of the explosion has proved to be the trapping of the radiation in zones around the Earth

There can be no doubt that both laymen and scientists are becoming more and more worried at what appears to be grave decisions concerning upper atmosphere and space research being made mainly for military advantage or political prestige Now is the time to question the desirability of so much scientific research coming directly under the sanction of Governmental or military authorities, for it is conceivable that many scientists are unable to criticize too objectively, since they depend on political and other authorities for financial support for their work Indeed, many scientists are directly employed by Governments, and they are in no position to express concern or raise objections This all takes us back to the many times (the most recent in *Nature* of August 25, 1962) that we have pointed out the grave danger inherent in allowing science and technology to become involved in power politics

The D. and F. Guggenheim Medal for Astronomy: Prof. J. A. Van Allen

THE Daniel and Florence Guggenheim International Medal for Astronomy has, for 1962, been awarded to Prof. J. A. Van Allen, director of the Department of Physics and Astronomy in the State University of Iowa (see also p. 939 of this issue). This prize, of the value of 1,000 dollars, is awarded each year by L'Académie Internationale d'Astronautique de la Fédération Internationale d'Astronautique to a person who has made outstanding contributions to the progress of astronomy over the past five years. Last year this prize was awarded to Sir Bernard Lovell, director of the Nuffield Radio Astronomy Laboratories at Jodrell Bank.

The Marconi Co. Chief of Research: Mr. G. D. Speake

MR. G. D. SPEAKE has been appointed chief of research of the Marconi Co. He is now responsible to the Director of Research for the execution of research policy, and to the general manager for the overall administration of the Marconi Laboratories at Great Baddow, Chelmsford. This appointment follows that of Dr. E. Eastwood, the company's director of research, to the post of group director of research for the English Electric Group of Companies, of which Marconi's is a member. Mr. Speake joined Marconi's Wireless Telegraph Co., Ltd., in 1950, when he was engaged in radar systems research. In 1954 he was appointed chief of the Vacuum Physics Section of the Research Division, and two years later became chief of the Microwave Physics Group. He has been deputy chief of research since 1960. Educated at Adams Grammar School, Newport, Shropshire, and St. Catherine's College, Cambridge, Mr. Speake graduated with first-class honours in physics. Prior to joining the Marconi Co. he was instrument manager of the Plastics Division of Imperial Chemical Industries, Ltd., during 1947–50.

Electrical Engineering at Saskatchewan: Prof. A. D. Booth

DR. A. D. BOOTH, head of the Department of Numerical Automation and reader in computational methods at Birkbeck College, University of London, has been elected to the chair of electrical engineering at the University of Saskatchewan. He leaves for Canada in September. Dr. Booth came to Birkbeck College in 1946, after a period at the University of Birmingham, during which he conducted research into methods of X-ray crystal structure analysis. The large-scale calculations involved in this aroused his interest in the design of computers and, after a period of study with von Neumann at Princeton on a Rockefeller fellowship, he returned to Birkbeck College and produced the first working computer with a magnetic drum store—the *ARC*, completed in 1949. This led to a later all-electronic machine, the *APE(X)C*, which formed the basis of one of the earliest successful computers, the *Hollerith 1201*. While in the United States, in 1947, Dr. Booth was responsible for the suggestion that the translation of languages could, in principle, be undertaken by a computer. Later he conducted pioneer experiments in this field using punched-card apparatus and, with a group of active young workers, including L. Brandwood, J. Cleave and others, produced programmes for the automatic transcription of Braille and for the machine translation of technical material. Dr. Booth's recent activities have included computer applications in law and in medicine as well as in translation and in classical numerical analysis. As chairman of the committee which set up the British Computer Society as well as a member of Council of several learned societies, he has also been actively associated with the Institute for Aviation Medicine, H.M. Underwater Defence Establishment and International Computors and Tabulators, Ltd. Accompanying Dr. Booth to Canada will be his wife (Dr. K. H. V. Booth) and their two children, to all of whom go the best wishes of their many friends and colleagues in Britain.

The Nuffield Institute of Comparative Medicine: Dr. L. G. Goodwin

DR. LEONARD G. GOODWIN has been appointed the first director of the Nuffield Institute of Comparative Medicine, which is being built by the Zoological Society of London in its Gardens at Regent's Park. Dr. Goodwin will take up his appointment on January 1, 1964; before that date he will act as consultant in the planning and launching of the Institute. The Institute was founded in 1960, with the aid of a grant from the Nuffield Foundation, in order that full use could be made, for purposes of comparative medical research, of the pathological and other material derived from the species of animals in the Zoological Society's Collections. Since 1958 Dr. Goodwin has been head of the Wellcome Laboratories of Tropical Medicine, having been a member of the staff since 1939. His research has been directed towards the discovery of new substances for the treatment of tropical and other parasitic diseases in man and animals, during the course of which he has conducted numerous expeditions to tropical areas.

Harwell Variable Energy Cyclotron

A NEW azimuthally varying magnetic field cyclotron to be constructed at Harwell will provide both internal and external beams of particles at high intensities and varying energies. External beam currents and maximum energies are expected to be 100 μamp for protons (50 MeV), deuterons (25 MeV) and helium ions (50 MeV), and several μamp for a variety of heavier ions at energies up to 10 MeV per nucleon. The machine, which is similar to others recently constructed at Berkeley and at Oak Ridge, will have a 200-ton magnet with 70-in. diameter pole faces; spiral ridges (3 per pole) will be used to shape the field to enable the higher proton energies to be attained. Extensive field surveys will be made, initially with model magnets, followed by a detailed survey when the magnet is installed late in 1963. The field will be variable from 6 to 17 kG and the frequency of the dee voltage over a three-fold range. The machine will be used principally for research in the fields of radiation chemistry, radiation damage and nuclear chemistry, and will be available for use by universities. It will aid the study of radiation effects at high rates of energy deposition, and of radiation damage involving changes in a large proportion of the sample, or where an individual atom suffers several successive displacements. The Rutherford Laboratory of the National Institute for Research in Nuclear Science is responsible for the design and construction of the machine, which will be operated by the Atomic Energy Research Establishment. The building and ancillary services are the responsibility of the Authority's Southern Works Organization, clearance of the site began on August 16 and the machine is expected to operate in 1965 The total cost of machine and building is estimated to be £1,600,000

The Institute of Physics and the Physical Society

THE second annual report of the Council of the Institute of Physics and the Physical Society, presented to and adopted at the annual general meeting on July 10, records a total membership in all grades of 9,120—an increase of 400 on the previous year (Pp 24 London The Institute of Physics and the Physical Society, 1962) The number of candidates entering for the graduateship examination in 1961 greatly exceeded that expected and of the 239 candidates 64 were successful The success of the appeal for the Physics Trust Fund made in 1960 has enabled the work directed at improving the teaching of physics to be increased greatly and some £25,000 a year is now spent on educational work At the request of the Royal Society, the Council appointed a British Committee on Physics Education, with Sir Nevill Mott as chairman, to consider all aspects of physics education in Britain A committee, under the chairmanship of Prof R V Jones, was appointed to prepare a report on "Problems facing University Physics Departments" and as part of its task has prepared evidence for the Council to submit to the Robbins Committee on Higher Education (see *Nature*, 193, 911, 922, 1962)

Units and Standards of Measurement used at the National Physical Laboratory

THE units and standards of measurements used at the National Physical Laboratory, Teddington, are described in the series of booklets entitled *Units and Standards of Measurement Employed at the National Physical Laboratory* (1 *Length, Mass, Time-Interval and Frequency-Angle, Volume, Density and Specific Gravity, Gravity, Force Pressure and Hardness* 2 *Light* 3 *Electricity* 4 *Temperature* London H M S O 1*s* 6*d* net each) A completely revised third edition of the first booklet, which deals with length, mass, time-intervals and frequency, has recently been published (1962) The fundamental mechanical units of measurement together with angle and the derived units of volume, density, force and pressure in both the International System of Units and the yard/pound system are discussed, and the standards and bases of measurement used in work involving the units are indicated In addition, the absolute value of the acceleration due to gravity at the Laboratory and the measurement of hardness are briefly dealt with The most up-to-date definitions of the metre, yard and pound are given The second booklet in the series, *Light*, published in 1956, deals with units and standards concerned with photometry, colorimetry and radiation, and the third, *Electricity*, published in 1960, describes the electrical units and the standards by means of which the electrical units are determined and preserved The fourth booklet, *Temperature*, published in April this year, is an English translation of the full text of the International Practical Scale of Temperature adopted by the International Committee of Weights and Measures in May 1960 It is emphasized that the scale is not a revision of the 1948 scale but only an amended edition of the same scale, the numerical values of temperatures remaining the same as in 1948 The departures of the 1960 text from that of 1948 are summarized separately in an appendix

Teddington Products Directory

A NEW directory of products is available from Teddington Autocontrols Ltd , Sunbury-on-Thames, Middlesex (*Products Directory* Pp 24 Sunbury-on-Thames Teddington Autocontrols, Ltd , 1962) Sectionalized under appropriate headings, the publication describes the complete range of automatic control instruments manufactured by the Company Each instrument is accompanied by the relevant data sheet

Scientific Research in France

THIS comprehensive reference work on scientific laboratories in France, first issued five years ago, is arranged under international establishments, then under the academic region of Paris, followed alphabetically by those of the several provinces (Office National des Universités et Écoles Françaises *Répertoire des Laboratoires Scientifique* Pp 578 Paris Services d'Édition et de vente des Publications de l'Éducation Nationale, 13 rue du Four) The international establishments comprise the European Organization for Nuclear Research and the European Community for Nuclear Energy (Euratom), and besides names of directors and heads of divisions the information given includes numbers of research workers, scope of present work and lists of recent publications Information given for the Academic Region of Paris includes first the professors and directors in the Faculty of Science of the University and in other university departments, then the staff of the National Centre for Scientific Research, followed by corresponding information on the establishments of the Prime Minister and of the other Ministries Some information on publications is included For each of the provinces the information is arranged similarly, that for the Faculty of Science being given first, followed by information for other scientific establishments There are an abridged index of scientific staff and alphabetical indexes of subjects and equipment

Population Statistics for England and Wales . First Quarter for 1962

THE Registrar General's *Quarterly Return for England and Wales—Births, Deaths and Marriages, Infectious Diseases, Weather, Population Estimates, quarter ended 31st March 1962*, shows that the increase in births is not slackening (No 453, 1st Quarter 1962 Pp 28 London H M S O , 1962 2*s* 6*d* net) 220,710 births were registered in the first quarter, giving a crude rate of 19 4 per thousand population, as against 17 9 in the previous quarter If this increase were to continue the number of births in 1962 is likely to be between 850,000 and 875,000, that is, nearly as large as in 1947 It is likely that an increase of this size is an indication of rising marital fertility the number of births having climbed steadily, while the number of marriages has remained constant Infant mortality at 23 1 is an all-time low for a first quarter Mortality for the first quarter of 1962 shows no noticeable difference from 1961

Educational Services Incorporated

EDUCATIONAL Services Incorporated (ESI) is a private non-profit corporation devoted to research and development in education and is supported largely by grants from private foundations and the Federal Government of the United States The Corporation has grown out of the activities of the Physical Science Study Committee and has developed along two distinct lines. The first line stems directly from the Physical Science Study Committee's high-school physics course and involves further explorations in the area of curriculum reform in the United States and abroad. An example of this is the Corporation's elementary science programme. The second type of activity undertaken by Educational Services Incorporated is represented by a group such as the National Committee for Fluid Mechanics Films, an autonomous organization operating under the ægis of the Corporation and using its facilities. While funds for the support of such programmes are in most cases granted to the Corporation the groups themselves determine their own policies and operate independently. Educational Services Incorporated has now launched its first *Newsletter,* which will appear at frequent intervals (1, No. 1, May 1962). It contains accounts of its work on improving curricula as well as a detailed description of the conference held at Accra during December 17–21, 1961, to examine ways in which the teaching of mathematics could be improved and extended. *ESI Newsletter* is published from 164 Main Street, Watertown, Massachusetts.

Cretaceous Cribrimorph Polyzoa

The first monographs devoted to the strikingly beautiful Cretaceous Cribrimorph Polyzoa were published in 1921 and 1922 by Dr. W. D. Lang, who considered that the concept of orthogenesis explained their evolution. His ideas were accepted by some but challenged by others, both at that time and afterwards. In a well-illustrated work, Dr. G. P. Larwood has re-examined the sub-family Pelmatoporinae (*The Morphology and Systematics of Some Cretaceous Cribrimorph Polyzoa (Pelmatoporinae), Bull. Brit. Mus. (Nat. Hist.), Geol.*, 6, No. 1. Pp. 1–285, pls. 1–23. London: Brit. Mus. (Nat. Hist.), 1962). The material dealt with is part of two large collections made by Mr. C. T. A. Gaster, but specimens in other collections especially that of Mr. R. M. Brydone, were also examined. As a result, Dr. Larwood has reached several conclusions different from Dr. Lang's, notably on the nature of the oral spines, pelmata and pelmatidia, which, in fact, he believes to be the same as the comparable structures in Recent Polyzoa. With the wealth of material available to him, he has also merged a few genera and several species, the ranges of which have thus often been extended. Naturally, the evidence of evolution in the group is affected, and Dr. Larwood concludes that, instead of progressing along confined and determinate, distinct lines, it has taken place in a diverging plexus of variation.

Health Laboratory Services

One of the essential aims of public health in the developing countries should be the co-ordination of all types of laboratories at different levels into a single national health laboratory service that would form an integral part of the national health service. Such a system, in which clinical and public health laboratory activities are closely associated and placed under one technical direction, presents great advantages from the point of view of economy and efficiency. In its third report the World Health Organization Expert Committee on Health Laboratory Services sets out a series of recommendations on the ways and means of achieving this integration, and thus offers guide-lines for developing countries wishing to improve their health laboratory organization. (*Technical Report Series* No. 236: *Planning Organization and Administration of a National Health Laboratory Service*. Pp. 46. Geneva: World Health Organization; London: H.M.S.O., 1962. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars.) The report opens with a description of the experience of developed countries in establishing a network of health laboratory services, and then defines the scope and functions of such services—microbiological, entomological, hæmatological, pharmaceutical and epidemiological, among others. This is followed by a full account of the many factors involved in the planning, organization and administration of a national health laboratory service in the context of long-term development programmes and in relation to the needs, health problems and resources of the country concerned.

Announcements

Dr. G. H. Metson, deputy director of research at the Post Office Research Station, Dollis Hill, London, has been awarded the Premium of the Institution of Electrical Engineers (valued at £50) for his papers "The Conductivity of Oxide Cathodes—Part 10: Spontaneous Generation of Negative Ions, and Part 12: Influence of Strontium Ion Migration on Matrix Conductivity", which appeared in the September 1961, and March 1962, issues of the *Journal of the Institution of Electrical Engineers*.

The annual convention of the American Oil Chemists' Society will be held in Toronto during October 1–4. Further information can be obtained from the American Oil Chemists' Society, 35 East Wacker Drive, Chicago 1, Illinois.

A symposium on "The Rheology of Emulsions", organized by the British Society of Rheology, will be held in Harrogate during October 1–3. Further information can be obtained from Mr. P. E. Knapp, 20 Wayside Avenue, Harrogate.

The third international symposium on "Batteries" will be held, under the auspices of the Inter-Departmental Committee on Batteries of the British Government, at Bournemouth during October 2–4. Further information can be obtained from Mr. I. B. N. Evans, 30 Fleet Street, London, E.C.4.

The national conference of the Institute of Personnel Management will be held at Harrogate during October 4–6. The theme of the conference will be "Economic Planning and Incomes Policy". Further information can be obtained from Miss W. A. Kilgour, the Institute of Personnel Management, Management House, 80 Fetter Lane, London, E.C.4.

The second Thomson Lecture of the Society of Instrument Technology will be delivered by Sir Harry Melville at the Royal Institution, 21 Albemarle Street, London, W.1, on October 4. The title of Sir Harry's lecture will be "The Effect of Instrumental Development on the Progress of Chemistry". Further information can be obtained from the Secretary, the Society of Instrument Technology, 20 Queen Anne Street, London, W.1.

The twenty-third national conference of the Council for the Preservation of Rural England will be held at Lowestoft during October 4–6. The theme of the conference will be "Post-War Developments in the Countryside". The conference is not confined to members of the Council. Further information can be obtained from the Secretary, the Council for the Preservation of Rural England, 4 Hobart Place, London, S.W.1.

BROADCASTING AND TELEVISION SERVICES IN BRITAIN

APART from its general interest, particularly its analysis of sound broadcasting and television over the past decade and its specific recommendations for future policy (see p 927 of this issue), the report of the Pilkington Committee on Broadcasting* is of considerable interest to scientists, and its usefulness is enhanced by the appendage of the report of the Television Advisory Committee, 1960 Memoranda were submitted or evidence given to the Committee by a number of scientific bodies, for example, on science broadcasting, by the London Area Science Policy Committee of the Association of Scientific Workers, the British Association and the Royal Society, which were concerned with the third television programme and the Scientific Advisory Committee, as well as with science programmes The Department of Scientific and Industrial Research submitted evidence on broadcasting of science and technology, recruitment and training of staff and the third television programme, the Institution of Electrical Engineers on research and on education and training of personnel, the National Research Development Corporation on the possibilities of band-width compression of television broadcast systems, the Nature Conservancy on natural history and conservation programmes, the Royal Horticultural Society on the need for planned instruction in horticulture, the Royal Society for the protection of birds on natural history programmes, the Scientific Film Association on educational broadcasting, treatment of medical programmes, effect of advertisements on scientific accuracy and objectivity, and the Television Society on a United Kingdom Communications Authority Co-ordinating Committee for research, line definition and colour, and educational broadcasting Evidence on educational broadcasting or special aspects thereof was also submitted by numerous other bodies, including the Royal Society of Arts, the Universities Council for Adult Education, the University of Birmingham and the Workers' Educational Association

While this list indicates well enough the range of scientific interest of the Report, the material of direct scientific interest is contained essentially in Part 4, which discusses the technical considerations, in Section 13 of Part 5, which discusses educational broadcasting, and in two short sections of Chapter 9, which discuss some particular kinds of programme Here, the forceful representations made by the Royal Society and the British Association are noted They pointed to the compelling need to present to the public the significance of science, and argued for more programmes on the varied aspects of science and technology and on the applications of developing scientific thought and knowledge, suggesting the appointment of a scientist to the senior production staff of the B B C to take charge of science programmes The Pilkington Committee thought it was no part of its task to advocate that particular subjects should find a place, or larger place, in programmes, and merely suggested that the two broadcasting organizations should consider what steps, or what additional steps, should be taken to see that these specific needs are met

On educational broadcasting the Committee observes that broadcasting is concerned with education in four ways First, through the broadcasts for schools and second through the educational broadcasts to those who have left school Third, there are programmes which, though they do not aim specifically to teach their audiences, are clearly educative, such as those on medicine and science Lastly, there is the generally educative effect of all programmes, of their diffused effect on knowledge, opinions and attitudes Apart from schools' broadcasting, however, there is no sharp break between educational and generally educative programmes

On schools' broadcasting three basic considerations were submitted to the Committee The service must be so organized as to fit into the national educational system, the purpose of the broadcasts is to help, not to replace, the teacher, and the broadcasting authorities have a duty to include education in their programmes From these considerations it follows that the broadcasting authorities, and not a central educational body, should provide the service, and because teaching is in the hands of teachers, the role of the broadcasting organizations is to be agents for the teachers All the educational bodies which gave evidence appear to accept these consequences as an essential part of the policies governing broadcasting for schools They are accepted by the B B C , but the Independent Television Authority has certain reservations about the first

There was little criticism of the programmes, and the standard of schools' broadcasts on both sound and television is high, though the Committee also concludes that there is need for more programmes, especially on television There was no criticism of either the B B C or the Independent Television Authority for not providing programmes for universities or technical colleges now, even by those who thought that such programmes should be provided in the future So far as they go—and in sound broadcasting they go a good way—the Pilkington Committee concludes that the adult educational programmes of the B B C are in themselves very good, but only a start has been made in television It finds it difficult to see how the growing demand for more television programmes can be met as long as each broadcasting organization has only a one-channel service within which to provide educational programmes as part of a comprehensive service

The idea of an educational service of broadcasting is discussed in a separate chapter, but is specifically rejected No specialized service of educational broadcasting, it concludes, should be introduced whether by a new organization or by either of the existing broadcasting authorities The Committee believes not only that the range of programmes should include educational items, the main purpose of which is specifically to teach, but also that generally educative programmes should have a right of place in all broadcasting It should also be acknowledged that almost all broadcasting should have an educative effect The development of a service specializing in education would strike at this concept of education as a purpose of all services of broadcasting, and for this reason the Committee rejected it and reasserted the principle that educational broadcasting in all its

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[*] Report of the Committee on Broadcasting, 1960 (Cmnd 1753) Pp xii+342 (London H M Stationery Office, 1962) 18s net

forms should continue to be the responsibility of the two broadcasting authorities and provided as part of comprehensive services It recommends that the broadcasting authorities should consult with professional bodies as to the practicability of broadcasting courses of adult education of a vocational nature and that they should try every possibility of improving arrangements for consultation and liaison with the recognized adult educational organizations

The technical considerations discussed in Chapter 16 are reasonably summarized in the Committee's recommendations on this section The B B C has so far built twenty-one very-high-frequency sound stations, which serve more than 97 per cent of the population, and, within the frequencies allocated, it is impossible to provide for more high-powered stations, although low-powered relay stations or satellites could be provided, and coverage extended or improved in areas where it is now unsatisfactory There were no representations that either the B B C or the Independent Television Authority has not executed its plans for expansion as fast as was reasonably possible The Committee concludes that short-term difficulties and disadvantages of change-over do not outweigh the long-term advantages to be derived from the adoption of the 625-line definition standard recommended by the Television Advisory Committee in its report of May 1960 Accordingly, it recommends that a change in the definition standard in the United Kingdom from 405 to 625 lines be authorized forthwith A compatible service of colour television should be introduced on this standard, and the further extension of television services should be by broadcast transmission in the V H F Bands IV and V This decision will involve building very-high-frequency transmitting stations, and the Committee recommends that these transmitting stations of the B B.C and the Independent Television Authority should be co-sited and that the Postmaster-General should be given appropriate powers of direction In allotting the uncommitted frequencies in Band III, it is recommended that first priority should be given to separating the B B C 's service to Wales from that to the English regions, and the second to improving the reception of the two existing programmes and completing their coverage

CENTRAL ELECTRICITY GENERATING BOARD

NEW RESEARCH LABORATORIES

A CEREMONY to mark the official opening of the Research Laboratories of the Central Electricity Generating Board at Leatherhead was held on May 10 After an introduction by Sir Christopher Hinton, the laboratories were declared open by the Right Hon Richard Wood, Minister of Power Dr J S Forrest, director of the Laboratories, replied

Reference was made by the speakers to the fact that research on a national basis by an electricity authority was initiated in Great Britain in 1930 by the Central Electricity Board At that time the National Grid was under construction and the need for the experimental investigation of certain problems in high-voltage transmission had been recognized Small laboratories were established at Croydon and Waddon, and it was of particular interest to note, in connexion with the opening of the new laboratories, that the first member of research staff to be appointed was Dr J S Forrest, the present director

The Central Electricity Board first set up a laboratory at Leatherhead on a temporary basis in 1940 following damage by enemy air attack at the Waddon laboratories This site, adjacent to the railway line, at Kingston Road, was afterwards developed, and between 1950 and 1955 had erected on it a number of permanent buildings, including a small high-voltage laboratory During the same period facilities had been secured on nearby land at Cleeve Road for the erection of a 400-kV experimental high-voltage transmission line This land adjoined the research laboratories of the Electrical Research Association. It is on a 20-acre site adjacent to the experimental high-voltage line that the new laboratories have been erected (Fig 1) These comprise a main building with a floor area of about 100,000 square feet, a separate high-voltage laboratory of 35,000 sq ft , together with a radiographic laboratory, a staff restaurant and recreation building The buildings at Kingston Road now house the materials division of the laboratories

The total staff of the laboratories numbers about 750, of whom roughly one-third are professional engineers or scientists

The Central Electricity Research Laboratories are organized on the basis of five research divisions described as. High Voltage, Physics, Engineering, Chemistry and Biology, and Materials, and in addition there are the supporting technical and secretarial services

The lay-out of the main block of buildings has been arranged architecturally in rather an interesting way The pond for cooling water and fire fighting has been made a central feature in front of the building and over part, on a sort of isthmus on stilts, stands the lecture theatre The main block, three storeys high, is backed by two wings each two storeys high, separated by an area at present used as a car park Behind the west wing is situated the canteen, and in fairly near proximity are a temporary workshop and stores Some 150 yards away to the north is the high-voltage laboratory and not far from it a small X-ray laboratory

The ground floor of the main block houses the library, one of the drawing offices, the photography section, together with the typing room, duplicating facilities and administrative offices The first and second floors are occupied by chemistry, physics, electronics and biology laboratories, the accommodation comprising for the most part rooms ranging in size from 200 to 1,250 sq ft

The design of the two wings was influenced by experience gained in earlier laboratories This had demonstrated the necessity of providing fairly large areas of floor space on ground-level on which heavy experimental plant could be erected Large-scale 'test rigs' require access to the major engineering services, suitable arrangements of foundations, head room, crane facilities and access and transport facilities Indeed, the provisions required are similar to those of the 'test bed' of a manufacturer of machinery or electrical plant One such area is provided

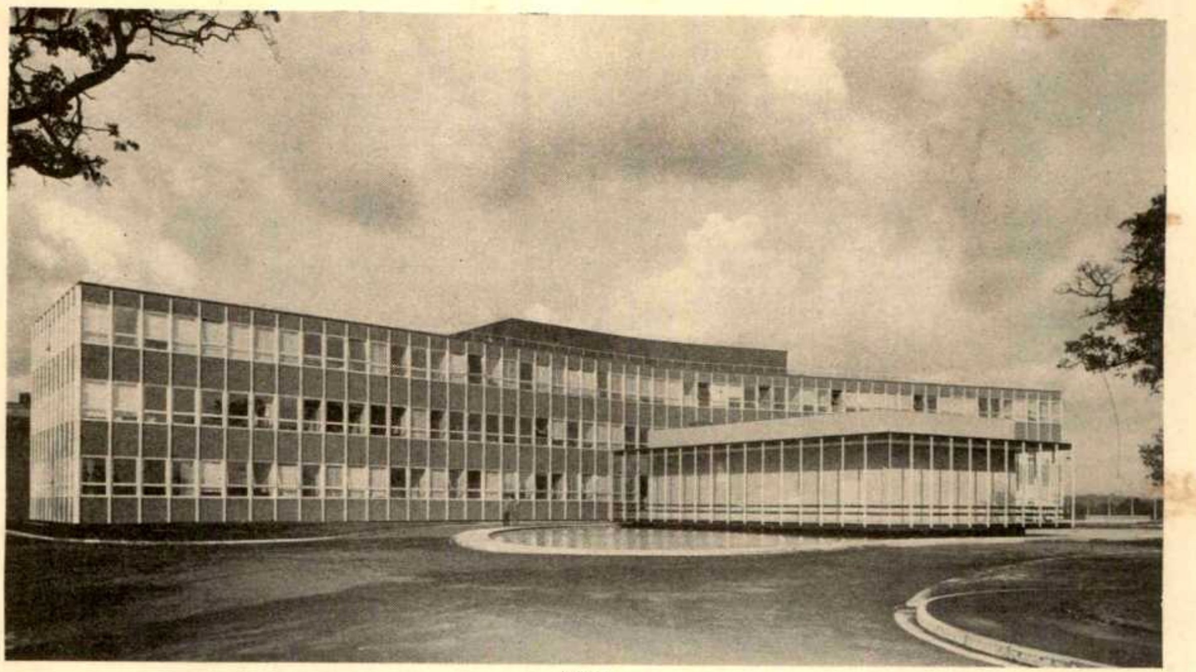

Fig. 1

in each of the wings, the dimensions being 200 ft. by 60 ft. These areas are flanked by laboratories and small rooms on two floors.

The high-voltage building has a total working area of 35,000 sq. ft., the main bay of the laboratory having an area of 12,000 sq. ft. and a height of 70 ft. The planning of these laboratories was completed in 1958, and parts of the new buildings were completed and occupied by January 1961. By the end of the year all the buildings were complete and in full operation.

Work in progress was demonstrated in all five Divisions of the laboratories. Terminating at the High-Voltage Laboratory is a new experimental line, a double-circuit single-phase line which will be used to study line corona and insulator losses and radio noise from multiple bundle conductors at system voltages up to the equivalent of 840 kV three phase. The equipment of the High-Voltage Laboratory itself will provide power frequency voltages up to 1·2 MV r.m.s. and impulse voltages up to 4 MV peak. Studies of the breakdown and deterioration of the dielectrics used in transformers, switchgear and cables form an important part of the work of this division. In addition, the division investigates the current-carrying capacity of conductors for overhead lines and substations, and for this purpose equipment is available for producing currents up to 100 k.amp. A particularly interesting group of investigations in the same division concerns magnetohydrodynamic generation. These comprise a 2 MW oil-burning jet, a shock-tube experiment and a plasma-jet experiment together with a mercury closed cycle equipment.

In the Physics Division work on solid-state physics deals with the fundamental electric and electronic properties of new materials which are likely to have application in the supply industry, for example, semi-conducting oxides, liquid semi-conductors and metals in the super-conducting state. Associated with this work is apparatus for the growth and preparation of single crystals, zone melting apparatus, and there is an automatic recompression system for the recovery of helium gas evolved during experiments using liquid helium. Localized electron states in solids are being studied by means of an electron spin resonance spectrometer.

The mathematical work of the Division is naturally much concerned with the expression of physical problems in mathematical forms suitable for analytical or numerical solution. The resources of the Board's Computing Centre situated at Gordon House in London are utilized for numerical computation. Typical of the physical problems which are encountered are: the diffusion of gases in the atmosphere, the scattering of electromagnetic waves by plasma and the oscillation of a freely suspended catenary in the atmosphere. There is, in addition, a range of important problems concerned with the automatic control of transmission line networks and of associated generating equipment.

In the laboratories as a whole there is a central instrument service which is provided by the Physics Division. For the most part, existing commercial equipment is utilized but a substantial effort is devoted to the development of new instruments and systems. The non-destructive examination of materials and of components is another of the Physics Division's responsibilities and the equipment provided for this purpose includes apparatus for the generation and detection of ultra-sonic waves and X-ray equipment giving complete coverage within the range 10–400 kV.

Mechanical and civil engineering, including specialized sections dealing with fluid dynamics and heat transfer, constitute the main fields of activity of the Engineering Division. A long-bed tensile testing machine provides facilities for the testing of transmission line conductors in lengths up to 77 ft. and heating current up to 3,000 amp can be circulated through the specimen. An equipment has been built for the tri-axial testing of concrete at elevated temperatures. This research relates to pre-stressed concrete pressure vessels. Strain-gauge measurements naturally play an important part, and considerable effort is devoted to the study of special techniques associated with the various uses of strain gauges. One such investigation concerns the practicability of using resistance strain-gauges for measuring the vibrations of turbine blades under service conditions. There is a vibration testing laboratory in which, at present, static vibration tests are being carried out on models of steam turbine-blading in order to determine their resonant modes.

Photo-elastic analysis is applied to the investigation of stresses in pressure vessels, steam headers and fuel element cans.

Measurements of particle size and other studies related to powders and dusts are carried out on materials such as fly ash and pulverized coal.

The work of the Division of Chemistry and Biology ranges from a group of researches on fuel cells to studies in marine and fresh-water ecology. The effects of power station operation on the ecology of marine and fresh-water organisms is a matter of increasing importance.

The analysis of flue gases, an important and traditional chemical measurement in thermal power stations, is now carried out by gas chromatography. Research is in progress on apparatus for effecting remote control of combustion by means of a computer handling automatically data provided by a gas chromatograph. The efficiency of different absorbent materials in extracting sulphur dioxide from flue gases is a related topic.

Internal corrosion in boilers is studied with the help of an experimental boiler, and work is also in progress on the fouling and corrosive effects of combustion products on the fire-side of boilers.

Although the scope of the Materials Division is in no way restricted to metals, the main effort is at present directed to metallurgical research, especially to performance at elevated temperatures in particular applications. Mechanical testing, involving high-temperature tensile, creep and fatigue tests, thus plays an important part. The effects of welding, stress relieving and other fabricating procedures are also studied. Much attention is devoted to austenitic steels used at temperatures above 550° C. Materials which may be of importance in high-temperature equipment for the direct generation of electricity are also the subject of investigation. Magnetohydrodynamic generation, for example, will require insulating duct materials and conducting electrode material capable of withstanding temperatures in excess of 2,000° C for long periods. Silicon carbide has potential importance in high-temperature rectification, and apparatus has been designed to grow single crystals of this material epitaxially.

The supporting technical services of the Laboratories were illustrated by an interesting series of exhibits in the central workshops and drawing offices. Machine and assembly work, profile cutting, welding, chassis wiring, armature rewinding and cable jointing were in progress.

Design and workshop drawings for mechanical and electrical equipment were shown in the drawing offices together with illustrative work.

In the photographic section examples included high-speed still and ciné photography, photomicrography and colour photography.

The opening of these new Laboratories serves further to demonstrate the will of the supply industry to maintain and to improve its position by its ability to absorb and to apply new scientific knowledge and, in addition, to make its own contribution to fundamental advances. It is important to realize that the Central Electricity Research Laboratories constitute only one of a group of major research projects operated by the Central Electricity Generating Board. The Berkley Nuclear Laboratories are exclusively concerned with the special problems of nuclear power stations. A further research station, designed to undertake large-scale experiments in the field of generation and transmission, is being established at Marchwood on Southampton Water. In addition, each of the five regions of the Generating Board has a regional research organization devoted primarily to the solution of problems in the operation of power stations and of the Grid System in its own area. All these organizations, with a research staff totalling some 1,300, contribute to the research effort devoted to improving electricity supply.

J. GREIG

ROTHAMSTED EXPERIMENTAL STATION

REPORT FOR 1961

THE diversity of modern agricultural research is well exemplified in the annual report of the Rothamsted Experimental Station for 1961*. It covers the activities of the twelve research departments at Rothamsted, the farms there and at Woburn, the experimental stations at Woburn and at Dunholme and the work of the Soil Survey of England and Wales. Of the 296 pages in the report, more than 200 describe investigations actually in progress or just completed and a short review can scarcely do justice to the year's work of this, Britain's premier, agricultural research station.

Of topical interest and importance are the results of a ley-arable rotation experiment designed to assess the effect of the ley on soil fertility and the performance of subsequent crops. Since Stapledon so forcefully and successfully advocated the use of the ley before and during the Second World War, it has been widely assumed that the benefits were automatic. Farm practice has often seemed to confirm this, but in fact critical experimental data, either for or against, are singularly few. For several years the Rothamsted experiment has been running in two parts, one on a field ploughed from old pasture and the other on land long under an arable rotation. The test crops were wheat, potatoes and barley, and the results, apart from a few interesting but minor variations, show that no definite improvement in yield has followed the ley that cannot, just as easily, be secured by the use of fertilizer in a purely arable rotation. Ley-arable rotations do not lend themselves very readily to really critical experimental control, and the danger of generalization from one set of experiments is obvious, but the work is valuable just because of this. We may well have overstated the case of the ley as a universal panacea and a well-based re-appraisal will certainly do no harm.

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* Lawes Agricultural Trust. Rothamsted Experimental Station, Harpenden. Report for 1961. Pp. 296. (Harpenden, Herts: Rothamsted Experimental Station, 1962.) 15s.

Another classical concept in agriculture is the role of farm-yard manure in maintaining and increasing soil fertility Again, farm experience would leave little doubt as to its value, but the reasons for this may not be those we have always assumed The Chemistry Department has continued its experiments to show just how farm-yard manure affects crops, and an interesting and essential development is a study of liquid manures resulting from the intensive methods of stock management now being practised Liquids from piggeries were analysed and shown to be rich sources of N P K, for example 1,000 gal of one sample supplied 90 lb nitrogen, 70 lb phosphorus pentoxide and 70 lb potassium monoxide The disposal of large volumes of such material is a major practical problem on farms to-day, but its value is such that new methods of conservation and disposal must be devised so that this potentially rich source of plant nutrients may be used to full advantage

The work being carried out by the Botany Department is concerned with matters ultimately related to crop yields The physiology and distribution of weed seeds and the competitive activities of the resultant plants have been studied, while the long-term investigations on the physiology of growth and yield continue to provide useful information One of the major advances in post-war agriculture has been the development of spring varieties of wheat almost the equal in performance to their winter counterparts An analysis of the photosynthetic processes in varieties of wheat suggested that the greater efficiency of grain production in Capelle and Jufy I was attributable to increased photosynthesis in the ears of these varieties as compared with Squareheads Master or Atle Studies such as these provide the plant breeder with vital information that may well open up new lines of cereal development Two new records are reported from the Plant Pathology Department For the first time blight lesions have been found on a potato tuber without any sign of the disease appearing above ground on the parent or neighbouring plants This adds to our scanty knowledge of what actually happens in the earliest stages in the development of this well-known disease For the first time, too, *Pithomyces chartarum*, the mould responsible for facial eczema in sheep in New Zealand, has been found in several areas in the Home Counties In New Zealand the disease is a serious one, the organism being produced in enormous quantities, especially on mown grass The discovery of the fungus in Britain is a direct, if unwelcome, consequence of the utilization of aerial spore-trapping procedures developed at Rothamsted

Rothamsted does not restrict its services to agriculture in Great Britain Overseas problems are studied the report lists the advisory visits, and secondments abroad, of members of staff. Of interest in this connexion is the work of the Biochemistry Department in devising and establishing leaf protein production units for use overseas. At home the Dunholme Field Station is now closed, and the sugar beet work will be carried on from a new base, Broom's Barn It is to be hoped that Dr R Hull and his staff will there find increased scope to continue the excellent and valuable work that has meant so much to the beet industry in Britain

J H WESTERN

CALCIUM REQUIREMENTS BY HUMAN BEINGS

MORE people fail to receive the amounts of calcium normally recommended as necessary than of any other nutrient, yet there is little convincing evidence of any disabilities specifically attributable to deficiency of calcium In many parts of the world only limited opportunities exist for the intake of the recommended amounts, and this has led to suggestions for the widespread fortification of foods with calcium This can be both expensive and technically difficult, and in the long run—according to a joint Food and Agriculture Organization/World Health Organization Expert Group on Calcium Requirements—nutritional improvements ought to be met through a better basic diet and not only by supplementation of this kind (*WHO Chronicle*, 16, No 7, July, 1962)

No supplementary calcium should be given without first obtaining reliable information about the calcium needs of different population groups Contrary to what has sometimes been believed, the development of rickets and dental caries is largely independent of the intake of calcium In adult life no clear signs of calcium deficiency in men have ever been described in any part of the world In women, among populations where the intake of calcium is habitually low, repeated pregnancies and long lactations may be associated in some regions with the development of osteomalacia, though this is not solely due to the low intake of calcium In some parts of northern India, for example, osteomalacia is frequent with higher intakes of calcium, whereas it is rare in southern India, where the intake is lower It would seem that if the intake of vitamin D is satisfactory, osteomalacia is not a problem even for women whose intake of calcium is low—though a low intake of calcium may be an exacerbating factor The common osteoporosis of old age is thought by some investigators to be associated with calcium deficiency but by others to be more related to endocrine disturbances Nor does present knowledge support the view that a low intake of calcium is necessarily prejudicial to normal physiological function, although certain pathological conditions, mostly rare, do exist in which increased intake of calcium may be advisable Among conditions in which calcium deficiency may play a part are the osteopathy of premature infants, various syndromes involving subnormal calcium absorption, such as steatorrhœa and certain forms of chronic renal damage with or without calciuria, and senile osteoporosis

In making recommendations on calcium needs, the Expert Group defined the minimum requirement of calcium for adults as the smallest amount that will maintain health and keep the body in calcium balance when the diet is otherwise adequate and the vitamin D status is satisfactory The available evidence, however, does not permit the specification of any particular figures for minimum adult requirements, for disorders often attributed to calcium deficiency have not been observed in large populations habitually subsisting on intakes of only 300–400 mg a day, and it has been shown that adults can successfully adapt themselves to intakes of 400–500 mg and even less

PENICILLIN-LIKE SUBSTANCES IN PREPARATIONS OF 6-AMINOPENICILLANIC ACID

By F. R. BATCHELOR, M. COLE, D. GAZZARD and G. N. ROLINSON
Beecham Research Laboratories, Ltd., Brockham Park, Betchworth, Surrey

EXAMINATION of preparations of crystalline 6-aminopenicillanic acid (6-APA) (refs. 1 and 2) has revealed the presence of three substances of a penicillin-like nature which have not been described hitherto. Two of these substances show substantial antibacterial activity, and were apparent on bio-autographs, while the other shows almost no activity and was located by continuous paper electrophoresis and assay by the hydroxylamine method[2].

6-APA itself shows a relatively low order of antibacterial activity[3,4], and, unless high concentrations are involved, this compound cannot be detected on bio-autographs without first reacting with a suitable acid chloride to form a penicillin. However, when relatively high concentrations of 6-APA were subjected to paper chromatography such that the amount of material on the chromatograms was sufficient to give rise to a zone of inhibition on bio-autographs without prior acylation, not one, but two zones of inhibition were seen. One of these zones was identified as being due to 6-APA itself. This was done by spraying the chromatograms with phenylacetyl chloride prior to development on the seeded plate[2]. As a result of this step the size of this zone of inhibition was very greatly increased, but, in contrast, the other zone on the bio-autograph was unaffected by the acylation. The solvent system used was butanol/ethanol/water, 4:1:5 top layer, and the test organism was *B. subtilis*. In this chromatographic system the unidentified compound ran with a lower R_F value than 6-APA. On the other hand, using the solvent system butanol/acetic acid/water, 60:15:25, the unidentified substance ran with a higher R_F value than 6-APA. These R_F values are given in Table 1.

Table 1. R_F VALUES FOR FACTOR 1 AND 6-AMINOPENICILLANIC ACID

Solvent sytem	Factor 1	6-APA
1	0.05	0.14
2	0.61	0.44
3	0.36	0.47

Solvent 1, butanol/ethanol/water, 4:1:5 top layer
Solvent 2, butanol/acetic acid/water, 60:15:25
Solvent 3, butanol/pyridine/water, 1:1:1

This unidentified substance, referred to here as 'Factor 1', has been found to be present in all preparations of 6-APA examined so far, including 6-APA isolated from fermentations of *Penicillium chrysogenum*[1,2], 6-APA prepared by enzymatic hydrolysis of phenoxymethylpenicillin[5,6] and 6-APA prepared by enzymatic hydrolysis of benzylpenicillin[5,7-9]. It would therefore seem unlikely that Factor 1 is an impurity carried through with the 6-APA during the extraction procedure. Factor 1 is an acid-stable compound, and cannot be detected in either the benzylpenicillin or phenoxymethylpenicillin from which the 6-APA was obtained. Furthermore, there is evidence that Factor 1 is formed from 6-APA in solution. Results are shown in Table 2 for the antibacterial activity of 6-APA solutions maintained under different conditions of temperature and *p*H over periods of 24 and 48 h. The activity was estimated by cup-plate assay against *Bacillus subtilis* and expressed in units of benzylpenicillin. It will be seen that over a period of 48 h at *p*H 6.0 and 37° C the antibacterial activity of the solution increased more than ten-fold, notwithstanding the fact that hydroxylamine assay of total β-lactam ring indicated a loss of 15 per cent. Paper chromatography showed that this increase in activity was due to an increase in the Factor 1 content of the solution.

6-APA and Factor 1 can be separated by paper electrophoresis using a pyridine/acetic acid buffer, *p*H 4.3, prepared by adding pyridine to 0.05 M acetic acid. In experiments using 17 V/cm for 4 h, 6-APA remained at the point of application, while Factor 1 migrated 6 cm towards the anode. Partially purified Factor 1 material was prepared by incubating a solution of 6-APA at *p*H 6.0 and 37° C for two days, and almost all the 6-APA was then removed by passing the solution through a column of cation exchange resin ('Amberlite *IR* 120 H') giving an acid effluent which was neutralized with sodium hydroxide and freeze-dried. This material was then subjected to preparative continuous electrophoresis using a Beckman Spinco apparatus run at 800 V with a residence time of 2 h. Before application to the electrophoresis curtain the material was made up as a 5 per cent solution in distilled water, de-salted electrolytically and diluted 2 in 5 with the pyridine/acetic acid buffer already referred to. Factor 1 was detected in the fractions by assay for antibacterial activity using *B. subtilis* as test organism. 6-APA was detected in a similar manner but only after first reacting with phenylacetyl chloride to convert to benzylpenicillin. It will be seen from Table 3 that 6-APA was obtained in Fraction 11, and Factor 1 in Fractions 17–20. Although the 6-APA content of the material fractionated in this way was too low to permit assay by the hydroxylamine method, the Factor 1 present could be estimated readily and is expressed in Table 3 as benzylpenicillin equivalents. The hydroxylamine assay method also revealed the second unidentified compound as being present in Fractions 24–29. This second compound, referred to here as 'Factor 2', showed no antibacterial activity either before or after phenylacetylation, but in terms of benzylpenicillin equivalents by hydroxylamine assay the amount present was greater than that of Factor 1.

Freeze-dried preparations of both Factor 1 and Factor 2 were obtained by pooling the relevant fractions above. Assuming both compounds to have a molecular weight similar to benzylpenicillin the purity of the preparations appeared to be of the order of 30 per cent. Hydroxylamine and manometric

Table 2. INCREASE IN ANTIBACTERIAL ACTIVITY OF SOLUTIONS OF 6-AMINOPENICILLANIC ACID MAINTAINED UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND *p*H

Time (h)	Antibacterial activity expressed as units of benzylpenicillin/ml				
	*p*H 7 5°C	*p*H 7 20°C	*p*H 7 37°C	*p*H 6 37° C	*p*H 8 37°C
0	5	5	5	5	5
24	7	10	26	52	17
48	7	14	28	60	17

Solutions of 6-APA at a concentration of 2 per cent were adjusted to *p*H values of 6, 7 and 8 and maintained at 5, 20 or 37° C assay[10] gave similar results for both Factor 1 and Factor 2, which is of interest since 6-APA itself is not amenable to manometric assay[2]

During attempts to prepare larger quantities of material rich in Factor 1 and Factor 2 by incubating a 15 per cent solution of 6-APA at 37° C overnight, most of the residual 6-APA was precipitated by adjusting the *p*H to 4 3 prior to stripping out further 6-APA by ion-exchange resin as already described Examination of this precipitate revealed that it contained a third unknown substance with antibacterial activity which migrated 1 cm towards the cathode (at *p*H 4 3, 17 V/cm) Control electrophoresis tapes using glucose revealed that this movement was entirely due to electro-osmosis and over a range of *p*H 2–8 Factor 3 would appear to have zero zeta potential As with Factor 1, the initial *p*H of the 6-APA solution affected the yield of Factor 3 With 6-APA kept between *p*H 1 and *p*H 5 no Factor 3 could be detected At *p*H 6 a small zone could be seen, but the greatest yields were obtained over the range *p*H 7–9 There is some evidence that Factor 3 is not, in fact, a single substance, but is a complex mixture of several substances When Factor 3 was subjected to two-dimensional separation, using paper electrophoresis in one direction to separate it from traces of 6-APA, Factor 1 and Factor 2 which contaminated the preparation, followed by paper chromatography using butanol/acetic acid/water in the other direction, the Factor 3 zone separated into at least four distinct zones The size of the zones, revealed by bio-autographs on agar plates seeded with *B subtilis*, decreased with the R_F values, which were 0 3, 0 23, 0 17 and 0 14.

Factor 1, Factor 2 and Factor 3 are readily destroyed by *B cereus* penicillinase and, like 6-APA, the three compounds are acid-stable

Results for the antibacterial activity of 6-APA, Factor 1 and Factor 2 are given in Table 4 The figures for Factor 1 and Factor 2 have been corrected for the purity of the preparation on the basis of hydroxylamine and manometric assays assuming a molecular weight equal to that of benzylpenicillin It will be seen that Factor 2 shows a very low order of antibacterial activity and is not likely to contribute significantly to the activity shown by preparations of 6-APA which contain this material On the other hand, Factor 1 shows considerable activity, notably against the Gram-positive bacteria Against *Sarcina lutea*, *B subtilis* and also *Neisseria catarrhalis*, Factor 1 appears to be about fifty times as active as preparations of 6-APA, and from the M I C (minimum inhibitory concentration) values for 6-APA it would therefore appear that the amount of Factor 1 present in the 6-APA samples could not be more than about 2 per cent Such a concentration of Factor 1 would, in effect, contribute all the Gram-positive activity which preparations of 6-APA display Against the Gram-negative bacilli, however, Factor 1 shows relatively little activity and with several organisms it is actually less active than 6-APA Consequently, it would seem that the activity shown by preparations of 6-APA against Gram-negative bacilli is, in fact, due to 6-APA and not to the impurity of Factor 1 This is certainly true if the content of Factor 1 in 6-APA is of the order of 2 per cent or less, since this amount would not make any detectable contribution to the Gram-negative activity of 6-APA

Table 3 FRACTIONATION OF PARTIALLY PURIFIED FACTOR 1 MATERIAL BY CONTINUOUS ELECTROPHORESIS

Fraction No	Hydroxylamine assay as µg/ml benzylpenicillin equivalents	Antibacterial activity prior to reaction with phenylacetyl chloride	Antibacterial activity after reaction with phenylacetyl chloride	
0–10	0	0	0	
11	0	0	+	6-APA
12–16	0	0	0	
17	40	–	+	Factor 1
18	70	+	+	
19	90	+	+	
20	70	+	+	
21	0	0	0	
22	0	0	0	
23	0	0	0	
24	30	0	0	Factor 2
25	110	0	0	
26	170	0	0	
27	140	0	0	
28	110	0	0	
29	50	0	0	
30	0	0	0	
31	0	0	0	
32	0	0	0	

Table 4 COMPARISON OF ANTIBACTERIAL ACTIVITY OF FACTOR 1, FACTOR 2 AND 6-APA

(Serial dilution method in agar)

	M I C (µg/ml)		
	Factor 1	Factor 2	6-APA
E coli	>100	>100	50
P vulgaris	>100	>100	250
S typhi	>100	>100	50
S typhimurium	25	>100	50
Sh flexneri	25	>100	50
Aer aerogenes	>100	>100	125
Kleb pneumoniae	>100	>100	250
Neiss catarrhalis	0 5	2 0	25
Sarcina lutea	1 0	100	50
Oxford Staph	10 0	>100	100
*Staph Russell**	>100	>100	250
*Staph aureus H**	>100	>100	250
Strep faecalis	>100	>100	>250
β-haemolytic *Strep*	10 0	>100	50
B subtilis	2 5	>100	100
Strep pneumoniae	5 0	50	100

* Penicillin resistant

No solid Factor 3 preparation has yet been obtained free from 6-APA, Factor 1 and Factor 2, and no quantitative information of antibacterial activity could be obtained From results where electrophoresis tapes were placed on agar plates seeded with a variety of organisms, Factor 3 appears to have a typical penicillin spectrum of high Gram-positive and low Gram-negative activity

It would appear, therefore, that 6-APA does possess antibacterial activity which is distinct from that exhibited by the compounds referred to here as Factors 1, 2 and 3

We thank Mr C Reading and Mr D Duke for technical assistance, and Mrs Shirley Stevens and her staff for the M I C determinations

Since this article was prepared, the paper by Grant *et al* [11] on poly-6-aminopenicillanic acid has come to our notice Although the substance reported by them may well be related to the Factors 1, 2 and 3 reported here, there are obvious differences in electrophoretic migration and antibacterial activity, and, furthermore, Factors 1, 2 and 3 are all readily dialysable under the conditions where poly-6-aminopenicillanic acid is retained

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CORRELATION OF OXIDATION-REDUCTION CHANGES OF INTRACELLULAR REDUCED PYRIDINE NUCLEOTIDE AND CHANGES IN ELECTROENCEPHALOGRAM OF THE RAT IN ANOXIA

By DR. BRITTON CHANCE and BRIGITTE SCHOENER

Johnson Research Foundation, University of Pennsylvania

A MICROFLUOROMETRIC method for the recording of the kinetics of oxidation-reduction changes of intracellular pyridine nucleotide in localized areas of tissue has been described[1,2]. One paper describes in detail the controls which have been used to evaluate the possible interference due to the oxyhæmoglobin–hæmoglobin change[2]. Another paper describes how emission spectra of localized areas of the tissues can be recorded by a modification of the microfluorometer used[3].

The purpose of this article is to correlate changes in electroencephalograms with changes in the intracellular oxidation-reduction state.

A rat, weighing approximately 250 gm., was anæsthetized with urethane (1.0 mgm./kgm.). A 20 mm.^{2} area of the cortex was exposed either with a high-speed trephine or a dentist's burr. (A number of these operations were carried out by Dr. W. S. Masland of the Department of Physiology.) The dura was left intact, and in successful preparations the blood-free field was obtained. The trachea and femoral vein were cannulated. The inspired gas was controlled with a gas-mixing apparatus. The potential changes were recorded with a 1-mm. silver ball in contact with a cortical area directly adjacent to the aperture and displaced from it by 0.5–1 mm. The recordings were monopolar, the reference electrode being the ear pins of the head holder. The frequency pass of the recordings at the half-power voltage points was 6–25 c.p.s. The microscope was focused on an area containing a minimum of visible blood vessels. The microfluorometer made use of a 1-kW. mercury arc, a 366 mμ primary filter, and a secondary filter giving a broad pass band (400–520 mμ), peaking at 450 mμ. Optical fields of 17 and 30μ were used.

Fig. 1 illustrates temporal relationships between fluorescence and potential changes. Time increases from left to right, and a fluorescence increase is indicated by a downward deflexion. In view of other experiments, the increased fluorescence is attributed to a reduction of intracellular pyridine nucleotide, to which the mitochondrial material contributes a major part[2]. The deflexion corresponding to a 20 per cent increment of total fluorescence is indicated as is the potential change corresponding to 1 mV. The rat, initially breathing 20 per cent oxygen and 80 per cent nitrous oxide, is transferred to 100 per cent nitrous oxide at the first event marker. Six seconds thereafter there is a 4 per cent decrease of fluorescence which reaches a maximum 12 sec. after the transfer to 100 per cent nitrous oxide. During this interval, the synchronous activity on the cortex increases somewhat[4]. At 12 sec. after the transfer to nitrous oxide, the fluorescence intensity increases over an interval of approximately 20 sec., and the electrical activity continues at a diminished level until the pyridine nucleotide reduction reaches approximately 80 per cent of the maximum value recorded. Thereafter, electrical activity can no longer be observed. At very nearly the same time, breathing can no longer be observed. Approximately 20 sec. after the cessation of breathing, the inspired gas is returned to 20 per cent oxygen and 80 per cent nitrogen, and the animal is ventilated manually two or three times. Approximately 5 sec. afterwards, the fluorescence intensity abruptly decreases and breathing is resumed. The fluorescence trace shows an overshoot in the recovery from anoxia. In the final phases of recovery, electrical activity can be observed on the cortex, and a few minutes after the end of the record illustrated here, electrical activity similar to that observed in the beginning of the experiment is observed.

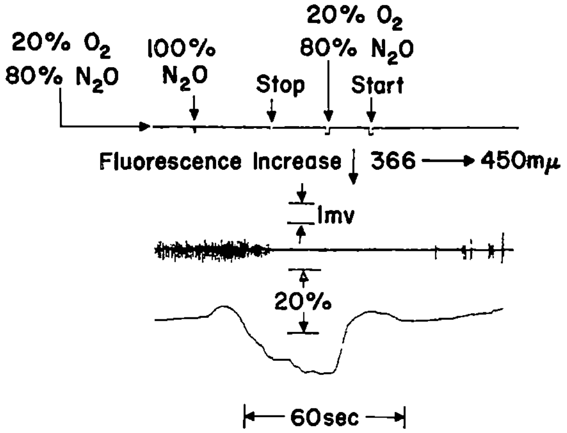

Fig. 1. Microfluorometer focused on 30-μ area in pre-occipital cortex. 1-mm. diameter monopolar electrode is placed approximately 1 mm. away from the observation point. The top trace represents the moments of changing the concentration of the inspired oxygen. In addition, the moments of starting and stopping breathing are indicated. The middle trace gives the electroencephalogram (6–25 c.p.s. band-width) and the voltage calibration. The lower trace gives the fluorescence changes with an increase indicated by a downward deflexion. The calibration mark represents a 20 per cent change of the total fluorescence emission, 366 mμ excitation, 450 mμ measurement

The most characteristic feature of this type of record is that both electrical activity of the cortex and breathing continue until reduction of pyridine nucleotide in anoxia has very nearly reached its maximum value. Two other characteristics of the record are the cyclic decreases of fluorescence on transfer from oxygen to nitrous oxide and vice versa.

If, instead of suddenly transferring the inspired gas from 20 per cent oxygen to zero as in Fig. 1, the inspired gas is slowly diminished as in Fig. 2, the sequence of events can be depicted somewhat more clearly. There the inspired gas as monitored by the Pauling oximeter is changed from 20 to 6 per cent and is accompanied by only slight reduction of pyridine nucleotide. The electrical activity increases but falls to a minimum at about 5.5 per cent inspired oxygen. Thereafter, there is a marked increase of electrical activity and a clear increase of pyridine nucleotide reduction. The electrical activity then achieves a very large amplitude as the inspired gas falls from 3 to 2.5 per cent and the pyridine nucleotide level becomes even further reduced. There is an abrupt halt to electrical activity, followed 6 sec. later by a cessation of breathing and a final increase of pyridine nucleotide reduction to its plateau value 15 sec. after cessation of breathing, 20 per cent oxygen is administered, and 6 sec. thereafter an oxidation of reduced pyridine nucleotide is observed together with evidences of very small electrical signals. As the pyridine nucleotide level rises toward a higher degree of oxidation, the electrical activity starts off at a very high level, which is maintained until the end of the recording.

A characteristic feature of this recording is that electrical activity ceases when the pyridine nucleotide level is about 70 per cent reduced, a smaller value than in the previous experiment.

With a fresh preparation in which only a few cycles of aerobiosis and anoxia have been carried out, and in which the transition from aerobiosis and anoxia is made rapidly, a continuation of localized electrical activity can be observed for 10 or more sec. after the pyridine nucleotide reduction has reached its maximal value. Such a record is indicated in Fig. 3. In this experiment, there is no initial diminution of fluorescence following the transfer from 100 per cent oxygen to 20 per cent oxygen plus 80 per cent nitrous oxide. The electrical activity is observed clearly to persist at 20 sec. after the pyridine nucleotide reduction has reached the maximal value and breathing can no longer be observed. In this record, the resumption of electrical activity can be observed at about the same time as reoxidation of the reduced pyridine nucleotide occurs after the changing from 100 per cent nitrous oxide to 20 per cent oxygen, plus 80 per cent nitrous oxide.

These records illustrate two types of correlations between the electrical activity of the cortex and the degree of reduction of pyridine nucleotide caused by the transition from aerobiosis to anoxia. In an experiment in which several cycles of anoxia have occurred, the sudden change from aerobiosis to anoxia causes electrical activity to cease simultaneously with an 80–90 per cent completion of the pyridine nucleotide reduction. A similar effect may be obtained if the animal proceeds in a number of stages to lower and lower oxygen tension. On the other hand, if a fresh animal makes a rapid transition from aerobiosis to anoxia, the electrical activity can be observed to persist after the completion of the pyridine nucleotide reduction.

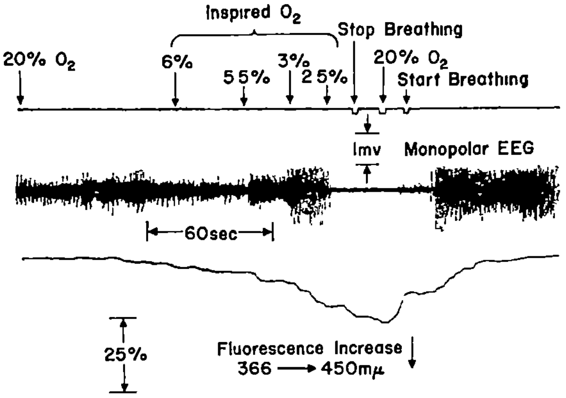

Fig. 2. Correlation between EEG and pyridine nucleotide reduction in the slow transition from aerobiosis to anoxia. The convention of recording is the same as that described in Fig. 1, except that the top trace includes the measurements of inspired oxygen concentration according to the Pauling oximeter.

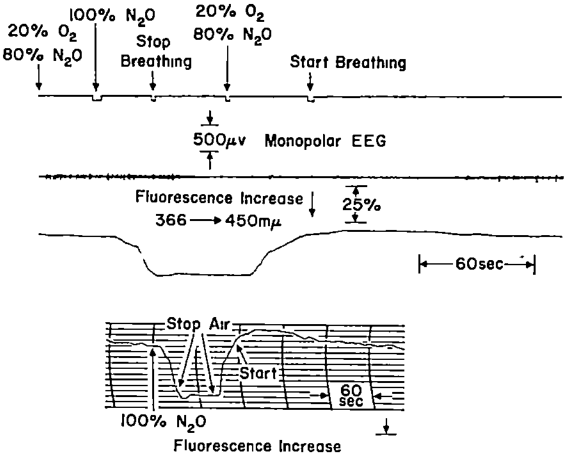

Fig. 3. Correlation between EEG and pyridine nucleotide reduction in a rapid transition from aerobiosis to anoxia. The recording follows the pattern described in Fig. 1, except that the time-course of the fluorescence change over a 7-min. interval is indicated by the insert below at the bottom of the figure.

It is appropriate to use the increase of fluorescence intensity in anoxia to a plateau value as an indication of the cessation of oxidative phosphorylation. Any electrical activity following this time must depend on stores of high-energy intermediates. The fact that these can be exhausted by repeated cycles of anoxia or by prolonged decrease of oxygen tension to anoxia is consistent with this view. Stores of high-energy intermediates consist not only of creatine phosphate but also of high-energy intermediates of the mitochondria themselves. Variations in the interval over which electrical activity can be maintained after the completion of pyridine nucleotide reduction may reflect changes in the level of such high-energy intermediates and be useful in their assay *in vivo*.

An interpretation of a diminution of fluorescence observed following the transition from oxygen to nitrogen and vice versa (Fig. 1) can be given in terms of the effects of adenosine diphosphate and phosphate on the steady state concentration of mitochondrial pyridine nucleotide[5]. We have found that activation of respiratory metabolism by an increase in the concentration of these compounds causes the oxidation of mitochondrial reduced pyridine nucleotide. Such increases in phosphate or phosphate acceptor could easily be a consequence of the increased electrical activity observed in hypoxia (Fig. 1). In Fig. 2 the oxygen tension was sufficiently low at the time when increased electrical activity occurred that no clear-cut diminution of fluorescence could be observed. The overshoot following the administration of oxygen is attributed to the accumulation of ADP and phosphate in the anoxic interval. It is hoped ultimately to be able to relate the phosphate potential to the electrical activity.

It is noteworthy that a re-oxidation of pyridine nucleotide is a necessary (Fig. 2) but not a sufficient condition (Fig. 1) for the resumption of electrical activity.

It is the primary purpose of this article to give a preliminary survey of a few types of correlations between localized electrical activity of the cortex and the kinetics of oxidation and reduction of pyridine nucleotide in the transition from the aerobic to the anoxic state and vice versa These results show a dependence of electrical activity and the oxidation-reduction state of pyridine nucleotide providing reserves of high-energy compounds have previously been depleted

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EVALUATION OF ANTIBIOTIC SYNERGISM

By Dr. DEBORAH KAPLAN and Dr. WALTER KOCH
Department of Microbiology and Public Health,
Chicago Medical School

ONE established method of determining antibiotic sensitivity consists in inoculating test tubes containing broth and antibiotic, and noting the smallest concentration of the antibiotic at which no visible growth occurs after an incubation of 18–24 h[1] If this amount is below the concentration attainable in the blood[2,3], the antibiotic may be useful in therapy There is a shortcoming in this method if the doses are not closely spaced, there is little certainty that the dose recorded really is the smallest which inhibits growth It has been found that, over a small range, logarithms of doses of antibiotics and logarithms of percentage transmission readings of bacterial growth are directly related As absence of growth is indicated by a percentage transmission reading of 100, the log of which is 2, the log of the smallest dose which would prevent growth can be estimated by extrapolation from two doses causing partial inhibition Let D_1 be the smaller dose, causing the percentage transmission reading R_1, D_2 and R_2 the corresponding values for the greater dose, and D_A the smallest dose causing absence of visible growth, then

$$\log D_A = \log D_2 + \frac{2 - \log R_2}{\log R_2 - \log R_1} \times (\log D_2 - \log D_1) \qquad (1)$$

An example should illustrate this test tubes with trypticase soy broth, containing different concentrations of streptomycin sulphate, were inoculated with 1×10^5 organisms of *Staphylococcus aureus*, type 81 per ml medium[4], and incubated for 18 h at 37° C The first tube showing no visible growth was that which contained 30 μg/ml The tube containing 20 μg/ml showed a percentage transmission reading of 91, while a tube containing 8 μg/ml gave a percentage transmission reading of 52 From equation (1) D_A, the dose causing absence of visible growth is found to be 23 34 μg/ml Though from this experiment it could be inferred that the organism is resistant to streptomycin, for which the limiting concentration is 20 μg/ml , repeated tests indicated a mean of 15 4 μg/ml

Using penicillin *G* potassium instead of streptomycin, a mean concentration of 70 5 units/ml was found to prevent visible growth, though in this specific experiment only 60 92 units/ml were needed This indicates that the organism is definitely resistant to penicillin, for which the limiting concentration is 10 units/ml

It has been pointed out that a 50 per cent endpoint reading has some advantages[1] In order to establish a 50 per cent end-point, several parallel inoculated trypticase soy broth tubes, without antibiotic, were set up After 18 h incubation, the growth is diluted with an equal volume of trypticase soy broth, the resulting tube contains 50 per cent of the bacterial number The percentage transmission reading ($R_{50\%}$) of this bacterial suspension is compared with the percentage transmission readings of the tubes containing antibiotic A tube with antibiotic will only by chance match the one containing 50 per cent of the bacterial number However, this dose, $D_{50\%}$, which would give 50 per cent growth inhibition, can be found by interpolation between two tubes, for which the doses, D_1 and D_2, and percentage transmission readings, R_1 and R_2, are known

$$\log D_{50\%} = \log D_1 + \frac{\log R_{50\%} - \log R_1}{\log R_2 - \log R_1} \times (\log D_2 - \log D_1) \qquad (2)$$

In an experiment, for example, the 50 per cent diluted control gave a percentage transmission reading ($R_{50\%}$) of 24 The tube containing 20 units/ml penicillin (D_1) gave a reading (R_1) of 12 8, the tube containing 60 units/ml (D_2) a reading (R_2) of 93 Using the equation (2), $D_{50\%}$ is found to be 28 33 units/ml In the same experiment 30 units/ml gave a percentage transmission reading of 25 Taking 20 units again as D_1 but 30 units as D_2, the result becomes 29 27 units/ml

Though it is preferable to interpolate for $D_{50\%}$, a good approximation can be obtained by extrapolation from two greater doses, D_1 and D_2, with readings R_1 and R_2

$$\log D_{50\%} = \log D_1 - \frac{\log R_1 - \log R_{50\%}}{\log R_2 - \log R_1} \times (\log D_2 - \log D_1) \qquad (3)$$

As stated here, $D_{55\%}$ for penicillin in one experiment was found by interpolation as 28 33 and 29 27 units/ml , respectively When extrapolating (equation 3) from $D_1 = 30$, $R_1 = 25$, $D_2 = 60$ and $R_2 = 93$, 29 36 units/ml is obtained

Combinations with different ratios of the two antibiotics were tried, and always run parallel to the controls and to the titrations of the single antibiotics If these two antibiotics were strictly additive, half the penicillin dose plus half the streptomycin dose should give the same effect as one full dose of either antibiotic In the above experiment visible growth was prevented by either 60 92 units penicillin/ml or 23 34 μg streptomycin/ml Consequently, 60 92 × 0 5 penicillin plus 23 34 × 0 5 streptomycin should yield the same effect as 60 92 units penicillin alone or 23 34 μg streptomycin alone In general, if simple addition prevails, this same effect should always be obtainable if the sum of the factors is one If smaller amounts than those expected are needed, synergism exists

Using penicillin and streptomycin in combination it was found, for example, that no visible growth occurred when the mixture contained 2·09 units/ml. penicillin and 4·18 μg/ml. streptomycin. This ratio (1 : 2) is of special interest, as it is the same as that of the maximum attainable concentrations in the blood, namely, 10 units penicillin/ml. and 20 μg streptomycin/ml. The above figures indicate that much less of either antibiotic is needed when they act in combination.

If there were only an additive effect, $\frac{60 \cdot 92 - 2 \cdot 09}{60 \cdot 92} \times 23 \cdot 34 = 22 \cdot 54$ μg/ml. streptomycin would be needed in order to complement the 2·09 units/ml. penicillin to the growth-preventing dose. It was found, however, that 4·18 μg/ml. streptomycin, instead of the expected 22·54 μg/ml. sufficed to complement the small dose of 2·09 units/ml. penicillin. The fraction: 22·54/4·18 = 5·39 is thus one measure of synergism.

Likewise, $\frac{23 \cdot 34 - 4 \cdot 18}{23 \cdot 34} \times 60 \cdot 92 = 50 \cdot 01$ units/ml. penicillin would be needed to complement the 4·18 μg/ml. streptomycin to the growth-preventing dose. The fraction: 50·01/2·09 = 23·93 becomes the other measure of synergism. Pooling both measures of synergism by taking their geometric mean, synergism is found to be: $\sqrt{5 \cdot 39 \times 23 \cdot 93} = 11 \cdot 36$.

Generally, let D_{A_1} be the dose of antibiotic 'one', causing a certain effect when acting alone, D_{A_2} the same for antibiotic 'two', $D_{A_1\text{ comb.}}$ and $D_{A_2\text{ comb.}}$ the effective doses of these antibiotics when acting in combination, then:

$$\sqrt{\frac{\frac{D_{A_1} - D_{A_1\text{ comb.}}}{D_{A_1}} \times D_{A_2}}{D_{A_2\text{ comb.}}} \times \frac{\frac{D_{A_2} - D_{A_2\text{ comb.}}}{D_{A_2}} \times D_{A_1}}{D_{A_1\text{ comb.}}}} \qquad (4)$$

or, simplifying:

$$\sqrt{\frac{(D_{A_1} - D_{A_1\text{ comb.}}) \times (D_{A_2} - D_{A_2\text{ comb.}})}{D_{A_1\text{ comb.}} \times D_{A_2\text{ comb.}}}} \qquad (4a)$$

is a measure of synergism.

If synergism obtains, the above expression (4*a*) is greater than unity.

The aforementioned experiments indicate that this penicillin-resistant *Staphylococcus* is rendered highly sensitive to penicillin by the presence of streptomycin; but also the amount of streptomycin needed is reduced to about one-third. Lower values for synergism are recorded when taking 50 per cent growth instead of the absence of visible growth as criterion. In the foregoing example, for example, 11·36 is reduced to 6·60.

The advantage of the combination over its two components acting singly becomes more pronounced with lengthening incubation time. As after 48 h incubation, in most instances, the tubes are either fully grown or show no visible growth, the results have to be based on these tubes from which growth is absent.

After 48 h, the mean growth-preventing dose is either 223 units/ml. penicillin, or 48 μg/ml. streptomycin.

The combination, which has the same effect, contains 4·5 units penicillin and 9·0 μg streptomycin; calculating synergism from formula (4*a*), 14·51 is obtained.

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A GENERAL SYSTEM FOR THE AUTOMATIC SELECTION OF AUXOTROPHS FROM PROTOTROPHS AND VICE VERSA IN MICRO-ORGANISMS

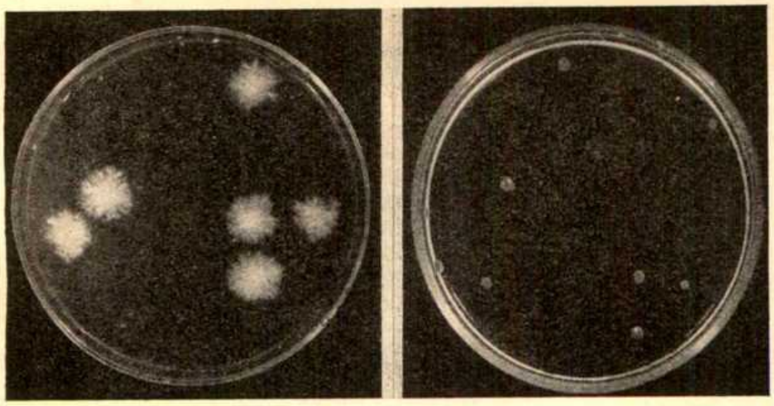
By D. APIRION

Department of Genetics, University of Glasgow

IN microbial genetics there have long been available techniques of high resolving power for the automatic selection of 'forward'-mutants, for example, in respect of resistance to drugs[1] and parasites[2], and of 'back'-mutants, for example, from auxotrophy to prototrophy[3,4]. The automation is based on establishing conditions such that cells of the parent strain —sensitive in the case of 'forward'-mutation to drug resistance, and auxotrophic in the case of 'back'-mutation to prototrophy—cannot grow while the 'forward'-mutants, or the 'back'-mutants, respectively, do grow.

It has been obvious for a long time that it would be very useful to be able to apply some such automation to selection in both directions in one and the same system. Most of the systems in which selection in both directions is possible have, so far, been only partially successful, mainly because of inadequate resolving power in either or both directions. To give a few examples: (1) Methods based on visual selection (for example, colour or morphology of the colony, enzymatic or other colour reaction, type of plaque, etc.) do select in both directions, but with low resolving power in both. (2) Methods based on drug resistance[1] or on host–parasite relations[5,6] or on reversion from auxotrophy[3,4] have a high resolving power but only in one direction. (3) Methods based on gradual enrichment[7-9] are not suitable for precise quantitative work. (4) Methods based on the fact that reversion from auxotrophy in respect of one growth factor sometimes involves mutation to auxotrophy in respect of another[10-13] are not sufficiently general.

The lack of a technique with high resolving power for the selection of mutants or recombinants in both directions in one system has been one of the main bottlenecks in genetic analysis at the intragenic level. Yet, the principles on which some such technique could have been based have been well known for a long time, and it is surprising that they have not been applied deliberately. There are examples in which these principles have been accidentally or incidentally used[14], and others[15,16] in which the results are likely to be interpretable on the basis of these principles. The principles are the following: consider a toxic analogue A' of a growth factor or metabolite A.



a b

Fig. 1. *a*, 5 × 10^6 conidia of a strain of *Aspergillus nidulans*, sensitive to fluoroacetate, plated on fluoroacetate medium (3 per cent, see text): six resistant colonies have developed. About 20 per cent of the resistants so obtained are auxotrophic, that is, unable to utilize acetate as sole source of carbon. *b*, 5 × 10^6 conidia of a strain resistant to fluoroacetate and unable to utilize acetate, plated on medium with acetate as sole source of carbon: eight prototrophic colonies have developed

Resistance to A' may—and often will—be based on failure to take up or further metabolize A', and therefore A. Resistant mutants of either type, that is, unable to take up or to utilize A, will be auxotrophic for one or more metabolites—for short, B—which the normal strain synthesizes from A. Thus, on a medium containing A' and B (or a substance which can replace B), 'forward'-mutants can be selected because they are resistant to A' (and by hypothesis, auxotrophic for B). 'Back'-mutants, capable of utilizing A and sensitive to A' can then be selected in the usual way, that is, by inoculating the auxotrophs in the absence of B but in the presence of A.

These very obvious considerations show how wide the field of search can be for systems in which selection in both directions at high resolving power may be possible. There is no need to stress how useful this possibility is for a variety of purposes: fine recombination analysis, specific mutagenesis at the intracistron level, etc. The search for suitable systems can start either from sensitive wild-type strains, selecting from them auxotrophs by virtue of their resistance to an analogue, or from auxotrophic strains, selecting those which are resistant to an analogue of a metabolite coming before the block which causes the auxotrophy. Clearly, as resistance can be achieved in a number of ways—of which the inability to take up or metabolize the toxic substance is only one—we should expect neither every resistant to a toxic substance to be auxotrophic in respect of a metabolite related to that toxic substance, nor every auxotroph to be resistant to a toxic substance related to the growth factor required by it.

The following is an example of a system of the first kind. It was based on selection of 'forward'-mutants by means of their resistance to an analogue (fluoroacetic acid). Some of these resistant mutants are auxotrophic, and more precisely unable to utilize acetate as the only source of carbon. 'Back'-mutants of these auxotrophic resistant mutants can be selected by plating them on a medium with acetate as the only source of carbon.

Most *Aspergillus nidulans* strains can grow on acetate as the only source of carbon. For the purpose of the present work a medium of the following constitution (per 1,000 ml.) was used: ammonium acetate, 12 gm.; sodium chloride, 2 gm.; magnesium sulphate (7 H_2O), 0·5 gm.; potassium dihydrogen phosphate, 3 gm.; ferrous and zinc sulphate, traces; agar, 12 gm.; pH adjusted to 6·1 by ammonium hydroxide or hydrochloric acid.

Fluoroacetate at high concentration prevents the growth of most *A. nidulans* strains. The fluoroacetate medium used for obtaining resistant mutants was as follows (per 1,000 ml.): glucose, 5 gm.; sodium nitrate, 4 gm.; potassium chloride, 1 gm.; magnesium sulphate (7 H_2O), 0·5 gm.; potassium dihydrogen-phosphate, 3 gm.; ferrous and zinc sulphate, traces; fluoroacetic acid (technical), 30 gm.; agar, 15 gm.; pH adjusted to 6·1 by concentrated ammonium hydroxide. On this medium sensitive strains do not grow, while resistant strains grow well (Fig. 1).

To select resistant mutants, suspensions of up to about 10^8 conidia/ml. from fluoroacetate-sensitive strains capable of utilizing acetate as the only source of carbon were spread in volumes of 0·1–0·2 ml. on the surface of the fluoroacetate agar medium (25 ml./dish). When mutagenic treatment was used, for example, nitrous acid[17], part of the suspension was treated before plating. After a few hours incubation a further thin layer of the same fluoroacetate medium (3–5 ml./dish) was poured on top. Resistant colonies began to appear 3–4 days later, and by the sixth or seventh day they were isolated on a complex medium with glucose as the main carbon source. These resistant mutants were then tested for their ability to grow on acetate medium. Of the 30 resistant mutants obtained after nitrous acid treatment (Table 1) 6 were also auxotrophic, that is, not able to grow on acetate as only source of carbon (Fig. 1), and required an alternative source, for example, succinate or glucose.

Back-mutants from these resistant auxotrophs were selected by plating the conidia on medium with acetate as the only carbon source. Table 2 shows, as an example, the results of plating on such medium three different suspensions of untreated conidia from one

Table 1. SELECTION OF 'FORWARD'-MUTANTS RESISTANT TO FLUOROACETIC ACID

Exp.	Treatment	Conidia per dish (× 10^3)	Total conidia plated (× 10^3)	Resistant mutants: No.	Resistant mutants: per 10^6 plated conidia
I	None	30	30	0	0
		300	300	0	0
		3,000	6,000	16	2·6
		17,000	85,000	105	1·2
	Total untreated		91,330	121	1·3
	Nitrous acid *	30	30	4	133
		150	300	26	87
	Total nitrous acid treated		330	30	91
II	None	5	5	0	0
		50	50	0	0
		500	500	2	4
		5,000	5,000	6	1·2
		10,000	10,000	11	1·1
			15,555	19	1·2

* 7 min. in: $NaNO_2$ (M 0·02) in buffer acetate pH 4·4: survival about 45 per cent.

Table 2 SELECTION OF SPONTANEOUS 'BACK'-MUTANTS ABLE TO UTILIZE ACETATE AS SOLE CARBON SOURCE FROM ONE 'FORWARD'-MUTANT (*f* 10) RESISTANT TO FLUOROACETIC ACID AND UNABLE TO UTILIZE ACETATE

Exp	Conidia per dish (× 10^6)	Total conidia plated (× 10^6)	Back mutants No	Back mutants per 10^6 plated conidia
I	45	135	19	0 14
II	120	240	112	0 47
III	60	180	37	0 20
			168	

resistant auxotrophic strain (*f* 10) Of the 168 back-mutants from this strain obtained in this way, 24 were tested on fluoroacetic acid medium; all were sensitive

As expected, not all resistant auxotrophs back-mutate, and those which do so may back-mutate both in respect of the auxotrophy and of the resistance, or only in respect of the auxotrophy So far, 29 resistant auxotrophs of independent origin have been tested for back-mutation (minimum per strain 2 × 10^8 conidia) Of these, six did back-mutate, and one of them back-mutated in respect of the auxotrophy but remained resistant to fluoroacetic acid

This attempt in one specific case shows that the general principles mentioned at the beginning are valid and that forward and back selection, based on resistance determined by auxotrophy, is possible with high resolving power in both directions

I thank Prof G Pontecorvo for guidance, Mr E Forbes for advice and Dr O H Siddiqi for a discussion which led to this work

This work was supported by a grant from the Hebrew University of Jerusalem out of the Sir Maurice Bloch Fund

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CATABOLISM OF DOUBLY LABELLED THYROXINE *IN VIVO*

By Dr L. G PLASKETT

Department of Biochemistry, University of Edinburgh

THERE is some evidence that the peripheral breakdown of thyroxine involves a reaction sequence in which the release of inorganic iodide from the 3′ . 5′ positions precedes similar release from the 3 . 5 positions This was first clearly indicated in the work of Roche, Michel and Tata[1], in which the rate of excretion in the rat of the iodine-131 of $[3'\ 5'{}^{131}\mathrm{I}_2]$ thyroxine was found to exceed that of the iodine-131 of $[3\ 5{}^{131}\mathrm{I}_2]$ thyroxine Clearly, metabolic separation of the iodine of the two rings had occurred, compatible either with diphenylether bond cleavage or with more rapid de-iodination of the thyroxine β ring than of the α ring These conclusions from experiments *in vivo* have received support from experiments *in vitro* with rat liver extracts in which a metabolite of thyroxine was identified which contained the iodine atoms from the thyroxine α ring only[2,3] The iodine from the β ring appeared as inorganic iodide The properties of this metabolite suggested that it may still contain the de-iodinated thyroxine β ring, which could be hydrolysed off by treatment with cold weak alkali, yielding diiodotyrosine This metabolite may be a hydroxylated derivative of 3 5 diiodothyronine

The special interest of this unknown metabolite arises from the long-standing hypothesis that partial de-iodination may be necessary in order to generate an active form of the thyroid hormone from thyroxine[4–7] Studies with thyroxine analogues have shown that substituted thyronines which contain no iodine (or bromine) on the α ring are physiologically almost inactive[8], whereas compounds devoid of iodine on the β ring are often active if the iodine is replaced (though not necessarily in the 3′ 5′ positions) by other substituents such as methyl or isopropyl[9] Therefore, if an active form of the thyroid hormone is generated by partial de-iodination of thyroxine, the de-iodination must presumably occur at positions 3′ or 5′, or at both The preferential de-iodination of the β ring is therefore a subject worthy of the closest possible scrutiny, and any metabolite from which some or all of the iodine of the β ring has been removed may be identical with or closely related metabolically to the active form of the hormone

The finding of partial de-iodination *in vitro*[2,3] conflicts with a report that thyronine is the product of the de-iodination of thyroxine in rat liver slices[10], and it has been commented that this conflict cannot be resolved on present evidence[11] The conflict probably arises, however, because of the different nature of the two *in vitro* systems which were used Liver slices, which fail to de-iodinate thyroxine at a rate comparable with that observed in liver extracts, produce none of the unknown metabolite which contains the α-ring iodine atoms only and which liver extracts produce in more than 50 per cent yield[12] Similarly, the findings of Yamasaki and Slingerland[13] that liver tissue catalyses the complete de-iodination of thyroxine were obtained using homogenates instead of extracts, and it is known that these two types of preparation differ markedly in their de-iodinating properties There is therefore no conflict of experimental findings, but the publication of two sets of data, one relating to an *in vitro* system which preferentially de-iodinates the thyroxine β ring, and the other which attacks both rings indiscriminately, calls for a re-examination of the early work on the excretion-rate of iodine-131 from thyroxine labelled on either ring Such re-examination has been undertaken by me in an attempt to confirm or refute the findings of Roche, Michel and Tata[1] The experiments of the French workers have been repeated with three main differences These were (1) the use of euthyroid experimental animals, (2) the use of doubly labelled $[3\ 5'^{131}I_2,\ 3'\ 5'^{124}I_2]$ thyroxine instead of the separate use of $[3\ 5^{131}I_2]$ thyroxine and $[3'\ 5'^{131}I_2]$ thyroxine, (3) the extension of the work to investigations in the guinea pig as well as the rat.

In spite of the obvious technical advantages to be gained by using thyroidectomized animals for de-iodination studies, it was decided to use euthyroid animals because thyroidectomy and hypothyroidism are now known to influence the activity of the peripheral de-iodinases[14,15]

The use of doubly-labelled thyroxine containing a different radioactive isotope of iodine on the two rings eliminated difficulties arising from the individual variations of laboratory animals the metabolic fate of both rings could thus be determined in the same animal The doubly-labelled material was synthesized by producing [^{131}I] 3 . 5-diiodothyronine as described elsewhere[3] and iodinating this with iodine containing iodine-124 It is known that in this synthesis the iodine which is introduced during the first and second iodination stages is confined specifically to the α and β rings respectively[16] The extent of labelling on the two rings was determined by the diazo-coupling method[17]

Into each of the animals (male Wistar albino rats and male guinea pigs) 2 μc of the doubly labelled hormone (4 μgm) was injected intraperitoneally Urine and fæces were collected for six days after the injection Each urine sample (collected over successive periods of 24 hr) was counted in a type *M*6 (20th Century Electronics) β counter immediately after collection and stored for exactly twelve days before being counted a second time The radioactivity of the different urine samples decayed at different rates depending on their content of iodine-131 (half-life 8 04 days) and iodine-124 (half-life 4 24 days[18]), and by measuring the rate of decay the proportion of iodine-131 and of iodine-124 present in each urine specimen could be calculated This was done as follows Where the count-rate of the urine specimen after collection is known, it is possible to calculate a figure, x, for the expected count-rate after 12 days assuming the original activity to have been entirely iodine-131 Similarly, a figure, y, can be obtained based on the assumption that the original activity was entirely iodine-124 If the observed count-rate after 12 days is z, then the percentage of iodine-124 present on the day of collection was

$$\frac{x - z}{x - y} \times 100$$

This relationship was shown to hold for the decay of control mixtures of iodine-124 and iodine-131 of known original composition It was found possible in this way to detect the presence of only 1 per cent of iodine-124 in a mixture of the two isotopes

Figures thus obtained for the isotope composition of the radioactive urines applied to the day of collection only A mixture of iodine-124 and iodine-131 continuously changes its composition towards a heavier preponderance of iodine-131 The isotope composition of the urine samples was therefore expressed as the composition which the samples would have had on the day of injection, when the administered thyroxine contained 34·6 per cent iodine-124, all located on the β ring, and 65 4 per cent iodine-131, all located on the α ring

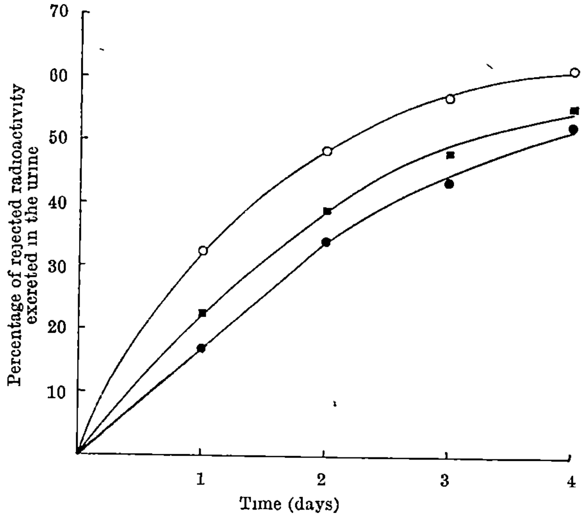

Fig 1 Urinary excretion of the iodine-124 (open circles), iodine-131 (filled circles) and total radioactivity (filled squares) of doubly labelled thyroxine in the rat as a function of time

The results showed that during the first 24 hr after injection of thyroxine the urine contained more than 50 per cent of its radioactivity in the form of iodine-124 It contained 33 per cent of the total iodine-124 administered and only 17 per cent of the administered iodine-131 There was no significant difference between the rat and the guinea pig in this respect During the second 24-hr period the two isotopes were excreted in the same ratio in which they were administered in the labelled thyroxine, the urines containing 34 per cent iodine-124 Thereafter the preponderance of iodine-131 increased During the fourth 24-hr period after injection the rats excreted urine containing only 27 per cent of its radioactivity as iodine-124 This was the last measurement which could be obtained in the rats on account of the low levels of radioactivity During the fifth 24-hr period the guinea pigs excreted urine containing 22 per cent iodine-124

Fig 1 illustrates the progress of the urinary excretion of the two isotopes with time

The work reported here thus confirms the findings of Roche, Michel and Tata[1] *In vivo* excretion of the iodine from the β ring, and therefore presumably de-iodination from that ring, is shown to occur more rapidly than from the α ring It follows that metabolic intermediates containing only the iodine from the α ring are to be expected in *in vitro* systems which truly reflect the *in vivo* happenings It cannot be denied, however, that there may be several de-iodinating pathways in the intact organism If this is the case then a pathway leading to the formation of thyronine and one generating partially de-iodinated metabolites may co-exist in the same tissue It is difficult to envisage what could be the function of the system which catalyses total de-iodination unless it be to remove excess thyroxine This hypothesis would presuppose either that thyroxine itself is the active form of the hormone or that the active form is generated by another metabolic pathway, since thyronine has no activity On the other hand, partial de-iodination holds the possibility that an active form might be produced, especially if the removed iodine is replaced by other substituents, such as hydroxyl groups The finding that 3′-hydroxy 3 5 diiodothyronine is physiologically inactive[19] does not necessarily conflict with this view because this substance is labile and may never reach the target organs intact The active form of the hormone may well be a labile compound which is formed *in situ* within the cell and which shows no physiological activity when presented to tissue extracellularly

Note Since the completion of the experimental work which is described in this communication several apparently conflicting reports have appeared on the subject of thyroid hormone catabolism Thus, further details of the kidney system which catalyses the monode-iodination of thyroxine to triiodothyronine have been published[20] By contrast with this a report has appeared which presents good evidence that the pathway from thyroxine to thyronine passes through intermediate stages of de-iodination in which the iodine atoms of the α ring are the first to be removed[21] This finding cannot be readily reconciled either with my work already referred to[2,3] or with that of Nunez and Jacquemin[22], who identified tritium-labelled iodotyrosines as metabolites of tritium-labelled thyroxine

These and other tracer investigations have brought the subject of thyroxine catabolism to the point where a very large number of the theoretically probable pathways can be shown actually to exist in *in vitro* systems In this situation, studies *in vivo* with thyroxine specifically labelled in different positions with more than one isotope may prove to be particularly valuable

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KINETICS AND OXYGEN REQUIREMENTS OF A LIGHT-INDUCED ELECTRON SPIN RESONANCE SIGNAL FROM WHOLE SPINACH CHLOROPLASTS

By W H STOREY, C. M MONITA and D. G CADENA, jun

Southwest Research Institute, San Antonio, Texas

ELECTRON spin resonance observations of suspensions of whole spinach chloroplasts performed in this laboratory have certain features which should contribute to a characterization of the structures giving rise to the signals The signals bear similarities to signals observed in other laboratories from spinach chloroplasts[1,2], from *Chlorella*[3], from both whole and fragmented red algae, strain *TX*27 (ref 4) and from crystalline chlorophylls[5,6] This article will be concerned chiefly with rise and decay kinetics of the rapidly decaying light-induced signal and with the dependence of its appearance on oxygen

Whole chloroplasts were prepared immediately following gathering of the leaves of spinach (*Spinacea oleracea*) grown by sand culture in this laboratory The preparation procedure was a modification of methods described in the literature[7,8] Sample temperature was held below 4° C and sample illuminance was maintained at a low level whenever possible A Waring blender operated for three 10-sec periods was used for the initial extraction The aqueous extraction medium was a 0 04 M sucrose solution containing 0 01 M potassium chloride and buffered with 0 05 M disodium hydrogen phosphate and potassium dihydrogen phosphate, yielding a *p*H of 6 8 After filtering the homogenate through cheesecloth, purification was accomplished by centrifugation first at 200*g* for 1 min, followed by centrifugation of the supernatant at 1,400*g* for 7 min The chloroplasts were re-suspended without washing in 5 ml of the same buffered solution used for extraction Microscopic examination showed the absence of large contaminating particles, but the absence of such small particles as grana and mitochondria was not determined The chloroplasts themselves appeared intact The concentration of chlorophyll *a* + *b* in undiluted preparations measured 1 7 mg/ml by a method described by Arnon[9] In measurements of Hill activity, 20 μmoles/ml chlorophyll/h of 2,3′,6-trichloroindophenol sodium salt (Eastman practical grade) were reduced, following a method described by Jagendorf[7] This consistently low value of Hill activity remains unexplained

Samples for electron spin resonance observation measured 8 × 23 × 0 37 mm and were contained in quartz 'Varian' electron spin resonance instrumentation operating at a modulation frequency of 100 kc/sec was used with the single modification that the slotted cover furnished for the cavity window was replaced by fifty 3-mil gold-plated tungsten wires, giving a transmission for light into the cavity of 83 per cent with negligible deterioration in cavity *Q* A Bausch and Lomb grating monochromator with incandescent light source, giving an output ranging from 6 × 10^{14} (550 mμ) to 1 4 × 10^{15} (730 mμ) photons cm^{-2} sec^{-1} at a band-width of

13 2 mμ, provided the illumination Sample temperatures were 25° ± 2° C

Two electron spin resonance signals must be distinguished There is a signal with no apparent hyperfine splitting (g = 2 0030 ± 0 0006 and line width = 7·0 gauss), so short-lived in the dark that it is obviously light-induced (*LS*, Fig 1*a*) There is a signal with apparent hyperfine splitting so long-lived in the dark (several tens of minutes) that its light dependence is not so obvious (*DS*, Fig 1*b*) The dark signal has not appeared with sufficient resolution to enable us to confirm Commoner's assignment of five lines[1] The similarity in appearance between our signals and those from crystalline chlorophyll *a* + *b* exposed to water vapour[6] is noteworthy. The action spectrum for effecting the light-induced signal has a single peak at 716 ± 5 mμ, with values of zero for the range 550–650 mμ and again above 760 mμ An action spectrum for a chloroplast paste reported in the literature is similar to that reported here at the long wave-length end, but action was reported from 500 to 650 mμ (ref 2)

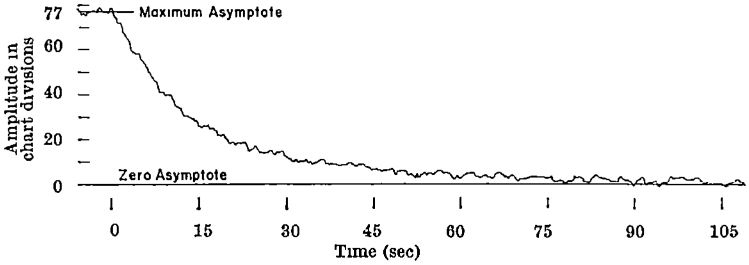

Fig 2. Decay curve for a suspension of whole spinach chloroplasts

Discussion of oxygen dependence requires that a process which inhibits the ability of the sample to give the light-induced signal be distinguished from the process by which the light-induced signal is given This inhibiting process is more rapid at 675 mμ than at 715 mμ, and it is more rapid for a sample examined within the first hour following preparation than for a sample aged overnight at 0° C with exposure to air Illumination of a sample exposed to air does not result in inhibition, but illumination with tank nitrogen bubbling through the sample does eliminate the light-induced signal Bubbling tank nitrogen through a dark air-equilibrated sample can result in a 13 per cent reduction in light-induced signal intensity, but not complete inhibition Following inhibition, with no light-induced signal observable in the range 550–760 mμ, the ability of the sample to give the light-induced signal is renewed by bubbling through it either air or tank oxygen Neither tank nitrogen nor tank carbon dioxide is effective Therefore, in order to effect the light-induced signal by 715 mμ illumination, the prior addition of oxygen to the suspension has been found a sufficient condition Since no Hill oxidants were added, there is a probable consumption of oxygen at 675 mμ through the Mehler reaction[10]. Furthermore, the dimensions of the sample cell are such that the sample can become anaerobic readily The dark signal apparently does not need oxygen

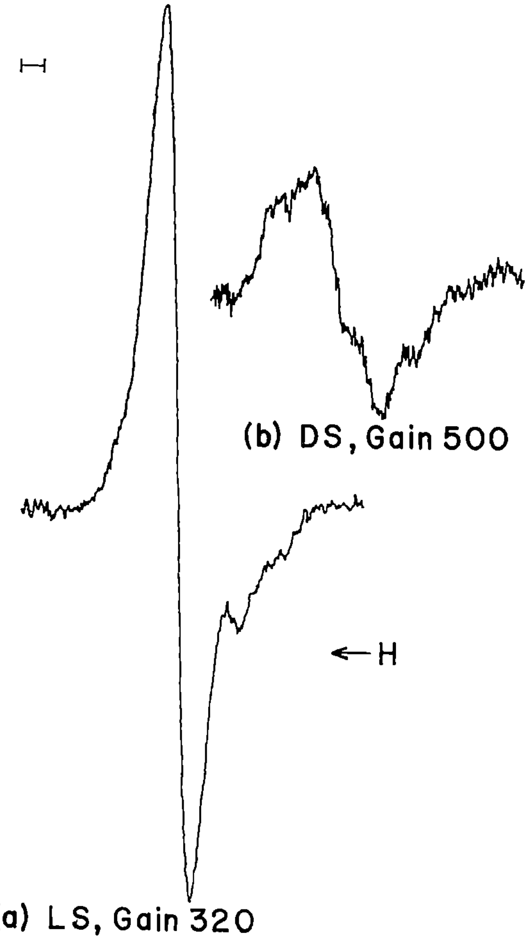

Fig 1 *a*, Light-induced (720 mμ), and *b*, dark electron spin resonance signals from whole spinach chloroplasts

Figs 2 and 3 show decay and rise curves obtained by adjusting the polarizing magnetic field H_0 to a value causing the light-induced signal to peak, and operating a shutter interposed in the light beam Analysis of the decay curves gives both a slow and a fast exponential component with a fast/slow amplitude ratio of 3/1 at zero time The decay half-times depend on the exposure of the sample to oxygen and to light Bubbling oxygen rather than air through a sample increases the decay half-times by at least one order of magnitude Exposure of the sample to 715 mμ light decreases the dark decay half-times, probably through oxygen consumption During the observation of four sets of rise and decay curves over a period of 18 min using a single air-equilibrated sample, the fast/slow decay half-times in seconds were as follows 7 4/31, 6 5/29, 5 3/27 and 4 5/25

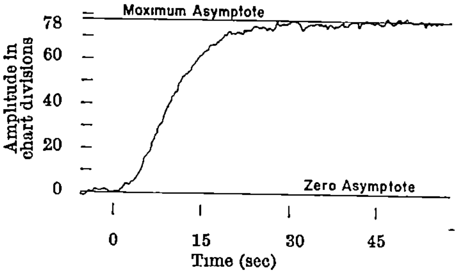

Fig 3 Rise curve at 720 mμ for a suspension of whole spinach chloroplasts

Figs 2 and 3 show the first set of decay and rise curves

During rise, the curves reached maximum slope only after several seconds of exposure to 720 mμ light In general, the slope at zero time is difficult to determine, but during its first 6 sec the rise curve shown in Fig 3 is parabolic Prior to obtaining this curve, the sample had been dark for 8 min Later curves taken after holding the sample in darkness for only 2–3 min gave briefer induction periods, as if a long-lived intermediate with no observable unpaired spin were present at zero time A reaction scheme fitting the rise curves precisely has not yet been obtained The necessity for including oxygen in such a reaction scheme cannot be asserted, since there remains the possibility that its only function in making the light-dependent signal observable is to alter the structure of some substance that is chiefly responsible for the rapid decay of the light-induced signal

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AEDES AEGYPTI (L.) AND *AEDES MASCARENSIS* MACGREGOR IN MAURITIUS: A CASE OF GENE SURVIVAL FOLLOWING SPECIES ERADICATION?

By G. A. H. McCLELLAND*
London School of Hygiene and Tropical Medicine, W.C.1
AND
RAYMOND MAMET
Medical and Health Department, Mauritius

THE successful hybridization of the mosquito *Aedes aegypti* (L) with *Aedes simpsoni* (Theo)[1], using a method of force-mating[2], has been followed by attempts using other species with genitalia closely resembling *A aegypti* and *A simpsoni* Among these, matings of *Aedes mascarensis* MacGregor and *A aegypti* were fully successful in both directions and yielded vigorous fertile hybrids

The latter F_1 hybrids resembled *A aegypti* except for partial expression of the strikingly pale mesonotal scaling of *A mascarensis* (Fig 1*A*, *C*, *D*) The F_2 and subsequent hybrids showed independent segregation of at least four of the characters used in distinguishing the adults of *A. mascarensis* from *A aegypti* Two of these (absence of femoral 'knee' spots and broadly crescentic anterolateral mesonotal spots) have been separately maintained on a background otherwise like *A aegypti* for up to five further generations The question immediately raised is that of natural hybridization

A mascarensis was first described in 1923 [3], and is known only from the island of Mauritius in the south Indian Ocean where *A aegypti* also occurred until its reported eradication in 1950 [4] Although the larva and male genitalia resemble those of *A aegypti*, the adults of the two species can be separated on nine distinct morphological characters of the head and thorax The genetic control of four of these characters at independent loci, as here reported, indicates that *A mascarensis* is not merely a polymorphic form of *A aegypti* What is known of its biology suggests that it is a species of distinct habits and distribution

A aegypti in Mauritius was restricted to coastal urban areas while *A mascarensis* is largely sylvan, larvæ of the two were never found in the same breeding place[3,5] *Aedes albopictus* Skuse, the commonest species in Mauritius and known competitor of *A aegypti* in Malaya and elsewhere[6,7], occurred in all the habitats of *A mascarensis*, larvæ of the two species often sharing the same tree-hole breeding place[3,5] *A albopictus* was moreover present in the semi-domestic habitat of the urban fringe[5] where it possibly interfered with the outward spread of *A aegypti* and contributed to the virtual isolation of that species from *A mascarensis*

The possibility of hybridization between *A aegypti* and *A mascarensis* in nature was first suggested as the possible origin of another Mauritian 'species' *Aedes vinsoni* Mattingly[8], combining superficially the pale mesonotum of *A mascarensis* with the pale abdomen of *A aegypti* var *queenslandensis*[8] In the present laboratory work, F_1 and F_2 hybrids with the palest variety *queenslandensis* from Saudi Arabia showed no approach to *A vinsoni* Furthermore, the type specimen of *A vinsoni* in the British Museum (Natural History) shows a clearer affinity with *A albopictus* than with either *A mascarensis* or *A aegypti* The recent capture on the island of a female mosquito intermediate between *A vinsoni* and *A albopictus* emphasizes this point Mattingly[9], who describes it as a second specimen of *A vinsoni*, again raises the possibility of hybridization There seems, however, no reason why '*A vinsoni*' should not be a rare variety of *A albopictus* genetically analagous to var *queenslandensis* of *A aegypti*

In 1961, out of a total of 120 adult *A mascarensis*, both wild-caught and reared in London from eggs, 45 lacked the mesonotal pale scaling so characteristic of the species (Fig. 1*B*) In all other respects these resembled the remainder, which agreed with earlier descriptions[3,5,10] In 1962, further adults reared in London, from eggs laid in Mauritius by wild-caught females, revealed three other characters of *A aegypti* occurring independently At the most only 4 males and 11 females out of 38 of each were true to type,

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* Present address Department of Biology, University of Notre Dame, Indiana, U S A

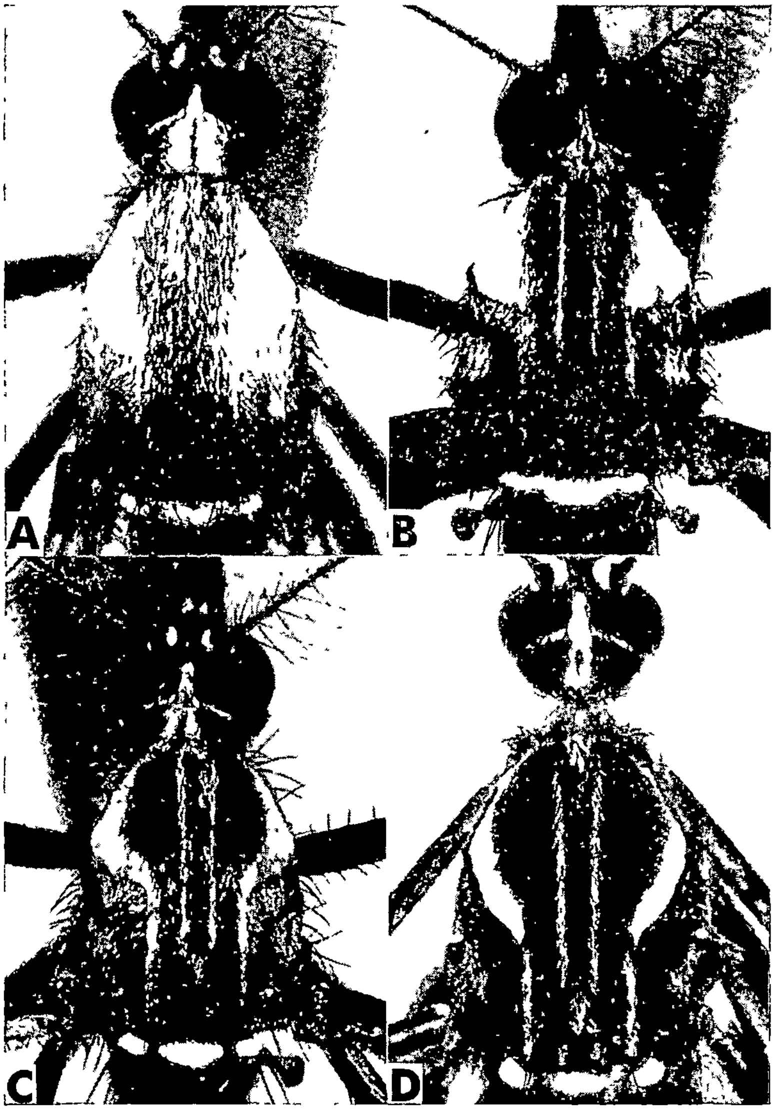

Fig 1 Dorsal aspect of the head and thorax of anæsthetized female mosquitoes *A*, typical *Aedes mascarensis* MacGregor reared from eggs laid by wild-caught female (1961), *B*, atypical form from same egg batch as *A*, *C*, F_1 laboratory hybrid of a typical *A mascarensis* male with a female of *Aedes aegypti*, *D*, typical *A aegypti*

and 3 females superficially resembled *A aegypti* Had such intermediate forms occurred three or four decades ago it is unlikely that MacGregor[3, 5] would have overlooked them

How can the presence of these '*aegypti* genes' in the population of *A mascarensis* be explained? It is scarcely plausible that so many dominant mutations, each coinciding with a character possessed by *A aegypti*, should have arisen to co-exist with high frequencies Alternatively, these genes might be relicts in a case of incipient speciation The recently eradicated population of *A aegypti* in Mauritius was probably one or both of the paler urban forms[6,8] which spread round the world with man but nowhere, outside the African mainland, evolved truly feral, let alone sylvan populations The possibility that not only did such speciation happen in Mauritius in less than a thousand years[11], but also that in the presence of *A albopictus* there arose a form as distinct from *A aegypti* as *A mascarensis*, would be remarkably rapid evolution If it had, it is even more surprising that the eradication of the initial form should seem to lead to a reversal of the process A third possibility will now be suggested

In the colour of its abdomen *A mascarensis* resembles not the type form or variety *queenslandensis* but the feral sub-species *formusus* of *A aegypti*[6] and other closely related species on the African mainland It seems possible that *A mascarensis* developed in isolation from an ancient population of *A aegypti* or some common ancestral form The reverse, that *A aegypti* and perhaps other group *A Stegomyia* in Africa might have arisen from something like *A mascarensis*, has been suggested[6] In either case, later re-introductions of man-adapted urban forms of *A aegypti* into Mauritius would have been virtually isolated from sylvan *A mascarensis* until recent urban expansion and perhaps the effect of control measures may have stimulated some overlap in their distribution Under these conditions some hybridization and consequent gene exchange is a distinct possibility

In the light of this point of view, it appears that although *A aegypti* has now been eradicated from the island, more than ten years of surveillance having failed to reveal it, its genes apparently still survive in *A mascarensis* at frequencies which may be increasing Although *A mascarensis* has in no way changed its habits since the eradication of *A. aegypti* it may now possess new genetic potential Selective forces might slowly bring about a recrudescence in the island of an urban *Stegomyia* not necessarily wholly like *A aegypti*, but capable of feral coexistence with *A albopictus* and probably less amenable to control Relative to *A aegypti* elsewhere the situation in Mauritius is almost certainly unique, but it suggests an additional type of hazard that may be met in insect control operations

A full account of the present work on hybridization in *Stegomyia* is being prepared for separate publication This work formed part of a research programme supported by the Colonial Development and Welfare Fund We thank Mr P F Mattingly for allowing us to read his paper in manuscript

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SOLAR FLARE EFFECTS ON LIVING ORGANISMS CONFINED IN MAGNETIC FIELDS

By W C. LEVENGOOD

Institute of Science and Technology, University of Michigan, Ann Arbor, Michigan

AND

M P. SHINKLE

Indiana University School of Medicine, Indianapolis, Indiana

SOLAR phenomena are known to influence living organisms on Earth The investigations of A E Douglass, for example, indicated a relationship between sun-spot numbers and the growth patterns in tree rings Solar flare activity, which is at present recorded on a diurnal basis, has received less consideration in terms of possible biological effects, even though active flares are known to be rather prolific sources of energetic particles Most evidence of the terrestrial effects of solar flares has been obtained through investigations of radio communication fade-out and magnetic field variations during periods of intense flare activity

It will be shown here that secondary effects from solar flares influence progeny variations in the fruit fly, *Drosophila melanogaster*, when these organisms are subjected to a magnetic field The present investigation came about as the result of earlier work[1], which disclosed that *Drosophila* productivity was affected by various environmental conditions A correlation was shown between barometric pressure recorded during the initial mating period and the resulting progeny Growth in an electric field reduced this correlation and produced a 35 per cent greater yield compared with control cultures In a later discussion[2], this electric field effect was attributed to variations in air-ion densities

The deflexion of charged particles by magnetic fields provides a possible tool for changing ionic concentrations within limited spatial regions It was postulated that organisms grown within a magnetic field would conceivably be exposed to a different environment of energetic particles than in control cultures Experimental work was initiated, and an influence of the magnetic field was indicated It was during a critical analysis of these experimental results that the possibility of solar flare influences was discovered

Drosophila were exposed to magnetic fields by placing culture bottles on the poles of permanent magnets, as shown in Fig 1 (Magnets were magnetizers (type *A*-1007) made by Indiana Steel Products, Valparaiso, Indiana) The field strength at the centre of each 4 5 cm square pole was about 1,000 gauss on the face and 350 gauss 1-in above (the separation between poles was approximately 8 cm from centre to centre) The magnets were set in one of four geomagnetic orientations (designated for each set of data), and this orientation was maintained throughout an entire series of generation matings For example, east (N)–west (S) gives the geomagnetic direction and the letters in parenthesis the direction of the poles of the magnet , in this example the N pole is oriented east and S pole west The culture bottles were about 5 cm in diameter and 11 5 cm high and covered the pole pieces Each bottle was held in position on the magnet with tape, and media was placed in the bottom to a depth of about 2 5 cm Room temperature was held at 22° ± 2° C

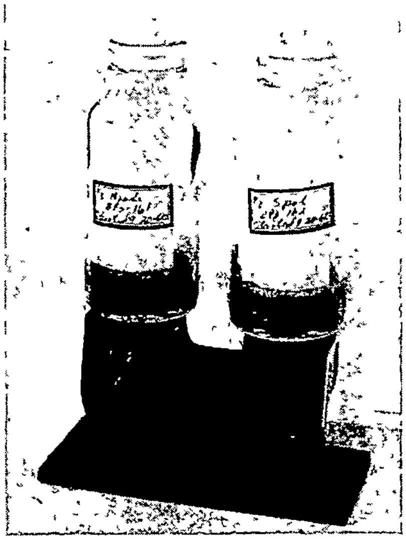

Fig 1 Test magnet with culture bottles in position

Culture bottles designated as (N) and (S) pole were compared as pairs in each generation, and after a given generation series was started in the F_1, repeated filial generation crosses were made Three adult pairs removed from the preceding F generation were left 10 days in the bottles, then removed before new flies emerged Several countings were made over an 8-day interval starting with the day the new generation flies were seen The cultures were left in the magnetic field except during the brief periods of counting Cultures designated as 'controls' were also simultaneously grown out of the magnetic field and all cultures, including the controls, were initiated as well as removed and counted on the same dates Flies exposed to the field were normal or wild-type (Flies were obtained from the Carolina Biological Supply Co , Elon College, N C regular banana cultures were used with 0 1 per cent mould inhibitor)

These data were statistically analysed with a previously used[1] correlation coefficient (r) for ungrouped data given as .

$$r = \frac{n\Sigma F_t \quad M - (\Sigma F_t)\ (\Sigma M)}{[n\Sigma F_t^2 - (\Sigma F_t)^2]^{1/2}\,[n\Sigma M^2 - (\Sigma M^2)]^{1/2}} \qquad (1)$$

where F_t represents the total number of flies per filial generation, n the number of generations and M the independent parameter being examined, such as barometric pressure (P), solar flare activity (S_F) or a factor involving both (to be discussed later) The results are considered significant at the 95 per cent confidence-level if

$$r > \pm 1\ 96/(n-1)^{1/2} \qquad (2)$$

Barometric pressures were recorded in the laboratory with a mercury barometer The solar flare index was obtained from preliminary and summary reports of solar activity issued by the High Altitude Observatory, Boulder, Colorado (Solar activity summaries prepared by D E Trotter and W O Roberts, High Altitude Observatory of the University of Colorado for the International Geophysical Corporation) The barometric pressure P and solar flare S_F values were averaged over a 72-hr period covering the day before, the day of and the day after the initial mating of each generation The use of this specific 72-hr period is based on two factors, one is the previous findings[1], which showed that this time-period produced the highest degrees of correlation with barometric pressure, and the second factor is the unsuccessful attempts (low correlations) in the present study when using solar flare activity values covering other time intervals within the generation periods

An analysis (using equation 1) of the progeny yields obtained from a number of generations grown in the magnetic field disclosed a low correlation of progeny with barometric pressure, with r values considerably below the level of significance given by equation (2) This finding was not in accord with the results from control cultures (grown out of the field), which showed positive, significant correlations between progeny and barometric pressure This reduced correlation between progeny grown in the magnetic field and barometric pressure indicated that the magnetic field altered the environmental conditions Solar effects were considered as possible factors in changing the environment within the magnetic field No obvious correlations were found between daily sun-spot numbers and progeny Diurnal solar flare activity values did, however, show interesting possibilities

Reported flare activity values may show day to day changes of several orders of magnitude, therefore the logarithm of solar activity was used for correlation purposes This logarithmic treatment of flare activity reduced the marked fluctuations to reasonable values for comparing with progeny and barometric pressure parameters, both of which generally disclose variations of less than one order of magnitude This solar activity parameter is

$$M = \log(S_F + 1) \qquad (3)$$

where ($S_F + 1$) allows, on a logarithmic scale, a data point for an occasional sequence of zero values for S_F over the 72-hr mating period

Progeny yields from a nine generation series raised in the magnetic field are shown in Fig 2 and compared with the solar flare activity The method of plotting the results in Fig 2 was chosen simply as a convenient way to illustrate the corresponding maxima and minima in the progeny and solar flare curves The environmental parameters (solar flare activity and barometric pressure) and progeny yields are time independent events (that is, no systematic variations with time), therefore, in the statistics used (equations 1 and 2), only the interdependence between the environmental parameters and progeny yields are considered The progeny curves in Fig 2 appear to vary inversely with solar flare activity (equation 3) Although both the N and S progeny curves in Fig 2 show similar variations, the S pole progeny variations disclose a slightly higher degree of inverse fluctuation with solar flare activity , the correlation coefficient for the N pole data in Fig 2 is $r_N = -0\ 78$, and the S pole $r_S = -0\ 82$, both significant at the 95 per cent level of confidence

Each generation series grown in the magnetic field was continued until the flies in a culture on one or both poles either died out or gave very few progeny, and termination (zero progeny) often occurred when flare activity was high, as happened, for example, in the series shown in Fig 2 The F_9 mating period in Fig 2 was on April 26, 1961, and the preliminary report stated that the flare activity on this date "caused a short wave radio disturbance"

In one series of tests, two magnets were oriented east (S) – west (N) and each new generation series was initiated at the same time on both magnets The progeny yields from the set of S pole cultures are shown in the middle set of curves in Fig 3 for a nine-generation sequence The S pole curves disclose similar fluctuations whereas the deviations in the N pole data (bottom curves in Fig 3) are more pronounced Also given in Fig 3 (upper curve) is the solar flare activity for the same generation periods Again it appears that increases in solar flare activity correspond with decreasing trends in progeny yields This effect may be especially noted in the S_2 curve in Fig 3, where the progeny variations follow the fluctuations in the solar curve more closely

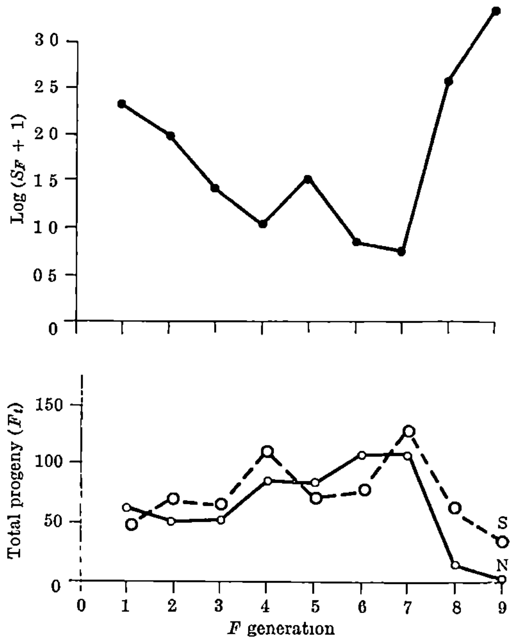

Fig 2 Generation series indicating inverse trends with solar flare activity (east (N)–west (S) orientation) Lower dashed line, progeny from culture on S pole, lower solid line, progeny from culture on N pole

than the S_1 curve The correlation coefficients listed in Table 1 also disclose greater negative r values for S_2 than S_1 data The series from magnet 1 is the only case (with east–west orientation) where the data from both poles disclosed r values below the 95 per cent level of significance Although S_1 shows a low r value a general inverse trend with solar activity is indicated in Fig 3

Table 1 CORRELATION COEFFICIENT FOR TEMPORALLY CORRESPONDING GENERATIONS ON TWO MAGNETS ORIENTED EAST (S) – WEST (N)-DATA SHOWN IN FIG 3

Magnet	$M = \log (S_F + 1)$ N	 S
1	—0 40	—0 23
2	—0 14	—0 65

The inverse correlation of progeny grown in the magnetic field with solar flare activity suggests a possible influence from ionizing particles on factors controlling the productivity of *Drosophila* Ions with known charges have been shown by Krueger and Smith[3] to affect various physiological processes In the experiments reported here the magnetic fields are of sufficient strength to alter the air-ion environment in the regions over the poles Magnetic fields of the strength used here can be shown to influence the paths of incoming particles over a range of energies[4] It is further suggested that charged particles may be differentially selected over the pole regions Whether a given particle spirals into or away from a magnetic pole region depends on the charge of the particle, its direction and velocity and the strength and direction of the magnetic field The density of ions within the bottles, placed on the magnets, may be increased by high-energy particles being drawn into the field and producing ionization along their path These penetrating energetic particles may conceivably be associated with solar flare activity

At periods of intense flare activity the density of ionizing particles in the atmosphere is known to increase[5] High barometric pressures may, however, compensate for increases in ionizing particles by absorption in a more dense atmosphere During periods of high barometric pressure fewer particles reach the Earth's surface, and because of the interrelationship both the solar flare and barometric pressure parameters should be taken into consideration when comparing control data with progeny from cultures grown in the magnetic field

This problem was first considered by using controls or progeny grown out of the magnetic fields Progeny from control cultures show a correlation with barometric pressure, but do not indicate a significant correlation with S_F (equation 3) An illustration of these differences in correlation is given by the control series shown in Fig 4 plotted with the barometric pressures to show the corresponding maxima and minima As shown by the r values in Table 2 the correlation between progeny and P is significant whereas there is no correlation with the S_F factor (equation 3)

Table 2 CORRELATION COEFFICIENTS FOR CONTROL DATA FROM 16 GENERATIONS SHOWN IN FIG 4

Parameter (M)	r
P, barometric pressure (av)	+ 0 73
Log (S_F + 1) (equation 3)	+ 0 06
[P – log (S_F + 1) /10] (equation 4)	+ 0 80

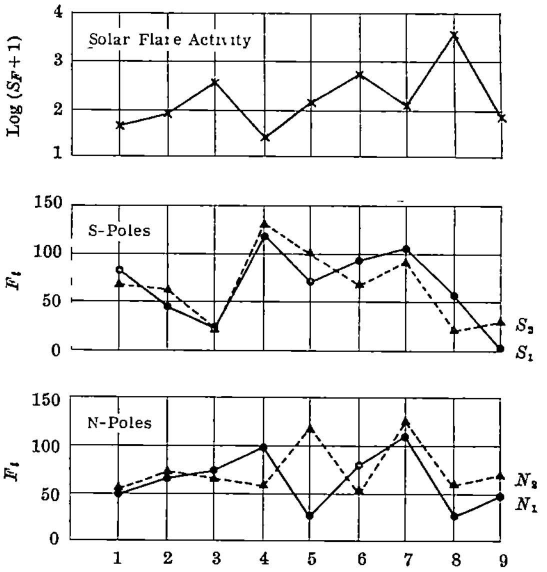

Fig 3 Progeny and solar flare data using two magnets (oriented east (S) – west (N)) on which cultures were grown simultaneously

An empirical relationship was worked out which incorporated both the P and S_F factors The equation is the result of various combinations and adjustments in the P and S_F parameters It is realized that with more exhaustive analysis this equation might be further modified and bettered , however, in its present form it did allow statistical comparisons to be made between the magnetic field and control data In this equation the flare activity is subtracted from the barometric pressure, the reason for this being the previously mentioned compensating effect of P on ionizing particles By subtracting the S_F function the effects are not minimized Expressed as a function of progeny this relationship is given by

$$F_t = K\,[P - \log (S_F + 1)/10] \qquad (4)$$

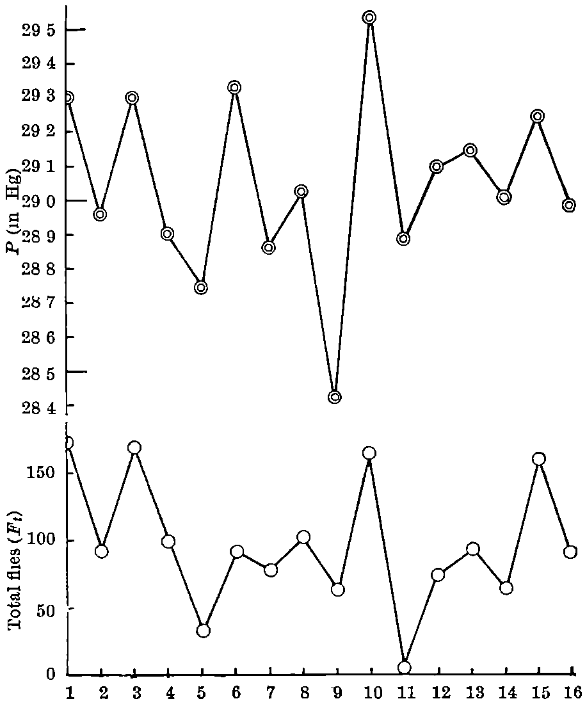

Fig 4 Control progeny (grown outside magnetic field) showing variations with barometric pressure (P is average of 72-hr period)

where K is a constant The solar flare function is divided by 10 simply to make the magnitude of the changes comparable to the tenths of an inch changes in barometric pressure As shown in Table 2 this equation gives about the same degree of correlation with progeny as when using only the P parameter

The application of the function within the brackets in equation (4) to magnetic field data is demonstrated in Fig 5 The solid line in the upper set of curves shows the flare activity function (equation 3) and the broken line the equation (4) relationship It may be seen that the empirical relationship (equation 4) varies directly with F_t whereas the flare activity, as in Figs 2 and 3, varies inversely Again the maxima and minima in the S pole curve in Fig 5 appear to correspond more closely with the maxima and minima in the upper curves than does the N pole results The correlation coefficients are given in Table 3 for the data plotted in Fig 5 and the t values substantiate the fact that the correlations are greater for the S pole results

Table 3 COMPARISON OF CORRELATION COEFFICIENTS USING FLARE ACTIVITY AND AN EMPIRICAL FUNCTION, MAGNET ORIENTATION EAST (N) – WEST (S) FOR DATA SHOWN IN FIG 5

$\log (S_F + 1)$		$[P - \log (S_F + 1)/10]$	
N	S	N	S
−0.36	−0.90	+0.04	+0.78

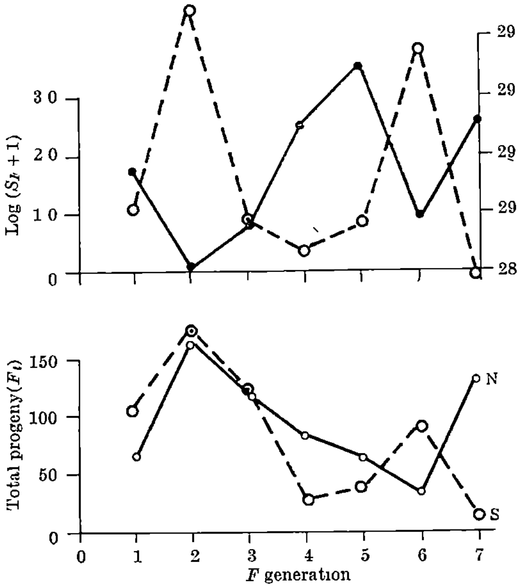

Fig 5 Magnetic field series (east (N) – west (S) orientation) Upper solid line, solar flare activity (equation 3), upper dashed line, empirical relation (equation 4) Lower dashed line, S pole progeny, lower solid line, N pole progeny

It should be pointed out that in all the magnetic field series, which have been discussed in detail the magnets were oriented in an east–west geomagnetic position Although many more series will have to be investigated, it appears that the inverse correlation between progeny and solar flare activity is much higher from the results obtained on east–west oriented magnets than with north–south Three series have been completed on magnets in the north–south position and the correlation with solar flare activity was below the level of significance in every case, in fact, the t values tended to be low and positive By using simplified conditions and the incorporation of the finding of an anisotropic distribution of particles arriving parallel to the orbital plane of the Earth[6] (general east-west direction), calculations suggest that the indicated orientation effect may be attributed to the manner in which charged particles encounter the fields surrounding the test magnets As these analyses become more refined and progeny data accumulate, it is hoped that the possible orientation effects, differences between the N and S pole results, etc, may be explained in a more rigorous manner.

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OCCURRENCE OF SPREAD-F AT MAGNETICALLY CONJUGATE POINTS

By D G SINGLETON

Department of Physics, University of Queensland

RECENT investigations have revealed striking similarities between the occurrence of such geophysical phenomena as auroræ[1], very-low-frequency electromagnetic emissions[2], and magnetic disturbances[3] and micropulsations[2] at magnetically conjugate locations These phenomena are all connected in some way with the Earth's magnetic field The existence of correlation between conjugate points therefore suggests that the individual lines of force, stretching from the northern to the southern hemisphere, play an important part in the physical mechanisms which result in these phenomena Spread-F (ref 4) is another geophysical phenomenon which is dependent on the Earth's magnetic field, its occurrence correlates with magnetic disturbance[5] and varies in a systematic manner with geomagnetic rather than geographic latitude[6] It is of interest, therefore, to see if spread-F occurs simultaneously at magnetically conjugate points The existence, or otherwise of such a correlation and the implied importance, or otherwise, of the individual lines of force will obviously have important implications with regard to the formulation of a successful theory of spread-F

Because of the difficulty of obtaining direct access to ionograms of suitably located stations, the spread-F occurrence data had to be obtained from f-plots in a manner described elsewhere[6] Examination of the world-wide distribution of ionosonde stations which produce f-plots on a routine basis[7] reveals that the

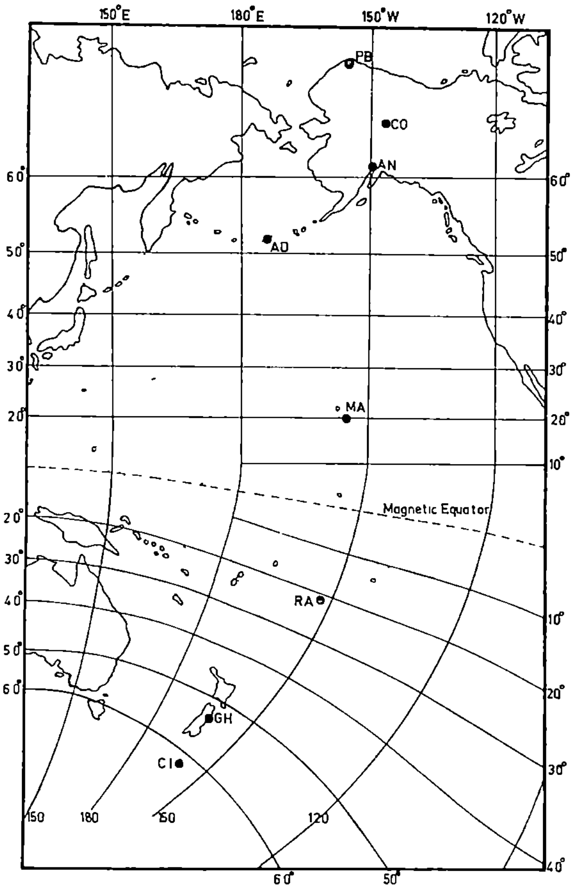

Fig 1

group of stations shown on the map of Fig 1 is the only group which contains stations which are nearly magnetically conjugate On Fig 1 the northern hemisphere grid of lines consists of the indicated lines of geographic latitude and longitude, while the southern hemisphere grid is the projection of the northern hemisphere grid to the southern hemisphere via the lines of magnetic force[8] How close Campbell Is (CI) and Anchorage (AN), Godley Head (GH) and Adak (AD) and Rarotonga (RA) and Maui (MA) are to being conjugate points can be gauged from an examination of Fig 1

Using the daily totals of hours in which spread-*F* occurred, Campbell Is , Godley Head and Rarotonga were correlated with their conjugate point stations and one or two stations north and south of their conjugate points The results of this analysis for the months of March, June and December 1959 are listed in cols 3, 6 and 9 of Table 1 With two exceptions (Campbell Is –Anchorage and Rarotonga–Maui both in December) the correlation between conjugate points is above the 10 per cent significance-level, whereas the correlation between each of the southern hemisphere stations and the stations neighbouring on its conjugate point station are either negative or positive and not significant These results suggest that the levels of occurrence of spread-*F* at conjugate point stations move together and that this connexion between the northern and southern hemispheres is limited to regions in the immediate vicinity of the conjugate points

The positive correlation of the daily totals of occurrence-hours at two stations does not necessarily imply that spread-*F* occurs simultaneously at the two stations It is, however, of importance from the point of view of the formulation of a successful theory of the formation of spread-*F* irregularities, to establish whether spread-*F* does occur simultaneously in the vicinity of magnetically conjugate points or not This has been attempted by applying a chi-square (χ^2) test to the night-time occurrence data Here the hypothesis is made that spread-*F* may occur in a purely random way at the two stations the occurrence data of which are being compared To test this, the presence or absence of spread-*F* on each hour of universal time at one station has been compared with that at the second in the usual way to obtain a value of χ^2 The resulting values of χ^2 for the station pairings used previously are listed in cols 4, 7 and 10 of Table 1 It will be noted that the values of χ^2 obtained are small except for the conjugate point stations for which they are, in the majority of cases, quite large The exceptions to this are Campbell Is – Anchorage in December and Godley Head–Adak in June The values in brackets in cols 4, 7 and 10 represent the probabilities that the departures of the observed distributions from the hypothetical random

Table 1 RESULTS OF AN ANALYSIS OF SPREAD-*F* AT MAGNETICALLY CONJUGATE POINTS

Combination of Stations		March 1959			June 1959			December 1959		
Station *A*	Station *B*	ρ	χ^2	χ^2 < 6 Mc/s	ρ	χ^2	χ^2 < 6 Mc/s	ρ	χ^2	χ^2 < 6 Mc/s
Campbell Is (CI)	Point Barrow (PB)	0 12	0 08 (0 78)	0 98 (0 32)	−0 10	0 90 (0 34)	1 6 (0 21)	−0 08	0 23 (0 63)	1 40 (0 23)
	College (CO)	−0 09	0 12 (0 72)	0 02 (0 89)	0 03	2 6 (0 11)	0 6 (0 45)	0 16	0 08 (0 78)	0 5 (0 49)
	Anchorage (AN)	0 44	4 2 (0 04)	0 07 (0 79)	0 46	19 9 (<0 001)	0 03 (0 86)	0 01	0 03 (0 86)	1 23 (0 25)
	Adak (AD)	−0 48	0 88 (0 35)	0 06 (0 80)	−0 04	2 8 (0 10)	0 09 (0 76)	−0 34	0 04 (0 84)	0 50 (0 49)
Godley Head (GH)	College (CO)	0 13	1 26 (0 26)	0 08 (0 78)	0 04	0 23 (0 64)	0 28 (0 60)	−0 20	0 01 (0 92)	+
	Anchorage (AN)	−0 56	0 07 0 79	+	−0 01	1 09 (0 30)	0 86 (0 36)	−0 02	0 59 (0 45)	0 63 (0 42)
	Adak (AD)	0 59	14 5 (<0 001)	0 23 (0 64)	0 33	0 5 (0 49)	0 01 (0 92)	0 36	12 5 (<0 001)	1 79 (0 18)
	Maui (MA)	0 10	0 17 (0 68)	+	−0 23	0 51 (0 48)	+	−0 27	0 35 (0 58)	0 89 (0 34)
Rarotonga (RA)	Anchorage (AN)	+	+	+	−0 01	0 01 (0 92)	0 66 (0 42)	0 04	0 68 (0 43)	+
	Adak (AD)	+	+	+	−0 01	0 15 (0 70)	0 46 (0 15)	−0 01	0 23 (0 63)	+
	Maui (MA)	+	+	+	0 68	22 9 (<0 001)	+	0 23	4 2 (0 04)	+

The correlation coefficients (ρ) have a 1 per cent significance-level of 0 45 and a 10 per cent level of 0 30 + No result because of no occurrences at one or both stations distributions ($\chi^2 = 0$) could have arisen due to chance. It appears, therefore, that the hypothesis that the incidence of spread-*F* at one point is random and independent of that of another is false in the case of conjugate point stations but may be valid for the other stations compared

It has been demonstrated[9,10] that high incidence of spread-*F* is most likely when the critical frequency of the F_2-layer is low. It may be, therefore, that the apparent association between the incidence of spread-*F* at magnetically conjugate points is due to similar variations of this parameter at conjugate locations To test this the above χ^2 analyses were repeated using the spread-*F* occurrence data for only those periods when f_0F_2 was less than 6 Mc/s at both stations The resulting values of χ^2 and their associated probability figures (in brackets) are listed in cols 5, 8 and 11 of Table 1 It is immediately obvious that the apparent association between spread-*F* occurrence at conjugate points is dependent on the range of critical frequency considered This suggests that the coupling of spread-*F* occurrence in the northern and southern hemispheres, via the lines of force, is primarily due to the simultaneous occurrence over limited areas about conjugate points of periods when the critical frequency is low That there should be such large-scale coupling of *F*-region ionization at magnetically conjugate points has been suggested on theoretical grounds by Farley[11]

A more detailed account of this and other aspects of the correlation between the occurrence of spread-*F* at spaced stations will be presented elsewhere

I thank Prof H C Webster and Mr J Crouchley for advice and Mrs D. Neilsen for assistance with the calculations The *f*-plot data were kindly supplied by the Ionospheric Prediction Service

This work is sponsored by the Radio Research Board of the Commonwealth Scientific and Industrial Research Organization

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EARTHQUAKE SERIES IN ST. KITTS-NEVIS 1961-62

By Dr G. R ROBSON and K. G. BARR

Seismic Research Unit

AND

G W. SMITH

Soils Research Section, University of the West Indies, Trinidad

In November and December 1961 strong earthquakes were felt in Nevis and many minor earthquakes followed in the following months A few are occurring at the present time

The island of Nevis is formed for the most part of material erupted from Nevis Peak, a strato volcano, 3,200 ft in height The peak has a breached summit crater a little more than 1 km in diameter within which there is a small dome of hornblende andesite lava The later eruptions from the peak appear to have been of the ash-flow or 'nuée ardente' type, though none has occurred in post-Columbian times Recent gravity faults cut the youngest rocks, the displacements being such as to depress Nevis Peak Soufriere activity now occurs on these faults

In 1952 P L Willmore[1] described in *Nature* the earthquake series in St Kitts–Nevis in 1950–51 In 1950 the earthquakes began on December 27, maximum intensities (VII–VIII on the Mercalli scale) being felt in the western and southern parts of Nevis More than 150 earthquakes occurred in January 1951, and minor renewals of activity occurred in March and May Willmore arrived in Nevis early in February and his shock recorders were used to locate some of the March tremors, which were found to lie within the island, roughly north-west and south-east of Nevis Peak Willmore concluded that the activity never reached the stage when emergency measures to deal with an imminent volcanic eruption appeared to be required

In 1953, two years after this earthquake series, a new soufriere began to form at Cades Bay in the north-east of the island, and in 1957 the heat flow from this was about 4 × 10^4 cal/sec

Following recommendations made by Willmore at the request of the Colonial Office, the Seismic Research Unit was set up in 1952 with headquarters in Trinidad and equipped with a network of vertical component Willmore–Watts seismographs, one instrument being installed in each of the larger British islands in the eastern Caribbean

The recent activity in Nevis began with a strong earthquake on November 2, 1961, at 19.04 local time The epicentre of this earthquake is shown on Fig 2, the focal depth was about 10 km and the magnitude 6 3 Intensities experienced were VI–VII in Nevis and IV–V north of the south-eastern peninsular of St Kitts More than 80 after-shocks were recorded by the seismograph in St Kitts during the following five days Many of these earthquakes were felt in Nevis and some of them were felt in St Kitts

A second strong earthquake followed on November 7 at 19 40 h (Fig 2) The focal depth once more was about 10 km and the magnitude 5 9 Intensities were similar to those on November 2 Twenty-six after-shocks were recorded in St Kitts during the following ten days At the request of the Government of St Kitts–Nevis–Anguilla, Robson visited St Kitts on November 21 At this time no near earthquakes

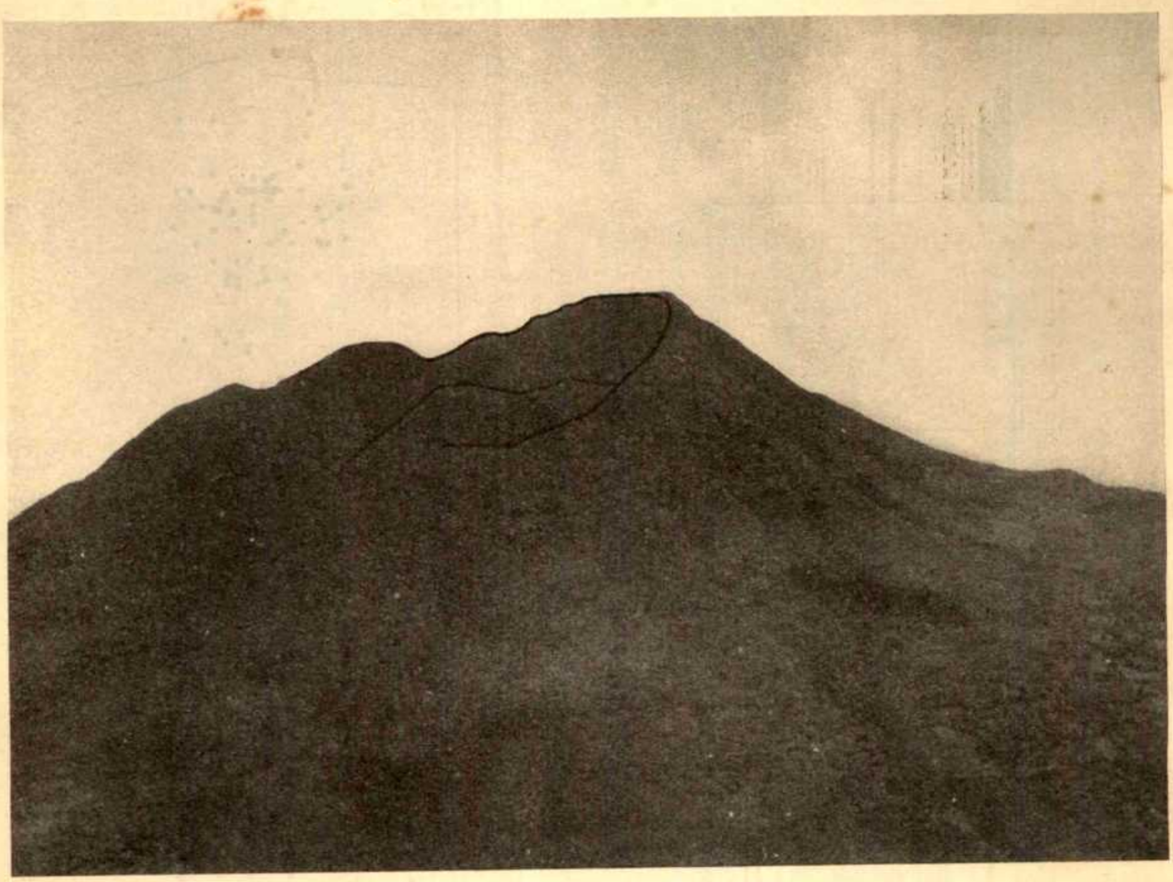

Fig. 1. Nevis Peak from the air looking north-east. The mountain is a strato volcano. A low dome of horneblende andesite fills the crater. The eastern rim of the crater forms the peak, while the western rim is breached and overlapped by the dome

were recorded in St. Kitts, and none was felt in Nevis. Cades Bay soufriere in Nevis was visited but there was no significant change in the heat flow from it. The Government was advised that no immediate action was required.

On December 14 a significant renewal of seismic activity occurred. Portable seismographs were flown to Nevis on December 19. These began recording on December 21 and are still in operation there.

The portable equipment consists of three Willmore 1-sec seismometers with low-frequency transistor amplifiers connected through telephone wires to $\frac{1}{4}$-sec galvanometers at a central recording station, which is equipped with a chronometer and radio. Differences in arrival times of earthquakes waves at the three stations can be estimated to 0·1 sec. Epicentre, focal depth and magnitude have been estimated for all earthquakes recorded at the three stations.

The shallow depth and short range of the earthquakes make it impossible to calculate magnitudes in the normal way. Instead, the observations of Carder and Cloud[2] and Romney[3] for explosions were used to compute magnitudes equivalent to Gutenberg's body wave magnitudes.

The results are shown in Figs. 3–5. The depth of the earthquakes varies from 1 to 11 km. It will be observed that the earthquakes recorded after December 21 do not occur close to the epicentres of the strong earthquakes of November 2 and 7 but are grouped about Nevis Peak. Furthermore, the energy released by these late earthquakes does not fall regularly with time as we have observed for after-shock sequences of strong earthquakes elsewhere in the eastern Caribbean. This has led us to suppose that these late earthquakes are not a simple after-shock sequence of the earthquakes of November 2 and 7 but that another factor is involved, in addition to strain release by the large shocks. It seems likely that this other factor is the presence of a body of magma beneath Nevis Peak, and that the Nevis Peak earthquakes are a result of changes in the shape

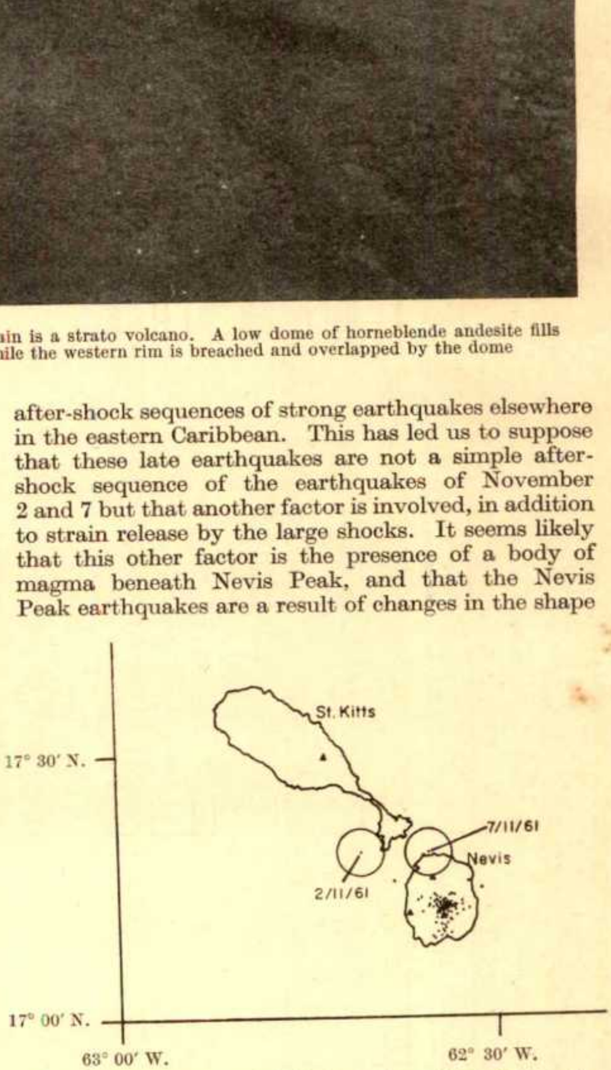

Fig. 2. Earthquakes recorded between November 1, 1961, and February 10, 1962. The circles indicate the probable error of the epicentres of the two strong shocks of November 2 and 7. Dots indicate epicentres determined by the portable equipment. The locations of the permanent seismographs in St. Kitts and the temporary stations in Nevis are shown by solid triangles

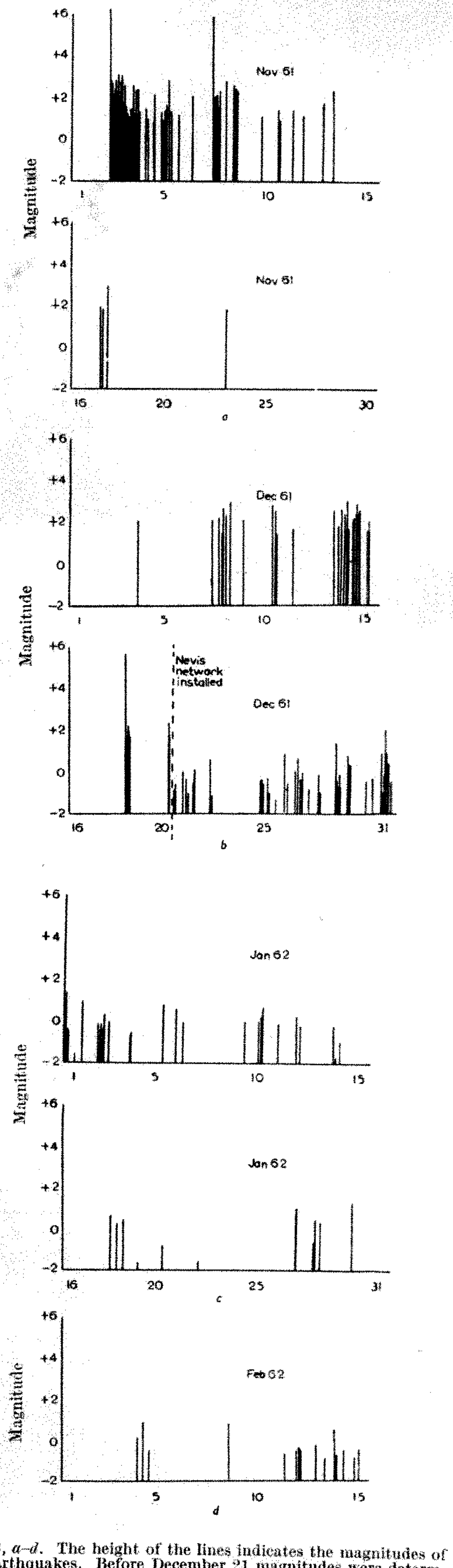

Fig. 3, *a–d*. The height of the lines indicates the magnitudes of the earthquakes. Before December 21 magnitudes were determined from records of the St. Kitts station or more distant stations

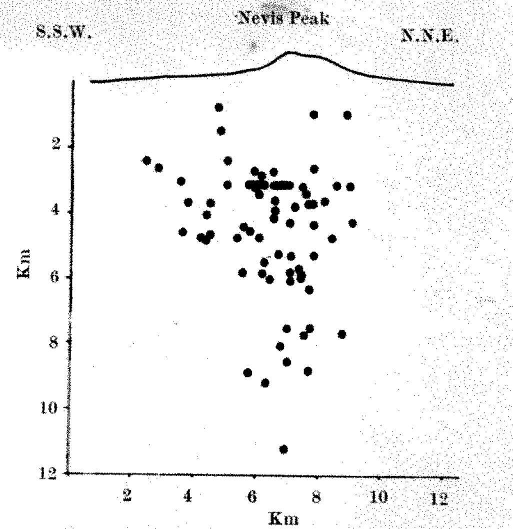

Fig. 4. All earthquakes projected on to a vertical north-north-east-south-south-west plane. Where depth could not be determined one of 3 km was assumed

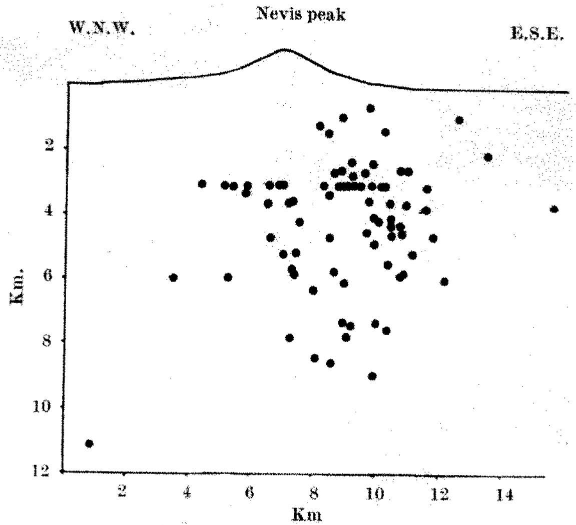

Fig. 5. All earthquakes projected on to a vertical west-north-west east-south-east plane. Where depth could not be determined one of 3 km was assumed

of this body in response to changes in the stress field within the surrounding rocks.

In its general features the present earthquake series resembles the earlier one of 1950–51. Both earthquake series began with strong shocks with epicentres west of the island which were followed by smaller shocks close to Nevis Peak.

We are now investigating the direction of first motion at the seismograph stations which recorded the large shocks in an attempt to determine the way in which the stress field in Nevis may have been changed. Further geophysical work, possibly gravity and magnetic surveys are desirable to test our assumption of the presence of a magma body and, if it exists, to attempt to determine its dimensions and depth.

We thank the Administrator of St. Kitts–Nevis–Anguilla, His Honour Lieut.-Colonel H. A. C. Howard, the Warden of Nevis, Mr. W. L. Maguire, and many Government officers for their assistance during the investigation.

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AGE OF LUNDY GRANITE, BRISTOL CHANNEL

By M. H. DODSON and Dr. L. E. LONG

Department of Geology and Mineralogy, University of Oxford

THE granites which form the greater part of Lundy, a small island in the Bristol Channel, have generally been regarded as either Hercynian or Tertiary in age. A Hercynian age, that is, 270–300 m.y., would be expected from the geographical location of Lundy near known Hercynian granites of the Cornubian province. On the other hand, a Tertiary age (≤ 70 m.y.) might be expected because the granites are intersected by a dyke swarm which closely resembles swarms associated with Tertiary granites in Scotland and Northern Ireland. Dollar[1] favoured a Hercynian age on geographical, structural and petrological grounds. His principal evidence was a statistical comparison of chemical compositions indicating a decided similarity of the Lundy granites to the Hercynian granites as contrasted with the Tertiary granites. Bott *et al.*[2], however, showed from gravity data that the structure of the Lundy complex was more like that of the Mourne Granite (Tertiary) than that of the Hercynian granites in south-western England. Palæomagnetic evidence is compatible with a Tertiary (possibly Eocene) age for the dyke swarm but gives no direct evidence for the age of the granite[3].

An unweathered specimen of the intrusive phase designated *G*1 by Dollar (the earliest part of the complex) was collected from the southernmost quarry on the east side of the island. Potassium–argon and rubidium–strontium isotopic age measurements were made on separated biotite, and a rubidium and strontium isotopic analysis was made on a representative sample of whole rock (Table 1). In addition, a K–Ar age measurement was made on a slate sample collected at the south end of the island several hundred metres from the granite contact (Table 1). The slate is lithologically similar to the Upper Devonian Morte Slates in North Devon[1].

Table 1. ANALYTICAL DATA

Material	K (per cent)	^{40}Ar* (p.p.m.)	^{40}Ar* / Total ^{40}Ar	Rb (p.p.m.)	Common Sr (p.p.m.)	^{87}Sr/^{86}Sr$_t$†
Biotite	7·83	0·0294 0·0311	0·53 0·51	4,145 4,235	0·1 16·1	90 1·31
Slate	3·45	0·0437	0·89			
Whole rock granite				586	10·2	0·858
Slate (composite of two samples				37‡	79‡	0·730

* Radiogenic component. † Measured today. ‡ Emission spectrograph analysis by N. Garton.

The Rb/Sr ratios in both biotite and whole-rock granite are extraordinarily large for such materials, suggesting that the granite is either an extreme differentiate or represents the first-melting fraction of country rock. Other petrological data support this view. (The large discrepancy between the two determinations of common strontium in the biotite may be caused by an inhomogeneous contamination with a strontium-rich constituent of the rock such as apatite or fluorite. The determination of radiogenic strontium is not affected.)

The K–Ar and Rb–Sr ages of 52 ± 2 m.y. on the biotite (Table 2) suggest that the granite is Tertiary. However, from the biotite data alone it is not possible to distinguish between the alternative possibilities of a granite emplaced in Tertiary time, or a Hercynian granite which was re-heated about 50 m.y. ago causing radiogenic argon and strontium to be lost from the biotite. The K–Ar age of 170 m.y. on the adjacent slate contrasts sharply with ages of 300–350 m.y. obtained on similar slates in Devon and Cornwall[4] which presumably date the main period of folding and recrystallization. The apparent age of the Lundy slate, falling approximately midway between its presumed original age and the granite biotite age, is best explained as the result of a local source of heat producing partial loss of argon. The granite seems to be the most probable source of heat, but there is also the remote possibility that the Tertiary dyke swarm alone could have accounted for it.

Table 2. ISOTOPIC AGES

Material	Method	Apparent age (m.y.)
Granite *G*1 Biotite	K–Ar	52 ± 2 54 ± 2
Biotite together with whole rock data	Rb–Sr	53 ± 3 51 ± 2
	Average	52 ± 2
Whole-rock maximum age	Rb–Sr	66 ± 3
Slate	K–Ar	170 ± 6

Decay constants : ^{40}K : $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$; $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$
^{87}Rb : $\lambda_\beta = 0{\cdot}147 \times 10^{-10}$ yr.$^{-1}$

The whole-rock isotopic data unequivocally confirm the hypothesis of a Tertiary age. By assuming that the non-radiogenic, or 'common' strontium present in the granite at the time of its initial crystallization had the lowest conceivable ^{87}Sr/^{86}Sr ratio of 0·700 (primordial value found in achondritic meteorites), we calculate a maximum age of 66 ± 3 m.y. This age is still just Tertiary. Actually the assumption of an initial ratio of 0·700 is an unrealistically low choice, but the substitution of any higher value would give an even lower maximum age.

The isotopic data for whole rock and the least contaminated biotite (first analysis) are shown graphically in Fig. 1, following the method of presentation of Compston *et al.*[5]. The abundance of strontium-87, conveniently expressed as the ^{87}Sr/^{86}Sr ratio, increases with time due to the decay of rubidium-87. From the ^{87}Sr/^{86}Sr and Rb/Sr ratios measured to-day, and the decay constant of rubidium-87, the ^{87}Sr/^{86}Sr ratio at any time in the past may be calculated; conversely, if the initial ^{87}Sr/^{86}Sr ratio is known, the age of the rock or mineral may be calculated. Fig. 1 shows that the biotite could not have an age approaching the maximum limit for the whole rock since extrapolation of the biotite curve to 66 m.y. would correspond to a negative ^{87}Sr/^{86}Sr ratio—obviously an impossibility. The simplest hypothesis is that the biotite and rock as a whole inherited the identical ^{87}Sr/^{86}Sr ratio at the time the magma

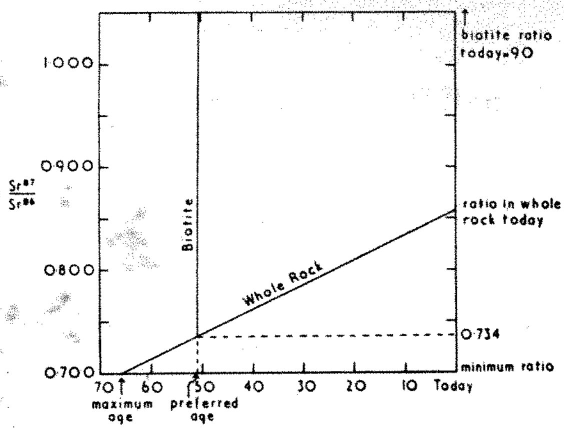

Fig. 1.

crystallized. This age, corresponding to the point of intersection of biotite and whole-rock curves in Fig. 1, is 51 ± 2 m.y., which is also in agreement with the biotite potassium–argon ages within experimental error. The granite could have crystallized earlier than that time, up to the maximum limit of 66 m.y., only if the radiogenic argon and strontium were expelled from the biotite slightly more than 50 m.y. ago. The intrusion of the granite *G*2 or the dyke swarm could produce a later heating which might accomplish such a recrystallization, but more likely for a small body such as the Lundy complex the time gap between intrusive phases was short.

The discussion so far assumes that the granite has been a closed chemical system for rubidium and strontium since its formation. A consideration of material balance demonstrates that if addition or subtraction of rubidium or strontium from the rock took place, the chemical change could not have occurred before the indicated maximum age of 66 m.y. Large-scale movement of rubidium or strontium could scarcely happen without corresponding movement of the associated major elements—in other words, the formation of a new rock in Tertiary time.

The $^{87}Sr/^{86}Sr$ ratio corresponding to the intersection of the biotite and whole-rock curves (Fig. 1) is 0·734 ± 0·005. Data regarding initial $^{87}Sr/^{86}Sr$ ratios in granitic rocks are meagre; nevertheless, this value of the initial ratio at Lundy is considerably higher than the typical values[6] of 0·71–0·72, and suggests that the parent material from which the Lundy granites were derived must have existed for a considerable length of time with a relatively high Rb/Sr ratio. We may therefore exclude the possibility that the Lundy granite was an extreme differentiate of a normal basalt magma, for basalts have very low Rb/Sr ratios, and $^{87}Sr/^{86}Sr$ ratios which are lower than the typical values for granites.

Considering the most probable alternative mode of origin for this granite, namely, partial melting of country rock, it is interesting to note the composition of strontium in the Lundy slate (Table 1). From this and the ratio of rubidium to strontium the $^{87}Sr/^{86}Sr$ ratio in the slate may be calculated to have been 0·728 ± 0·003 at the time of crystallization of the granite. This is not significantly different from the calculated initial ratio for the granite.

We conclude that the age of the granite is unquestionably early Tertiary, while on balance the evidence favours a date of emplacement of 52 ± 2 m.y., that is to say, Middle Eocene. Since *G*1 is the earliest part of the complex, the whole must be Tertiary. Therefore, Lundy represents the most southerly known Tertiary igneous centre of the North Atlantic Province. The petrological affinity between the Lundy granites and the Hercynian granites may perhaps be explained in terms of their geographical proximity.

We thank Drs. R. StJ. Lambert and N. J. Snelling for their assistance in collecting the material and in potassium analysis, and Dr. A. T. J. Dollar for helpful discussions. The work was performed as part of the programme of age determination in the Department of Geology and Mineralogy, Oxford University, under the general direction of Prof. L. R. Wager.

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MEASUREMENT OF OCEAN BED CURRENTS

A NEW CHEAP METHOD AND A SUGGESTED PLAN

By Dr. J. N. CARRUTHERS

National Institute of Oceanography, Wormley,
Godalming, Surrey

THE increasing wish to learn everything possible about water movement on deep ocean floors may perhaps lend interest to a simple and cheap method recently used with complete success from the research vessel *Sarsia* of the Marine Biological Association of the United Kingdom. The method, which provides for single observations only, is easily operable from a drifting ship and has been developed with a further step in view of which some account is given here.

The deepest of three observations made on June 5 and 6, 1962, was at the position 46° 7′ N.–8° 25′ W. beneath 2,250 fathoms (about 2½ land miles) of water. The position, which was fixed by Decca, lies about 160 miles from Ushant Light in the direction 240°. Earlier, the two equally successful single observations of on-bottom current had been made on the shelf edge, the first at the position 47° 49′ N.–8° 13′ W. below some 950 fathoms of water, and the second at a station sounding some 1,400 fathoms at 47° 20′ N.–8° 15′ W. The echo soundings quoted are approximate averages for the initial and final positions of the drifting ship.

At the deepest station where the echo sounding changed very little indeed during the ship's drift, a bed current running towards north-north-west (mag.) at 5 cm./sec. was observed. At both the other stations which lay alike on a markedly sloping bottom, the current was towards south-south-west (mag.) at the speed of 4 cm./sec.

It is very easy indeed to make quick single observations of sea-bed currents down to depths of about 60 fathoms from a drifting ship with virtually no concern at all for weather[1], and, for a few years back, there has been no difficulty while drifting, in making single observations of on-bottom current below depths ten times as great (and more). This has been possible by using a simple leaning-tube current indicator which is operable without more dependence on weather than applies to routine water-bottle work[2]. It was because that earlier leaning-tube device had a depth-of-use limit that the new method to be described was developed. What the earlier depth limit in question is has not so far been precisely determined, but it lies at between 1,600 and 3,000 metres submergence. With the previous method[2] the single observations made on bottom were easily repeatable hourly at depths around 600 fathoms. If they had been made as deep as possible, the repetition-rate would necessarily have been much slower.

The new device, which produced the three results already given, is also a leaning-tube current indicator —of great sensitivity—but its use need have no depth restriction at all. The functioning of its precursor depends on the setting of an oil-surmounted jelly which leaves surface very hot in a 'Pyrex' bottle surrounded by an envelope of hot water. This latter has to remain warm enough to keep the jelly mobile until it (the envelope) is voided on bottom being reached. The new all-depths device functions after the solution of a strange kind of tablet, which is protected from the sea-water during descent. This protection is given by an initial surround of kerosene to be later voided. The perfect tablet for the purpose needs to preserve great strength until such time as water is allowed to get at it. It must also have the desirable solution times for all temperatures in which it will be used. After much delay due to unsuccessful trials with various kinds of expensive solution tablets in vogue, the perfect tablet was found to be a common sugar-lump painted in a certain way Using celluloid enamel, four sides of the lump were painted over to leave two opposite sides only white. For this, a small bottle of crimson nail varnish with its own little brush was very convenient.

Many dozens of the painted cubes were prepared, and an adequate number of them were tested for solution timings under the exact conditions in which they would be used. Tests in sea-water varying from 0° C. to 13° C. showed excellent repeatability with a range of about 12–6 min. Next, it was proved that the painted lumps were unaffected by submission to pressures simulating 4½ miles of submergence—this while immersed in kerosene.

All that needs to be said in this first description of the new simple device and its operation can conveniently be related to the two accompanying illustrations. The main item is a 40-in. long polythene tube 4 in. in overall diameter and of wall thickness ¼ in. This is sealed near one end (to become the base) by means of a welded-in disk of polythene a couple of inches or so up. A tether of thin strong cord is tied into a peripheral hole very near the base below the sealing. Into a second hole dead across from the tether hole, one end of another light cord is secured and its free part passed through a short length of brass tubing. This is of just the right weight to make the buoyant tube (loaded in a way as will be described) stand vertical when flooded with and submerged in still sea-water under the following circumstances. The tether is secured at a length of about 6 in. to a weight easily able to sink the flooded tube, and, at the same time, the cord passing through the brass tubing is led upwards, is cut short to about 6 in., and is then tied at its free end into the bottom loop of a highly stretched rubber band the upper end of which is fastened into a hole in the top rim of the polythene tube 180° away from the basal tether hole. Thus, the brass counterpoise tubing becomes a sort of spine to the tube when it stands erect in motionless sea-water. If, after careful adjustment for erect posture in still sea-water, the tube tethered to a sinker were thrown into moving sea-water just deep enough to submerge it, it would be seen to have fore-and-aft properties; it would lean over with the spine away from the current. Secured into the top of the tube is a directional inclinometer the nature of which can be made out from Fig. 1. The basal disk of this, which is a neat fit within the tube, has a few holes bored in it to permit the passage of water through it smoothly and slowly. The side bars of the inclinometer can easily be tied at their tops into the polythene tube by means of a thin twine passed through facing holes in them and the tube top. The fore-and-aft direction of the inclinometer has, of course, to match that of the tube when secured into it.

The inclinometer possesses a free-hanging pendulum of directional nature. This is contrived from a terylene thread which runs loosely through a small plastic sleeve forced jam-tight at one end into a vertical 'hole' running centrally through a powerfully magnetized ceramic disk flashed across the diameter. The emergent end of the thread is tied securely around a tiny bar of wood which is 'glued' tight to the disk bottom with a dab of waterproof cement. The magnet disk, enamelled white overall, carries four coloured stripes as indicators of direction—red for north-seeking, blue for south, green for east and yellow for west. The stripes run across both faces of the disk and down its side. The free end of the thread passes up through and is secured into a smooth hole bored downwards at the mid-point of a non-turning 'axle' which carries two non-turning disks made (as is the entire inclinometer), of white hard and buoyant polypropylene. These plastic disks are spring-loaded on their sides nearest to the upright side-bars—the springs being powerful and of phosphor bronze. Unless these disks are held apart, they securely grip the plastic sleeve on which the circular magnet cannot turn in the least degree. In practice they are forced apart as far as possible and are held fully agape by means of a small snatch-away bar (also of hard plastic) which is inserted between the disk rims at their topmost part. The length of the terylene thread is cut to ensure that the circular compass hangs free—but only just free—of the rims of the gripper disks at their lowest part. To ensure that the pendulum length remains constant—as it must—special provision is made to obviate small changes in length when the inclinometer is tilted. But for such provision the length would shorten a little when a portion of the thread bore on the non-turning 'axle'. Constant length is arranged for by making a suitable cut into the axle bar below its mid-point just beneath the hole up through which the

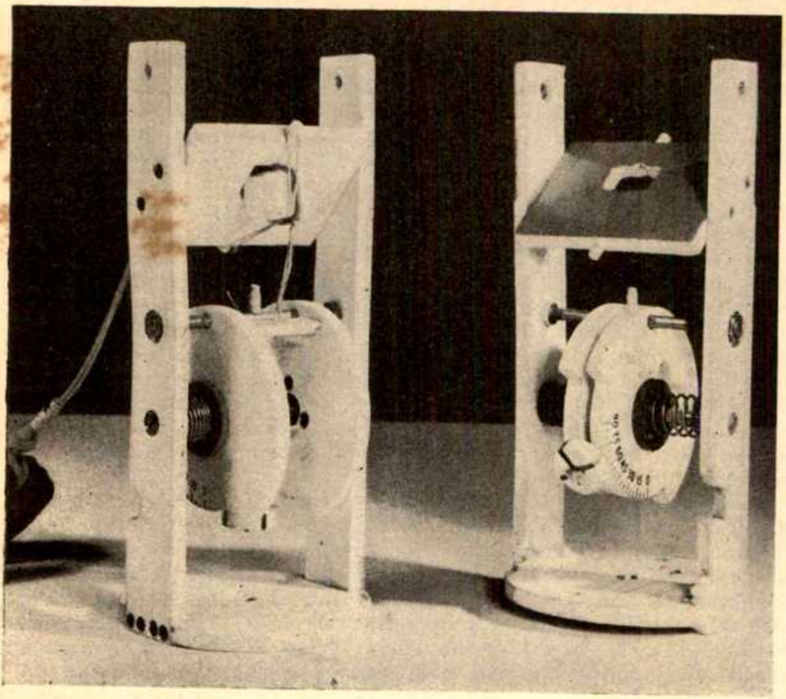

Fig. 1. The directional inclinometer. Cocked on the left; sprung on the right

terylene thread passes. The latter is easily fixed for precise length because, above the hole in the non-turning 'axle', there is a tiny soldered-in brass tubelet standing upwards. The thread is led up through this and is then easily secured in position by leading it down the side of the tubelet and snugging over it a short length of tight-on plastic sleeving.

With the gape-bar in position and the pendulum consequently free to stay always vertical, the first step is to cock the inclinometer. Near the top of the side-bars, a plate is fixed between them sloped at 45°, as seen in the photograph. In this is a rectangular hole into which (at its lower side) a painted sugar-lump is pressed edge-on to leave the two opposite white sides in the fore-and-aft line. The lump is held in position by means of a tiny rubber band during the preparations. Through the rectangular hole passes down from above a loop of thin polythene braid. This, which is placed around the sugar-lump, is in strong tension due to its being in connexion with the spine cord already referred to. This cord, which passes over a nick in the top rim of the polythene tube, is bent into a rubber stretcher which runs externally down the spine of the tube and serves not only to exert a strong pull on the sugar-lump but also to keep the brass tube counterpoise weight vertically up the spine. At the top of the brass tube there is a hook which provides that tension can be put on at the last moment. The drag loop can be put around the sugar-lump while the cord is loose because the tiny rubber band keeps the lump from falling out of position during the cocking.

Before the inclinometer in its fully gaped state is secured into the tube, a pull thread is tied slack between a hole in the small gape-bar and the underside of the loop which passes around the sugar-lump. When the gaped inclinometer has been secured into the top of the tube, the tension link is stretched and the cord hooked on at its far end above the brass counterpoise tube. The situation now is, of course, that there is such a strong pull on the tablet that if it suddenly sublimed the loop would be snatched up through the rectangular hole and would pluck up the gape-bar so suddenly that the pendulum would be instantly clamped.

Before the tube assembly as so far described was taken to sea, calibration tests were kindly made at the National Physical Laboratory to relate tilt-angle to current speed, and in this connexion it should be mentioned that the inclinometer carries a graduated scale. The device is very sensitive indeed and cannot serve in its present form for currents above one knot; its competence effectively falls away at less than that speed in fact. Visits were paid to a river affording a wide variety of flow directions, and checks were made that the pendulum of the leaning tube indicator in its new form always clamped to show the known current direction. Subsequent tests in a very turbulent mill stream were also most reassuring in affording evidence that the closure of the inclinometer, now that it is so sudden, takes place without the falsification of direction which characterized an early model when, with slow closure, the pendulum could be rolled out of direction as the tube surged.

It is now time to say that the tube is sent down into the sea with most of its length filled with sea-water dipped from overside. The top portion, of length equal to that of the inclinometer, is filled with common kerosene. Then, using a 1 ft.^{2} of high-quality rubber sheet, the latter is placed on top of the tube and stroked tightly down the sides using both hands. Next, a high-quality long flat rubber band is stretched by a helper and affixed under strong tension around the rubber capping in three winds. At this stage, the tube itself is ready for use. Because of the fact that kerosene attacks natural rubber, it is advisable always to use a new rubber capping and band for each observation. It is not satisfactory to use oil-resistant artificial rubber because of its inferior stretch properties, and there would be disadvantages in the use of vegetable oil instead of kerosene in order to avoid the attack.

The way in which the device is used can be made out from comments on Fig. 2. The customary thimble is chopped off the end of the ship's hydrowire and this is then led through a snug hole at the finely coned steel top of a long narrow iron weight. It is passed on through a hole running the full length of the latter and the emergent end is bulldogged into a loop too big to pass the hole, but small enough to snug into a basal recess in the weight. The weight used from R.V. *Sarsia* weighed about 1 cwt. and was about 5 ft. long without any whip at all. The illustration shows an alternative plummet. This is a length of old chain weighing about 10 lb. per foot, and a 10-ft. length of it was carried for use in case of loss. If such a cheap ready-to-hand plummet be used it has to be fitted with a nosecap at its top to surround the attachment to the hydrowire smoothly. The nosecap shown covers a link or more of the chain.

and the wire fits snugly into the hole drilled through it. The practice is to pay out the bare hydrowire until the heavy plummet will be hanging freely at a suitable distance above the ocean bed. With a long length of wire veered, it is, of course, far from easy to place the plummet at a desired height above the ground—especially when working from a ship which cannot steam up to the wire to keep the visible portion of it sensibly vertical. This is the case with R.V. *Sarsia*. If the work had been in train from a vessel which can edge into the wire, the decision what length of wire to pay out would have been easier.

There is, of course, a modern pinger method which makes possible the keeping of a suspended instrument at a desired small height above a deep ocean bed, but that is an expensive accessory usable only from a sophisticated ship. For the new operation being tried for the first time, it was not necessary to know with any precision how close an approach was made to hanging the plummet some ten fathoms above the ocean bed. It did not matter what the distance was—5–50 or more fathoms. In the event, we had proof that we had chosen our wire-length successfully—as will be told.

If time had served, it would, of course, have been easily possible to make a preliminary lowering with a waterbottle attached just above the plummet. The reversal of the usual thermometers would have resulted in the achieved depth becoming known. Alternatively, one could have measured the shape of the hydrowire at a number of points down its length using suitable messenger-operated inclinometers.

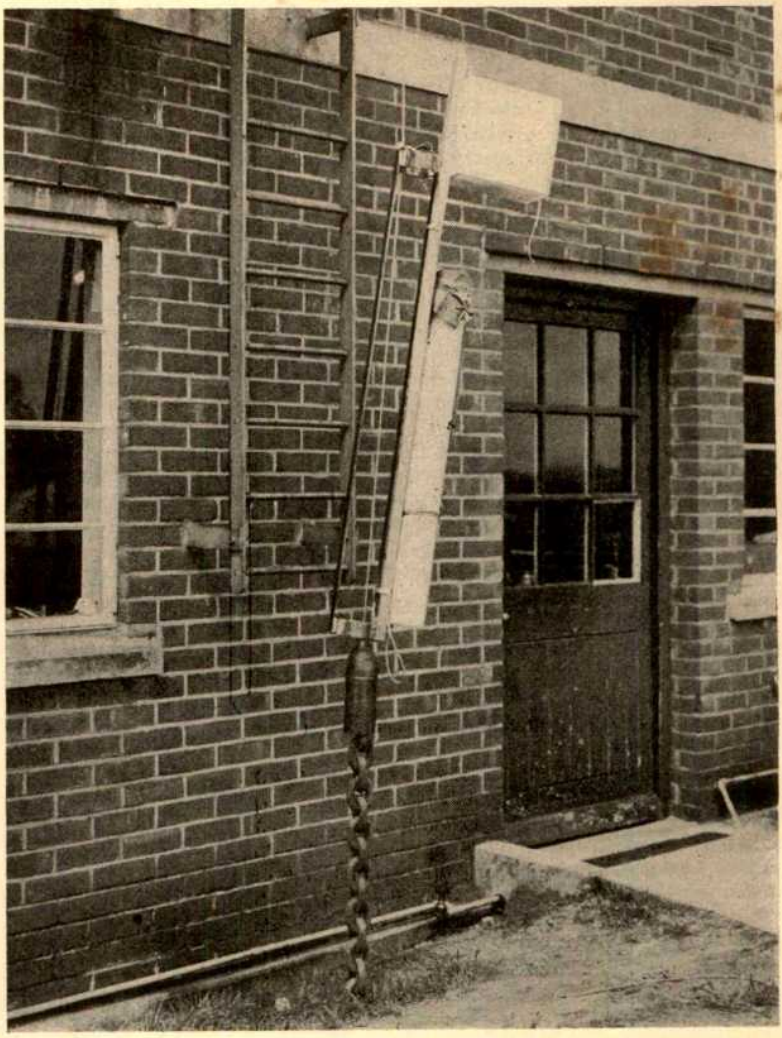

Fig. 2. The total assembly sent messenger-wise down the veered hydrowire. The frame is shown resting on the nose-capped chain plummet over which it passes freely in practice

Once the plummet is down hanging alone and freely at the end of the chosen length of veered wire, everything else used is slid down the wire like a giant messenger. The total assembly is shown in Fig. 2. A couple of 2-in. rings cut from a steel pipe of strong wall and 4-in. aperture are welded terminally on to two steel bars in such a way that the rings are about 4½ ft. apart and the bars 180° apart. The bars are on the outside of the rings so that the latter have no projections within. Half-way between the bars and on the same side, each ring has a saw-cut made in it of size sufficient to admit the hydrowire. At either side of each cut there is a hole. Having made ready a couple of square-bent staples of stout common galvanized wire, it is possible to place the simple framework on to the hydrowire and then to pass the legs of a staple out-from-in through the holes. Having bent the emergent legs over flat against the exteriors of the rings, the framework could not leave the hydrowire if allowed to run freely down it. Fastened tightly to that steel bar, destined to be the uppermost when bottom is reached, is a long square-section bar of substantial hardwood the function of which is to make screw-in and screw-on attachments easy. On to this wooden bar near what is to be the bottom end of the framework a small brass eyeplate is screwed. Into this eye the tether of the polythene tube is tied at a length of some 6 in. Near the top of the wooden bar, a rectangular free-flooding (because holed) box welded up from polypropylene sheet is screwed on. This box contains a large smooth spool made entirely of the same material but without an axle. It is just shake-free within the box and on it is wound something more than 400 yd. of nylon braid of breaking-strain about 600 lb. One end of this braid emerges from a slot cut in the side of the box nearest the top, and is passed through a smooth brass eyeplate fixed near the top of the wooden bar.

When the frame is first placed on the hydrowire it is, of course, held up at rail-level by a castaway rope until all is ready for slipping. Because of the weight of the box of cord, the frame leans on the hydrowire in such a way that the edge of the steel frame ring away from the box presses tightly on it. This situation permits that a heavy steel shackle of aperture too small to pass over the plummet can be fixed round the wire at that point without any risk of it falling down within the frame when this latter will slide freely down the wire to shoot easily off the plummet and dive for bottom. Into this shackle the emergent end of the nylon cord is tied after passing through the brass eye spoken of. Of course, the nylon cord is secured firmly to the spool at its other end. On the tether side of the polythene tube there is a small welded-on plastic eye open up-and-down. This, about 1 ft. from the top, comes between two brass eyes screwed into the wooden bar when the tube is pressed against the latter. These eyes are also open up-and-down. Using a yard-long piece of stout but bendable smooth steel welding-wire, a loop twisted in its top is secured to the shackle. For convenience I shall refer to this wire as the 'skewer wire'. A seized curl is now made in it a distance down it (as it hangs) to come up against the polythene tube just above the position of the upper screw-eye seated in the wooden bar. The length of skewer wire below this curl is passed down through that upper screw-eye, down through the plastic eye, and on down through the lower screw-eye to project some 6 in. below the latter. The tethered, cocked and filled tube is now held securely against the bar to keep it under control during descent.

Using a 2-ft. length of thin but strong slippery polythene braid, one end of this is tied round the outer wind only of the rubber band which holds the rubber capping on to the tube top. It is pulled down somewhat to keep the rubber band bowed down a little, and is then knotted while drawn tight round the projection of the skewer wire below the lower screw-eye. The free end is then led up and tied slack into the seized curl of the skewer wire.

All is now ready for letting the entire assembly slip away to run freely down the hydrowire. With the arrangements described there is no problem such as would exist if two large units had to separate at the bottom. By putting everything on the bottoming frame apart from the shackle, there is no risk of premature separation during descent. There is also no need to provide an infallible mechanism to achieve separation between what has to go down to the ocean bed and what has to stay up on the hydrowire. Most important of all, the arrangements described, besides being very simple, avoid the risk of two distinct units separating somewhat during descent with the consequent likelihood of fouling due to unequal or unlike rotation round the hydrowire.

When the assembly reaches the plummet, the framework runs easily over it. Because the shackle cannot pass over the plummet, it results that during the free dive to the ground a length of nylon cord is pulled out from the spool box. It will doubtless have been realized that any small risk of the shackle being left behind somewhat during the descent of the assembly down the hydrowire is prevented by the fact that the skewer wire which constrains the tube to the wooden bar cannot lift. Because of the way in which the tie from the rubber band is made, there is enough hold-down of the skewer wire fastened at its top into the shackle to ensure that this latter stays in its initial position until the plummet holds it back.

From the descriptions given here it will be seen that when the framework passes over the plummet two distinct things must happen. The constraint of the tube against the wooden bar is released, and the rubber cover is pulled off the top of it. This, of course, voids the paraffin oil, whereupon the sugar-lump is no longer protected against solution. The rubber cover cannot fall into the tube to cause trouble because a small crocodile at the free end of the twine tied to the rubber band is clipped to a corner of it initially.

During the free fall of the framework to bottom after passage over the plummet, the kiting effect of the released but tethered tube will keep the wooden bar uppermost. The box will also contribute to this. Once the thing is on bottom the nylon cord will pay out from the box as the ship drifts because the frame acts as an anchor. Before all the cord has run out the sugar-lump will have dissolved and the inclinometer will have functioned to record slope and direction before the leaning tube is disturbed. At the end of the run-out, the travel of the hanging plummet through the water above will, of course, impose a drag on the bottomed gear, but there will be a lifting component in this. It results that the frame is not towed bodily along the ground with serious risks of loss. It commences by being towed lightly on its heel, that is, on the edge of the steel ring remote from the cord, and finishes without doubt in then being towed harmlessly through the water off bottom altogether. That this desirable state of affairs is not guesswork was tested in an age-old way; the frame was coated liberally with greasy paint before use. As regards this likelihood of harmless off-bottom kiting when the cord draws tight on the bottomed gear, it has to be mentioned that when the cord box has only the empty spool left within it, it and the spool become buoyant enough in conjunction with the buoyancy of the polythene tube to exercise enough lift on the wooden bar to urge it upwards. Overside tests made with no cord in the box and with the frame hung from both ends, showed that just before drag comes on it as it lies on the ocean bed, the wooden bar side must be resolutely upwards. It was a fortunate circumstance that the ship was light during the short cruise. This gave her pronounced leeway assessed at 1 knot during the least-deep observation and at $\frac{1}{2}$-knot during the other two. In all three cases the drift decided from Decca fixes was towards south-south-west. Had the cord not paid out properly during the free dive of the gear on to the ground, the inclinometer would have evidenced a 'current' equal to the drift speed and in a north-north-easterly direction. Equally, if, after reaching ground, the frame had been pulled into movement before the sugar dissolved, there would also have been evidence of a 'current' towards north-north-east.

Everything pointed to correct functioning and to the fact that weights, timings, dimensions and cord-length had been happily chosen.

It was, of course, essential to know that the gear reached the plummet in the same state as it left the surface, that is, that the rubber cover had not washed off during descent. To check on this a stop was put atop the plummet over which the frame could not pass. The made-ready gear was then allowed to run down freely to different depths. Because the shock of arrival at the plummet could now be felt in the hydrowire, we also got our descent timings in this way and learnt that 45 min. should be allowed for a run-down of 4,000 metres. Each time that we brought up the un-tripped gear it was seen that the rubber cover was still secure on the tube top and that the sugar-lump had remained in its pristine state surrounded by kerosene of initial volume. Tests were also made to learn the time of free-fall of the gear after its overshooting of the plummet The entire assembly with the cord bôx full was tossed overboard on a measured line and was found to fall through the water at about 1 metre per sec This means, of course, that a free fall of 100 metres below the plummet would cut the life of the sugar by well less than 2 min because the kerosene would first have to be flushed from the tube and forced out of the sugar-lump as well

Clearly, adaptations could easily be made to suit the individual tastes of would-be users of this method of observing ocean bed current The run-out cord could be made as long as wished within reason In these first trials we pulled the cord in by hand after having hauled in the plummet, but, with a powered reel it could easily be brought in to any length chosen If the intention were to work the method under conditions of very brisk leeway indeed, it would be useful first to moor a buoy on tight scope in the modern way Then, if the ship were not allowed to fall further away from the buoy than a distance represented by the length of the run-out cord, all chance of dragging would be avoided For the observations under report we had allowed for at least a 12-min drift at 1 knot

Particular usefulness for the new simple technique is seen in connexion with observations on canyon bottoms and on rough difficult bottoms generally where there might be natural reluctance to use expensive gear likely to be lost For such work in restricted areas there would be particular need for a tightly moored buoy so that repeat observations could be made at just the same place each time There remains need for a remark on the time taken to make such observations as those reported

At the deepest station sounding 2,250 fathoms, the time occupied between plummet away and tube inboard was about 3½ hr , but this could presumably have been cut to 3 hr if the cord had been wound in on a powered reel

Future Possibilities

Though it is intended to use the method here described when knowledge of deep-bed currents is wanted on the spot, so to speak, an obvious next step of great promise seems indicated

The method described here provides only for single observations, and perhaps only very few repeats per day would be possible in the deepest water The likelihood of making much use of continuously recording instruments beneath the greatest depths is scant, and, even if such use were possible, expense will be only one drawback Always there looms the great question of space coverage The oceans are so vast that to learn everything about the bed currents at just one abyssal spot would be of sorely limited value though a great achievement of compelling interest

It is possible to suggest with confidence a scheme of work which would achieve great coverage in both time and space at very modest cost and but little trouble in this important field of studying ocean bed currents There is a proviso, one would have to forget the clock and would have to be content not to know what happened at precise hours. This accepted, the scheme to be suggested could, over great areas of ocean, build up data towards revealing what speeds and directions of bed current *ever* exist

It would be easy to make a cheap expendable form of the leaning-tube current indicator This would be made of the cheapest possible material resembling polythene in point of maintenance of *in situ* buoyancy under great pressure There would be no wires nor lines, no kerosene, and no rubber cap, and the simple instruments would be supplied to all manner of ships merely to be thrown overboard at desired positions of which a record would be kept The solution tablet would be a very long-lasting one to the end that there would be such a wide and random spread in the clamping times of the devices that all fluctuations more or less might be expected to be covered in time The tether could be a short length of string tied to a brick (for easy stowage) and each tube would have an identity number for record Once tossed overboard it would sink to bottom to put on its single record after quite a long sojourn there It would stay on the ocean bed until, consequent on the 'rotting' of the string, it rose to surface to float freely until such time as cast ashore Many would certainly be found, and the requisite information sent in as with drift bottles There would be provision that the record clamped into the inclinometers for direction and speed could not be destroyed by the finders A multi-language questionnaire sheet of printed polythene could be enclosed telling the finder to take his finding to the nearest police station The tubes could, of course, be vividly striped to facilitate discovery of them after stranding

There would seem to be no real difficulty in sowing far and wide over the oceans from ships of every sort and description, large numbers of a cheap version of the leaning tube indicator which would sink to the deepest ocean bed, there to put on a registration of water movement, speed and direction, to be later brought up to the surface for onward transmission by 'Bottle Post' Great boons of such a method of investigation would be the complete escape from expensive ship time, the fact that sowings could be arranged for wherever ships of any kind ply, and the absolute certainty that the records would not be falsified from above since there would be no attached lines whatsoever It could be expected that if enough such instruments were thrown over on enough occasions at such a interesting place, say, as the Puerto Rico Deep, then, with the passage of years, revelation would come as to what speeds and directions of bottom current ever exist there Quite certainly adequate returns would come from north-west Europe One attraction of the suggested plan is that there would be no concern at all for weather It seems reasonable to suppose that as much money as would run a big research vessel for a day could be rewardingly applied to what has just been advocated

There seems no reason to doubt that the scheme could be profitably tried in any area of ocean

The pursuit of knowledge always benefits from a leaven of excitement, and who would not be excited to learn from an object found stranded on an Icelandic beach, what the bottom current had been a couple of years earlier at a spot in the Caribbean Sea ?

Thanks are tendered to Dr F S Russell for kindly placing R V *Sarsia* at my disposal, to Captain C A Hoodless and his ship's company for much help generously given, and to my colleagues Messrs L A Baxter, C L Gulliver and J W Bicknell, on whose help at sea the work depended

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FARADAY ROTATION OF POLARIZED GALACTIC RADIO EMISSION

By R. WIELEBINSKI and DR. J. R. SHAKESHAFT

Mullard Radio Astronomy Observatory, Cavendish Laboratory, Cambridge

RECENT work[1-3] has revealed the apparent existence of a small linearly polarized component in the 408-Mc/s radiation from certain areas of the sky. Linearly polarized emission is associated with the acceleration of relativistic electrons in a magnetic field, the mechanism which is generally believed to be responsible for most of the galactic radio emission.

The methods of observation both at Leyden Observatory[1,2] and at the Mullard Radio Astronomy Observatory, Cambridge[3], makes use of two mutually perpendicular dipoles rotated about the axis of symmetry at the focus of a paraboloidal reflector. The degree of polarization is then determined by measuring either the correlation between the voltages induced in the dipoles (Leyden) or the difference between the powers intercepted by the two dipoles (Cambridge).

Both methods suffer from limitations set by irregularities in the reflectors and dipole supporting structure, and by the reception from off-axis directions of radiation from the ground and sky; such radiation may in some circumstances appear to be almost completely polarized, and careful corrections have been necessary in both series of observations in order to isolate the true polarized signal from these spurious and often larger effects.

Although the substantial agreement between the two sets of observations strongly suggests the presence of a true polarized component, the corrections required are sufficiently great to make an independent test desirable. A series of observations has therefore been made to detect the Faraday rotation of the plane of polarization due to changes of the electron content of the ionosphere. This method was first used by Thomson[4] with inconclusive results and has also been applied at Leyden[2,5]: not very convincingly in the former paper, somewhat more so in the latter.

The three areas of sky listed in Table 1 were selected as showing comparatively large polarized components and each was observed regularly at transit over a period when there was a large variation to be expected in the total electron content of the ionosphere along the line of sight.

Table 1

Region	Centre of region	Magnitude of polarized component	Period of observation
a	α = 02h 54m δ = +67·5°	2·5 °K	Jan.–May 1962
b	α = 03h 54m δ = +67·5°	2 °K	Jan.–May 1962
c	α = 06h 07m δ = +45°	2 °K	Nov. 1961–April 1962

The instrument used was that described in reference 3 and the observations were made at transit in order that the spurious contributions due to galactic and ground radiation would remain unaltered throughout and only a true linearly polarized signal would be modified as the electron content of the ionosphere varied.

Most of the observations were made at night to avoid the problem of solar interference, but some were extended into the period just before sunset. The reception pattern of the aerial was known to be such that on these occasions any solar radiation was below the limits of detection.

The two regions at δ + 67·5° were observed at both upper and lower culmination. In the latter case the inclination of the geomagnetic field is nearly perpendicular to the line of sight and very little rotation would be expected.

The results obtained for each of the three areas are shown in Fig. 1, which gives the magnitude and direction of the electric vector of the apparent polarized radiation, the magnitude is expressed in terms of a 'polarization temperature' which is defined as the difference in aerial temperatures when the receiving system is rotated to receive first maximum then minimum radiation. The vectors shown are made up of (i) a genuine polarized signal; (ii) off-axis radiation from the ground; (iii) off-axis radiation from the Galaxy; (iv) instrumental effects.

Components (ii) and (iv) are independent of sidereal time and may therefore be determined by averaging the apparent polarization vector over a period of 12 h, since (i) and (iii) are likely to have an average close to zero over such a period. Component (iii) was computed from the known reception pattern and the distribution of brightness over the sky. It was found to be less than ½° K for each of the three regions and has been ignored.

The sum of (ii) and (iv) is shown in each of the figures by the vector S; the circle indicates the uncertainty in this vector. It can now be seen that the observed polarization vectors can be represented by the addition to S of a nearly constant vector the direction of which varies during the periods of observation; such a result is to be expected if the latter vector represents the true polarized signal (i) and the varying direction is related to the Faraday rotation in the ionosphere.

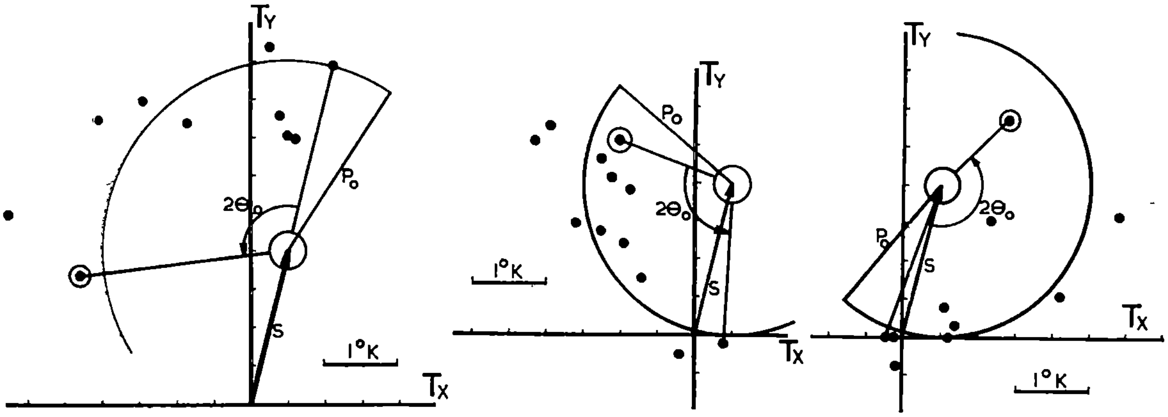
A comparison has therefore been made between the observed angle of this vector (θ_O) measured from an arbitrary zero and the predicted Faraday rotation angle (θ_P) computed from the ionospheric data for each night. θ_P is given[6] by the expression

$$\theta_P = \frac{A}{f^2} \int NB \cos\varphi \sec\chi \, dh \text{ degrees}$$

where $A = 1·4 \times 10^{-6}$, f = frequency in Mc/s, N = number of electrons per cm^3, B = magnetic induction in gauss, φ = angle between B and ray direction, χ = angle between zenith and ray direction, dh = element of height in cm, or approximately, with f = 408 Mc/s

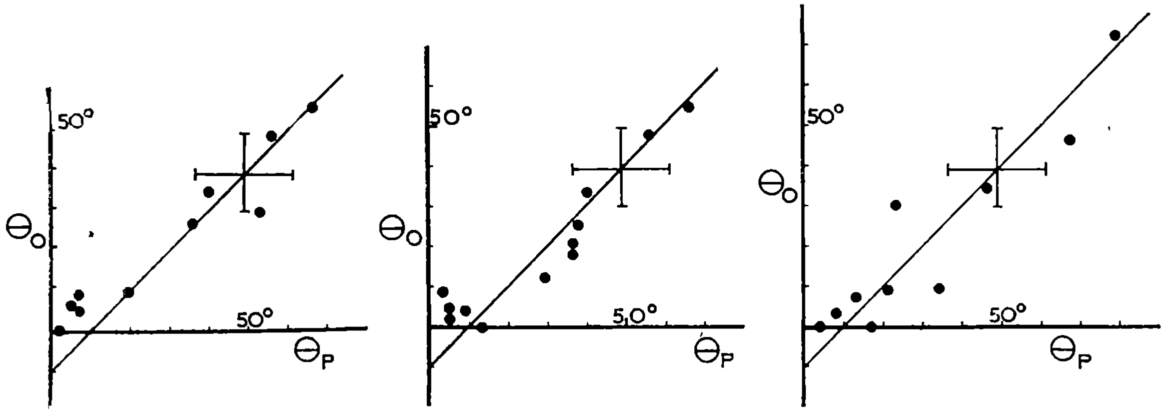
$$\theta_P = 8·1 \times 10^{-12}\, \overline{B \cos\varphi \sec\chi} \int N dh \text{ degrees}$$

The values of total electron content, $\int N dh$, have been derived by the application of ionospheric critical frequency data (f_0F_2) measured at Slough at the appropriate times to an ionospheric model[7] based on previous observations of total electron content[8-10].



a *b* *c*

Fig 1 Vector representation of observed polarization temperatures for the regions *a*, *b* and *c*



a *b* *c*

Fig 2 Comparison of the observed (θ_O) and predicted (θ_P) angles of the plane of vibration of the electric vector for the regions *a*, *b* and *c*

The results of plotting θ_O against θ_P for each of the three areas of sky are shown in Fig 2, the probable errors for an individual point are indicated

It can be seen that there is extremely good agreement between the observed and predicted rotation angles, this result and the relative constancy of the vectors P_0 in Fig 1 confirms the conclusion that P_0 must represent a true polarized component in the emission By projecting a 45° line drawn through the points in each of Figs 2*a*, *b* and *c* to $\theta_P = 0$ (corresponding to zero Faraday rotation) the position angle of the electric polarization vector outside the ionosphere can be found These directions are indicated on the sketch maps shown in Fig 3 It is noteworthy that the directions of the plane of vibration of the electric vector tend to be perpendicular to the galactic plane

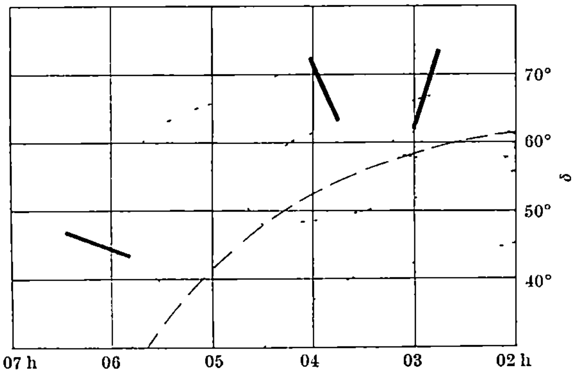


Fig 3 Sketch showing the direction of the plane of vibration of the electric vector for the regions *a*, *b* and *c* in relation to the galactic plane Background temperature contours at 404 Mc/s are also indicated

There is clearly the possibility that measurement of the direction of the plane of polarization, perhaps at some frequency other than 408 Mc/s, could be used as an alternative method to lunar radar and satellite observations for study of the variation of total electron content in the ionosphere

We thank Miss M E Davies for discussions, the Director of the Radio Research Station, Slough, for critical frequency data and the team at Leyden for the communication of their results prior to publication

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LETTERS TO THE EDITOR

GEOPHYSICS

Half-life of Radiocarbon

AFTER full discussion of the new determinations[1-3] of the half-life of carbon-14, the Fifth Radiocarbon Dating Conference, meeting at Cambridge (see p 943 of this issue of *Nature*), adopted the following resolution

"We commend the three groups of research workers on their new determinations of the half-life of carbon-14, and upon their joint presentation of a figure to be used as a most probable value We agree that these results represent a significant improvement in our knowledge of this important physical constant, and that the mean of the values given, 5,730 ± 40 yr is now the best value available

"Inasmuch as further experiments may lead to an even more reliable result, we recommend, as a temporary expedient, that radiocarbon age results continue to be reported on the basis of the 'Libby half-life' 5,568 yr [4], used heretofore We anticipate that upon the conclusion of the experiments mentioned, the question of adopting a new procedure for reporting will be considered In the interim, published dates can be converted to the basis of the new half-life by multiplying them by 1 03, without appreciably altering the standard errors as quoted

"The delay in revising the basis of reporting will also allow time for evaluation of the carbon-14 content of dendrochronologically and historically dated samples "

A further action of the Conference was to confirm the practice of using A D 1950 as the reference year of 'zero age B P ' for purposes of reporting radiocarbon measurements

H GODWIN
(Chairman)

Botany School,
University of Cambridge

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'Scale Frequency' of the Exosphere

WHISTLER measurements of nose frequency (f_n) and time delay at this frequency (t_n) give a f_n, t_n distribution well fitted by a 'gyrofrequency' model of electron density in the exosphere[1] one for which the electron density is everywhere proportional to the magnetic field strength or gyrofrequency (f_H) Since the electron density is directly proportional to the square of the plasma frequency (f_0) and uniquely determined by it we can express this model by the relation

$$f_0^2 = f_a f_H$$

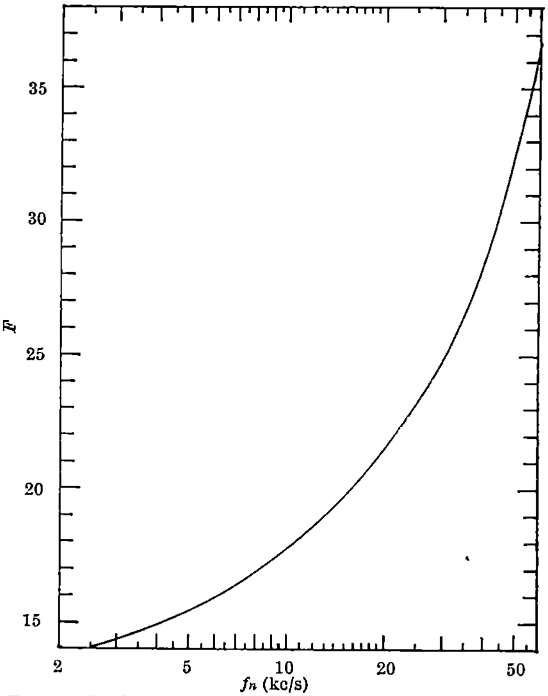

Fig 1 The function F for calculation of scale frequency from nose frequency and nose delay time

The constant of proportionality, f_a, has the dimensions of frequency and so might be called the 'scale frequency' of the exosphere It is typically around one megacycle per second (as deduced from Smith's work[1])

To the extent that the gyrofrequency model fits the real exosphere the scale-frequency is constant But the point I wish to make in this communication is that, regardless of this fit, the parameter, 'scale frequency', is a very useful one in exospheric studies. It is in the same form (frequency) as the other parameters (f_0, f_H, f) describing emission and propagation in the exosphere The low-frequency dispersion $D_0 = f^{1/2}t$ becomes $D_0 = s f_a^{1/2}$ where s is the half-length of the field line in light-time units (light-seconds) For f_a constant along a field line the nose whistler integral can be solved analytically[1] Since whistler data show that the scale frequency is at least quasi-constant then variations in space and time can be sensitively expressed by it This would be particularly useful in discussing magnetic disturbance effects

Scale frequencies are readily deduced from f_n, t_n data from nose whistlers A recent extension of whistler analysis[2] makes it possible to obtain f_n and t_n from perhaps all well-defined whistlers even when the nose is not directly observable In this work[2] it was shown that the nose frequency dispersion (D_n) is related to the low-frequency dispersion (D_0) as

$$D_n = 1\ 456\ D_0$$

Thus

$$t_n f_n^{1/2} = 1\ 456\, s f_a^{1/2}$$

Since the field line (and length s) is uniquely determined by the nose frequency, a function of f_n can be substituted for s Thus

$$f_a = F\,t_n^2\,f_n^{5/3}\ \mathrm{kc/s}$$

$$F = (1\ 456\,s f_n^{1/3})^{-2}$$

where t_n is measured in seconds, f_n in kc/s and the slowly varying function F is shown in Fig 1

It is interesting to note that a theoretical model[3] of electron density in the exosphere based on the premise that the electrons are controlled by the Earth's magnetic field rather than gravity gives a quasi-constant scale frequency Thus, this is largely a consequence of geomagnetic control of exospheres just as a quasi-constant scale height in the Earth's atmosphere is a consequence of gravity control

R L DOWDEN

Ionospheric Prediction Service,
University of Tasmania,
Hobart

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PHYSICS

Special Theory of Relativity

Two years ago I pointed out[1] what appears to be an inconsistency in the kinematical part of Einstein's special theory of relativity. I repeated this in a slightly different form in a volume published in December last[2]. No comment has been made on the former publication, either spontaneously or in response to individual requests, and in none of the many reviews of the latter has even an oblique attention to the criticism appeared In view of its profound and far-reaching consequences if it is valid there can be no justification for leaving a twice-published criticism without a published refutation if it is not

All experimental evidence yet obtained for the special theory is electromagnetic, and this would equally support Lorentz's earlier theory of an ether in which moving bodies are physically changed, and possibly other theories also No test of the kinematical requirements of Einstein's theory has ever been made—doubtless because it has so far been impossible to reach sufficiently high velocities except by the action of electromagnetic forces on charged particles The essence of the theory, however, lies in its kinematical requirements, and if these are falsified the whole theory collapses

Einstein's special theory is so intimately interwoven with modern nuclear physics that its disproof would have revolutionary theoretical effects, but there is another aspect of the matter which is, if not ultimately more important, at least practically more urgent Apart from this theory there is no reason to suppose (among other things) that the velocity of light, c, is unsurpassable, though if a higher velocity were reached in the laboratory the equations of the theory would necessarily register it as lower than c All velocities would be under-estimated and, with the increasingly high speeds now being attained, the consequences of this might be extremely serious In these circumstances the duty of publicly answering or accepting, at the earliest opportunity, a published criticism of the theory becomes compelling I therefore appeal for an answer or acceptance before further risks are taken

The alleged inconsistency lies in the fact that the argument used to prove that 'moving clocks run slow' (with which all the kinematical implications of the theory are bound up) proves, with exactly the same validity, that moving clocks run fast Both cannot be right, so the basis of the theory must be faulty To show this in the most fundamental manner possible, and at the same time to make the task of refutation as simple and direct as possible, I quote here the passage from Einstein's original paper[3] which purports to prove that moving clocks run slow, together with a parallel passage which similarly proves the opposite

In the former passage two clocks are introduced, one of which is at rest in an arbitrarily chosen 'stationary' co-ordinate system, K (co-ordinates x, y, z, t), while the other is at rest in a 'moving' co-ordinate system, k (co-ordinates ξ, η, ζ, τ) The transformation equation between the time co-ordinates has previously been given in the algebraically equivalent forms

$$\left.\begin{aligned}\tau &= \beta(t - vx/c^2)\\ t &= \beta(\tau + v\xi/c^2)\end{aligned}\right\}$$

where $\beta = 1/\sqrt{(1 - v^2/c^2)}$

Here is the passage from Einstein's paper "We imagine one of the clocks which are qualified to mark the time t when at rest relatively to the stationary system, and the time τ when at rest relatively to the moving system, to be located at the origin of the co-ordinates of k, and so adjusted that it marks the time τ What is the rate of this clock, when viewed from the stationary system ?

"Between the quantities x, t, and τ, which refer to the position of the clock, we have, evidently, $x = vt$ and

$$\tau = \frac{1}{\sqrt{(1 - v^2/c^2)}}\,(t - vx/c^2)$$

Therefore

$$\tau = t\sqrt{(1 - v^2/c^2)} = t - (1 - \sqrt{(1 - v^2/c^2)})t$$

whence it follows that the time marked by the clock (viewed in the stationary system) is slow by $1 - \sqrt{(1 - v^2/c^2)}$ seconds per second, or—neglecting magnitudes of fourth and higher order—by $\frac{1}{2}v^2/c^2$ "

And here is the parallel passage, leading to the opposite conclusion We imagine one of the clocks which are qualified to mark the time t when at rest relatively to the stationary system, and the time τ when at rest relatively to the moving system, to be located at the origin of the co-ordinates of k, and so adjusted that it marks the time τ What is the rate of this clock, when viewed from the stationary system ?

Between the quantities ξ, τ, and t, which refer to the position of the (other) clock, we have, evidently, $\xi = -v\tau$ and

$$t = \frac{1}{\sqrt{(1 - v^2/c^2)}}\,(\tau + v\xi/c^2)$$

Therefore

$$t = \tau\sqrt{(1 - v^2/c^2)} = \tau - (1 - \sqrt{(1 - v^2/c^2)})\tau$$

whence it follows that the time marked by the clock (viewed in the stationary system) is fast by $1 - \sqrt{(1 - v^2/c^2)}$ seconds per second, or—neglecting magnitudes of fourth and higher order—by $\frac{1}{2}v^2/c^2$

I should point out that this contradiction has nothing to do with the mere paradox that, if there is an observer with either clock, he will find the other's clock running slower than his own There are no such observers, and if there were they could be ignored The situation, as presented by Einstein, is entirely objective All the necessary readings could be recorded impersonally and the records studied later Nor is it a matter of change of co-ordinate system There is no such change the same clock (A) is chosen as the 'stationary' clock, and the same clock (B) as the 'moving' clock, in both cases, and every symbol has exactly the same meaning in both cases The conclusion of the first passage is that each reading of B is behind the corresponding reading of A, and that of the second passage is that each reading of A is behind the corresponding reading of B Thus, if $\sqrt{(1 - v^2/c^2)} = \frac{1}{2}$, and $t = 12$, Einstein's equation gives $\tau = 6$, and when $\tau = 6$ the other equation gives $t = 3$ Hence, when B reads 6, A read both 12 and 3 That is a contradiction

To avoid this outcome it must be explained not why the two cases are different—that is obvious—but *why, consistently with the theory, the former result must be accepted as true while the latter must be rejected as false*

HERBERT DINGLE

Purley,
Surrey

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Sizing of the Fall-Out at Vienna

DURING December 22, 1961–January 8, 1962, the occurring aerosol at Vienna was sampled for 403 5 hr continuously on filter paper *F* (Schleicher und Schull chromatographierpapier 2043 *Mgl*) by a Goetz aerosol spectrometer *AS*[1] at the site of the First Physics Institute of the University (window on the second floor) The spectrometer effects the quantitative separation and size selective deposition of the airborne particulate matter The deposit pattern yields the size distribution The operating conditions of the spectrometer for this run were 10,000 r p m and 40 mil limiting orifices, that is, the spectrometer did sample all the airborne particles with Stokes's diameters δ between 0 3μ and 5μ at a rate of 3 0 l /min (total volume of the sample 72 6 m³)

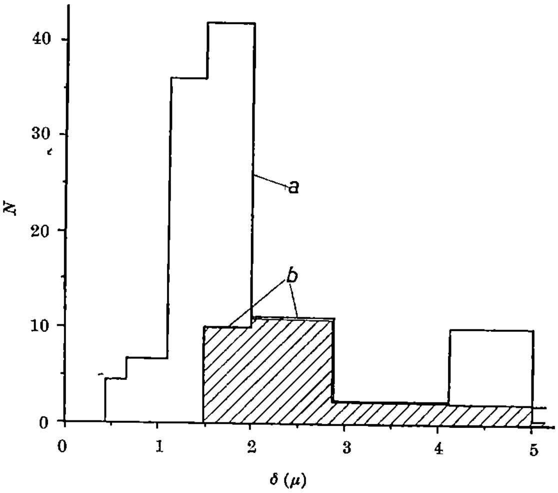

Fig 1 Size distribution of fall-out particles *N*, Number of particles in the sample of 72 6 m³ for the diameter-range 1μ versus *δ*, Stokes-diameter Curve *a*, Each particle bearing an activity 0 5 × 10^{-12} c , curve *b*, each particle bearing an activity between 0 5 and 0 07 × 10^{-12} c

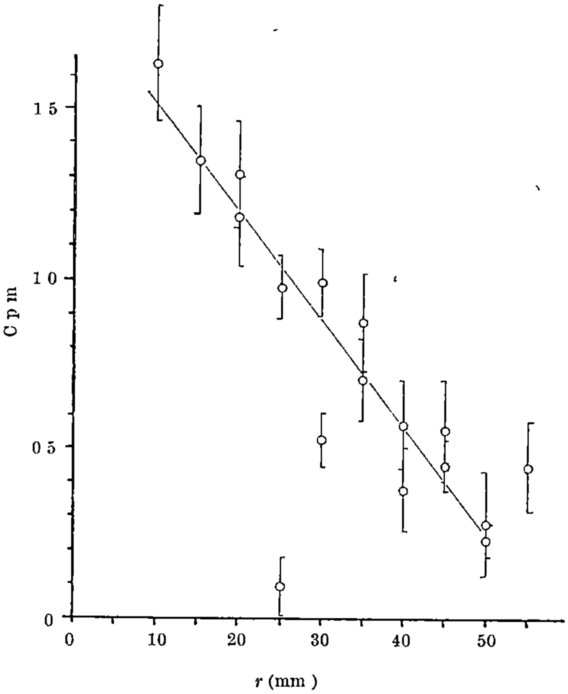

Fig 2 β-activities at February 22, for the sample of 72 6 m³ in c p m for the disks of 6-mm diameter versus position parameter *r* in mm

After the run the filter paper was removed from the spectrometer and two autoradiographs *m* and *n* were taken on Kodak X-ray blue brand, *m* 20 hr and *n* 168 hr exposure-time, both developed in 'Ixal' automatic (Daniel, Wien) for 4 min at 18° C The autoradiographs showed all the single particles bearing a β-activity larger than approximately 0 5 × 10^{-12} C (potassium-40 equiv) for *m* (ref 2) and 0 07 × 10^{-12} C for *n* The first value was estimated by measuring the activity of single particles, the second one by comparing the exposure-times On the autoradiograph *m*, 24, and on *n*, 102 active particles were observed, but, on *m*, 20, and on *n*, 81 particles could be used only for calculating the size distributions given in Fig 1

The size distributions of Fig 1 should be considered as estimates, for the number of observed particles was rather small (The sizings were based on statistics of the deposition loci of the particles)

About two months later the sample was further investigated Disks of 6-mm diameter were cut out of the filter paper at given positions—position parameter *r*—avoiding particles shown at the autoradiographs so far as possible The position parameter *r* is the distance between the point on the filter paper under consideration and the origin of the Archimedian spirals defining the deposit pattern of the spectrometer minus a constant It proved to be more convenient than the spiral-length *L* used in former work[3-6] These disks were mounted at the centre of the planchets of the Tracerlab low-background-β-counter *CE*14 The resulting β-activity in c p m was measured and the activity of the samples calculated

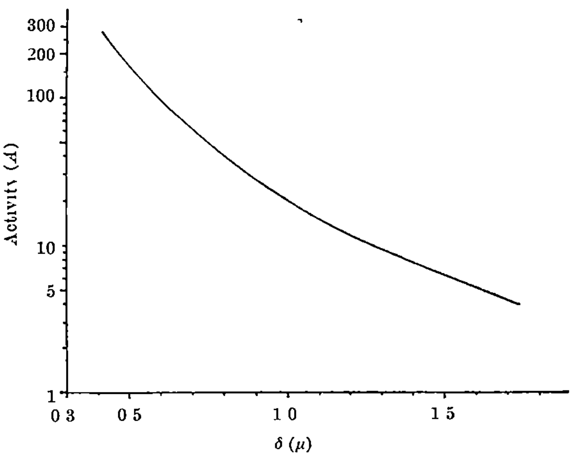

Fig 3 Distribution of the β-activity A versus Stokes diameter δ

for the reference date February 22, 1962 (Time necessary for counting one single disk up to 48 hr , several disks were counted twice for estimating the decay during the counting period) Fig 2 shows the β-activity versus position parameter The range given to each activity in Fig 2 corresponds to 1 96σ (σ = standard deviation), this range was calculated from counts only, errors from sampling as well as cutting and mounting the disks were not considered

From this curve the distribution of the β-activities versus Stokes-diameter (Fig 3) was calculated in the usual manner[4] The activity A is given in c p m for the whole sample of 72 6 m[3] and Stokes-diameter intervals of 1μ

Fig 1 and Fig 3 yield the following information the β-activity was mainly attached to particles of Stokes-diameters smaller than about 0 7μ In most of the cases one single particle does not bear enough activity to show up in autoradiographs unless the particle is larger than 1μ Only a relatively small activity seemed to be attached to particles of Stokes-diameters of 0 7–1 0μ

F ERNST
O PREINING
M SEDLACEK

Institut fur Medizinische Physik und
I Physikalisches Institut,
Universitat Wien

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Experimental Evidence for the Concept of the Displacement Spike

THE mechanisms by which fast, heavy, and charged particles lose energy to crystalline matter are generally explained in terms of either thermal[1] or displacement[2] spikes The mere fact, however, that a majority of the energy is dissipated in the excitation of orbital electrons and the creation of a thermal spike does not necessarily imply that the resulting radiation damage is so formed In uranium, for example, the existence of a continuous band of energy-levels above the Fermi surface permits a temporary excitation of the valence electrons, since these may fall back to their original levels and so deliver their energy to the lattice as heat For such cases, the bulk of the remanent radiation damage may well derive from the Coulomb interactions which the particle makes with lattice atoms in the crystal Brinkman[3] has shown, by use of the atomic interaction potential

$$V(r) = (AZ_1Z_2e^2)\frac{\exp(-Br)}{1-\exp(-Ar)}$$

which approximates to a Coulomb repulsion for close interactions and a Born-Mayer exponential repulsion for large separations, that, for a light uranium fission fragment travelling through uranium, atomic dis-

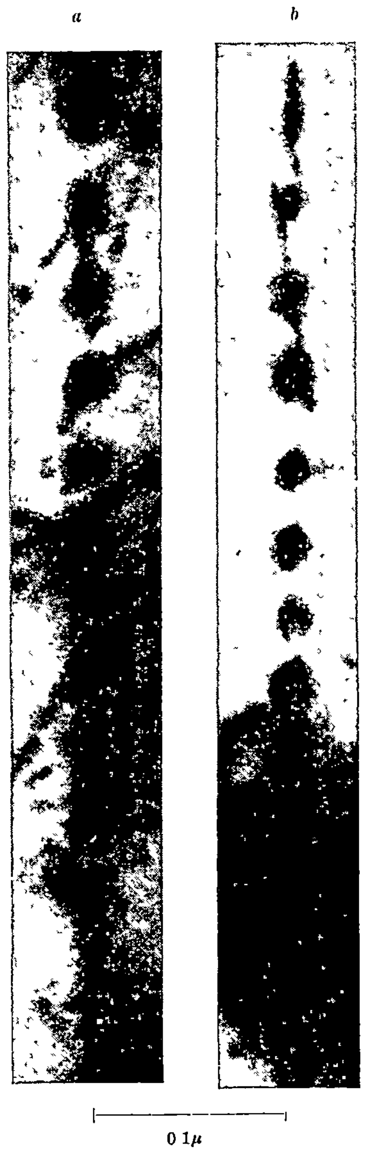

Fig 1 *a*, Electron micrograph showing the discontinuous nature of fission fragment tracks in molybdenum disulphide, *b*, electron micrograph of a fission fragment track in molybdenum disulphide The segments are resolvable as dark contrast loops placements are produced every 40 Å on the average Occasionally the displacement produces a highly energetic ($\sim 10^5$ eV) primary knock-on which proceeds to lose energy by hard-sphere collisions In such cases a displacement spike can be formed Atoms are driven into the surrounding lattice to occupy interstitial positions on a closed shell which surrounds a core of vacant lattice sites

A number of electron microscopists have examined the tracks which fission fragments produce in crystalline matter, and it has generally been concluded that thermal spikes are responsible for the observed effects Experimental evidence for the concept of the displacement spike has so far been equivocal In crystals of molybdenum disulphide, however, the observation of intermittent dark contrast tracks suggests a discontinuous release of energy by the fission particle[4] There can be no doubt that some of the intermittent contrast effects are due to dynamic diffraction effects at inclined continuous and linear regions of strain Such phenomena are similar to those seen at inclined dislocation lines in metal foils and can be partially explained by an application of the kinematic theory of electron diffraction In the majority of cases, however, the contrast effects survive a tilting of the specimen about an axis perpendicular to the electron beam and these are therefore real in nature An example is given in Fig 1*a* It will be observed that the separation between the segments gradually decreases In some of the tracks the segments are clearly resolvable as dark contrast loops (Fig 1*b*) and there is evidence[4] that these are closed dislocation lines

It is possible to explain these observations by adopting the ideas of Brinkman[3] If it be assumed that the energy which is delivered to the electrons in molybdenum disulphide is spread throughout the lattice as heat (the electrons are highly mobile between molecular layers) then the damage-creating mechanism is that of displacement As the particle loses energy the separation between successive displacement spikes decreases until a merging results in the formation of a continuous track For isolated displacement spikes the configuration is unstable and collapse can result in the formation of a closed vacancy dislocation loop[5] In the continuous region a dislocation dipole[6] or a long dislocation loop can be formed —there is basically no difference between these two types of extended defect

It may be concluded that the concept of the displacement spike is useful for visualizing the dynamic processes which occur when displacement cascades are produced by heavy particles The energy which is communicated to the electrons, however, may result in the initiation of a thermal spike, and this may be the dominant mechanism of damage creation in other types of crystal[6]

I thank Dr F P Bowden for his advice

LEWIS T CHADDERTON

Physics and Chemistry of Solids,
Cavendish Laboratory,
Cambridge

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ENGINEERING

A 5-Mc/s Klystron Amplifier using Positive Ions

AN experimental investigation of the principles of microwave klystrons is rendered difficult by the inherently high operating frequencies at which it is not feasible to measure such quantities as input and output voltages An important parameter in the klystron is the length of the drift space In the normal operating range this is usually less than 1 cm It is therefore difficult to carry out quantitative experiments in which the drift-length is varied The drift-length depends on the transit time of the electrons and its relation to the high-frequency period Thus lowering the operating frequency gives longer drift-lengths Cullen and Stephenson constructed a special demountable klystron to operate at a frequency of 300 Mc/s, in which the optimum drift-length was about 5 cm and it was fairly easy to investigate the effect on performance of varying this length Voltage measurement at 300 Mc/s still presented some problems

By using beams of positive ions instead of electrons, transit times are changed in the ratio $\sqrt{(m_i/m_e)}$ where m_i, m_e are the masses of the ion and the electron respectively Thus it becomes possible to investigate velocity modulation effects at much lower frequencies Using argon ions we have constructed a klystron amplifier to operate at a frequency of 5 Mc/s Voltage gains up to five times have been obtained The optimum drift-length is about 5 cm with currents of 3 mA and accelerating voltages of 2–4 kV Preliminary investigations have shown that the performance gives good quantitative agreement with the small signal space-charge wave theory of Ramo and Hahn[2,3] It appears therefore that the positive ion klystron is a good analogue of the microwave klystron and the possibility arises of investigating microwave electronic problems at low frequencies, where measuring techniques are relatively simple

M R GAVIN
K CHANDRA
L J LLOYD

Department of Electronic Engineering,
University College of North Wales,
Bangor

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Thermocouple Electric Motors

FURTHER to the recent publications[1,2] on the subject of electrostatic motors running at high voltage and low current, I gave consideration to design possibilities of a thermocouple-driven motor operating at a few mV but a heavy current

A pilot model was made and ran satisfactorily, due to the voltage drop of normal brush gear being too high, mercury commutation was tried This motor ran well, but considerable splashing of mercury occurred which was unsafe from the health angle A totally enclosed design was adopted (Fig 1) The motor was powered by a single massive thermocouple of iron-constantan

The main particulars are (all resistances in μohms)

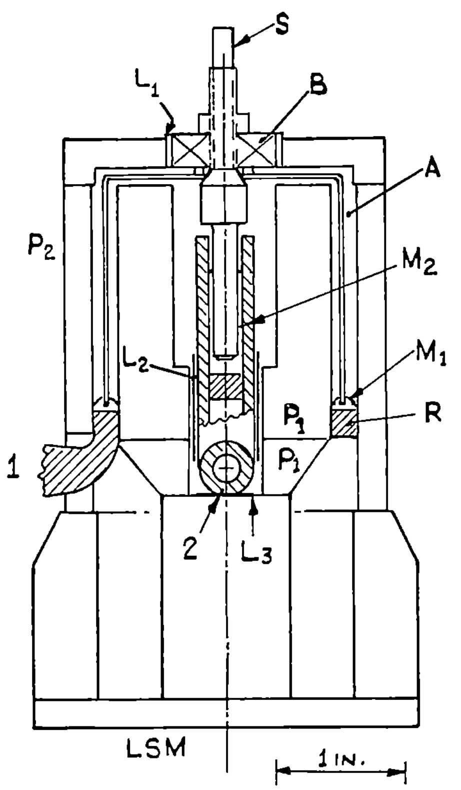

Fig 1 Schematic section of thermocouple motor P_1P_2, Soft iron concentric pole pieces, *LSM*, loud speaker magnet, *A*, copper ring armature 0 03 in thick carried by copper shaft *S* running in ball-bearing *B*, rotates in annular air-gap, 1,2, connexion points for iron and constantan limbs of thermocouple (not shown), M_1, annular mercury contact ring for *A*, resting on copper ring *R* and connected to 1, M_2, mercury pool affording connexion between *S* and 2, L_1,L_2,L_3, insulating liners Vertical anti-eddy current slots are provided in the armature *A*, slots not shown Thermo element sizes, constantan, one piece *c* 0 16 in thick by 1 in wide by *c* 2 in long, iron, 4 rods in parallel, *c* 0 25 in diam × *c* 4 2 in long Water cooling is incorporated but omitted from the drawing for clarity

Motor total copper resistance, *c* 125, mercury contacts, *c* 5, total, 130

Thermocouple total iron resistance, *c* 130, constantan resistance, *c* 390, total, 520

Running the motor just starts to run comfortably at about 100° C hot junction temperature, say 4 mV With a total circuit resistance of 650 μohms the current would be a little over 6 amp, representing frictional losses of about 0 025 W

Output with a hot junction of 700–800° C, $E = c$ 30 mV, $I = c$ 46 5 amp, power = 1 4 W

Improvement it would balance the design if the couple resistance were lowered to equal that of the motor 4 thermocouples in parallel would do this At 700°–800° C, as before, we might expect $E = c$ 30 mV, $I = c$ 115 amp, power = a little less than 4½ W

A test stand incorporating a parabolic mirror is being made It is hoped to make some trials using solar energy as the heating medium, when suitable weather comes along

J E CRAWFORD STRINGER

Lincluden,
Preston Park,
North Shields,
Northumberland

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X-ray Evidence for Cement–Clay Interaction

LARGE quantities of Portland cement are used annually for the stabilization of soils for roads, airfields, and other engineering works While the improvement in engineering properties effected by cement treatment is believed due mainly to the hardening of cement, reactions between hydrating cement and clays leading to the formation of additional cementitious material have been suggested[1]

Several factors would appear to be significant in the formulation of a hypothesis for cement–clay interaction

(1) Hydrating cement is a partly crystalline, multiphase, alkaline system containing calcium hydroxide, calcium silicate hydrates, and hydrating aluminates, and lime is released during hydration[2]

(2) Silica and alumina may be dissolved from clay minerals by alkalis or calcium hydroxide[3]

(3) Ion exchange and flocculation occur rapidly when lime is mixed with clay minerals in the presence of water Excess lime may react with the mineral through pozzolan-type reactions, forming products that are initially amorphous but may later become crystalline X-ray investigations have indicated a destruction of montmorillonite mineral structure and moderate attack on kaolinite in the presence of calcium hydroxide[4]

Consideration of these factors suggests that in hardening clay–cement mixtures, hydration and hydrolysis of the cement could be regarded as primary reactions Usual cement hydration products would form, the *p*H of the pore solution will increase and fresh, highly reactive lime would be generated The clay phase will enter into secondary processes Calcium ions produced by cement hydration convert the clay to the calcium form and cause flocculation Calcium hydroxide initiates attack of the clay particles Silica and alumina dissolved in the high *p*H environment could combine with calcium and form additional cementitious material which would form on or near the surface of clay particles and tend to 'glue' them at points of contact Even stronger bonds would develop between hydrating cement grains and the clay particles coating these grains The resultant structure would consist of a high-strength cement–clay skeleton and clay matrix Units of the skeleton would contain a core of hydrated cement gel to which layers of degraded clay particles are bonded Less-decomposed clay would be trapped in the intercellular space

Kaolinite – cement and montmorillonite – cement specimens containing 15 and 30 per cent cement by weight were prepared by kneading compaction and cured for different periods at 60° C in a 100 per cent relative humidity atmosphere Compaction water content was slightly greater than optimum for the compactive effort used Unconfined compression tests on specimens soaked in water for 24 h after curing periods of 1, 7, 28, and 84 days gave strengths several times those for unsoaked, untreated specimens indicating very effective stabilization X-ray diffraction traces were obtained using copper radiation on samples of powder prepared from specimens cured for similar times

X-ray spectra for hydrating neat cement pastes were dominated by the formation of calcium hydroxide lines at 1 92, 2 62, and 4 93 Å, having intensities that increase with curing time The formation of calcium silicate hydrate (CSH) was observed

Tests on kaolinite–cement mixtures revealed no calcium hydroxide lines except in the case of kaolinite plus 30 per cent cement cured for one day. On the other hand, 4·93 and 2·62 Å lines were easily identified on traces for kaolinite blended with 30 per cent of ground cement that had been hydrated for 3 months. This indicates that the absence of calcium hydroxide lines is not due to masking effects by kaolinite. But since the compression tests indicate large strength increases with curing time and calcium hydroxide was identified in the high cement content specimen cured for one day, it would appear that the cement hydrated normally and formed calcium hydroxide. Since calcium hydroxide is not present in a crystalline form in the kaolinite–cement, it must be present either in an amorphous form or used up by a secondary process. Some weakening of kaolinite reflexions was noted at the higher cement content, suggesting an attack on the kaolinite structure by cement hydration products.

No calcium hydroxide lines were noted on the diffraction patterns for montmorillonite–cement. However, as in the case of kaolinite–cement, calcium hydroxide peaks were pronounced for a mixture of montmorillonite plus 30 per cent ground hydrated cement, indicating that 'dilution' of cement by montmorillonite could not have masked the appearance of calcium hydroxide lines. At 15 per cent cement content the 14 Å montmorillonite basal reflexion increased in height and sharpness during the longer curing periods. A similar intensification of montmorillonite basal reflexions caused by a small amount of lime added was noted by Eades and Grim[4]. At a cement content of 30 per cent the montmorillonite reflexions weakened with increased curing time suggesting major structural breakdown.

Chemical analyses performed in conjunction with the X-ray tests indicated continual decreases in *p*H, free calcium hydroxide content, free electrolyte content, and extractable calcium content with curing time. Values of these quantities were consistently lower than values computed by proportion on the basis of measured values for hydrating neat cement pastes. These results also support the existence of secondary processes involving the lime generated by the hydrating cement and the clay.

New lines of low intensity were observed in some X-ray patterns for specimens containing 30 per cent cement at long curing times. This suggests the formation of new crystalline reaction products. These products have not, as yet, been positively identified.

ANTHONY HERZOG*
JAMES K. MITCHELL

Institute of Transportation and Traffic Engineering,
University of California,
Berkeley

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* Present address: University of New South Wales, Sydney.

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METALLURGY

Yield Point in Mild Steel

NUMEROUS investigations of the factors controlling the lower yield point have shown that the lower yield point (σ_{LYP}) varies with grain size (d) according to the equation

$$\sigma_{LYP} = \sigma_0 + k_y d^{-1/2}$$

Several investigators have shown that k_y is independent of composition and does not depend to any great extent on temperature. The main source of temperature dependence and of composition dependence arises from the factor σ_0, which may be regarded as containing the frictional effects opposing the motion of dislocations during the Luder's extension.

Cracknell and Petch[1] investigated the frictional effects of carbon and nitrogen on σ_0. Their results indicate an equation of the type

$$\sigma_{LYP} = \sigma' + k'(C+N) + k_y d^{-1/2}$$

Heslop and Petch[2] examined the effect of manganese and derived a similar formula to explain their results

$$\sigma_{LYP} = \sigma'' + k''(Mn) + k_y d^{-1/2}$$

In both cases, k_y was relatively unaffected by composition variation.

In the course of a multiple regression analysis of the properties of mild steel, the regression coefficients between the lower yield strength and the composition variations for manganese and silicon were determined as being 2·1 t.s.i. and 2·7 t.s.i. respectively per 1 atomic per cent of solute. In a similar investigation to that carried out by Cracknell and Petch, it was found that the corresponding regression coefficient was approximately 90 t.s.i. for carbon and/or nitrogen. Once again it was found that k_y was uninfluenced by variations in composition and that these elements contribute to the yield strength through the frictional component of σ_0.

The regression coefficients are plotted in Fig. 1 as a function of the distortion produced in binary iron alloys per 1 atomic per cent of solute. The data of Heslop and Petch[2] for manganese and of Cracknell and Petch[1] for carbon and/or nitrogen are included in Fig. 1. A logarithmic scale has been used for convenience, the slope of the line was assumed to be unity to conform with a straight-line relationship on a linear scale graph.

Although Fig. 1 shows a relationship between the increase in yield strength per 1 atomic per cent solute versus the mean lattice distortion, it must not be inferred that this lattice distortion (as measured

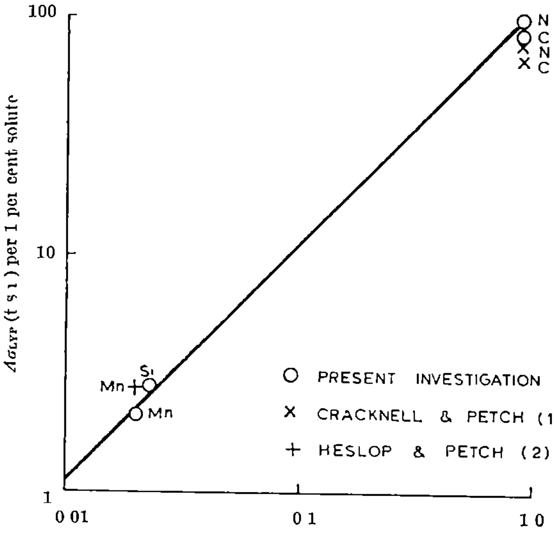

Fig. 1. Change in lower yield strength versus lattice distortion per 1 atomic per cent

by the increase or decrease of mean lattice parameter) is the controlling factor. If this were the case then silicon, which contracts the lattice, should counteract the effect of manganese, which expands the lattice. This is not so, for the effect of these elements is additive. The average dilation of the lattice is merely an average measure of the local distortion around each solute atom, and hence a measure of the binding energy between dislocation and solute atom[3].

One of the difficulties associated with this model is the fact that carbon and nitrogen give rise to an anisotropic strain and produce localized tetragonality, whereas substitutional elements such as silicon and manganese produce an isotropic strain. Nevertheless, it is interesting to observe that the change in yield strength, or more particularly, the change in the frictional contribution to σ_0, is directly proportional to the concentration of solute, at least in dilute solutions, and that the ratio of the change in lower yield point to the lattice distortion is approximately the same for both substitutional and interstitial solutes.

I thank Dr. F. H. Saniter for permission to publish this communication.

T. GLADMAN

Swinden Laboratories,
United Steel Companies Ltd.,
Moorgate,
Rotherham.

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Flow of Cadmium under Very Small Stresses

AT room temperature cadmium is very much more resistant to plastic deformation than lead, as can easily be proved by trying to bend permanently by hand rods or bars of the two metals, of like dimensions. For example, a bar of cadmium 18 cm. long by 1.5 by 0.6 cm. cannot be perceptively bent by hand with ordinary effort, while a similar bar of lead bends as much as is desired extremely easily.

However, under very small stresses applied for a long time, which have no effect on lead, cadmium yields markedly. This is, perhaps, most strikingly shown by arranging a bar as a horizontal cantilever so that it is subject to a bending movement due to gravity alone. The photograph shows a bar of lead and a bar of cadmium, of the dimensions given above, which were clamped at the right over a distance of 3 cm. and left for 14 weeks at room temperature. There is no perceptible distortion of the lead, but the cadmium has yielded in the manner shown and is still yielding.

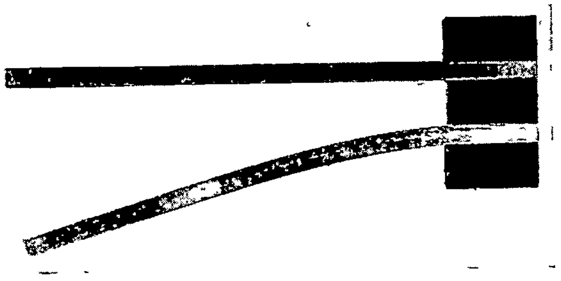

Fig. 1. Lead (above) and cadmium (below) 14 weeks under gravity

A sixpence left between two horizontal plates of cadmium, the upper of which supported a weight of 55 kgm., made little indentation in 20 hr., but in the course of 6 weeks effected a clean cut intaglio of both sides of the coin.

Dr. D. Aboav, who is working on the creep of cadmium under simple shear by the method already described[1], has found flow at a rate of 4×10^{-3} per cent shear strain per hour under a shear stress of 100 gm. wt./mm.^{2} and has detected flow at stresses as low as 10 gm. wt./mm.^{2}. The magnitude and the type of flow, whether $t^{1/3}$, linear with time or compounded of both, is intimately connected with grain size.

Zinc is being investigated for the effect.

E. N. da C. ANDRADE

Department of Metallurgy,
Imperial College of Science and Technology,
London, S.W.7.

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CHEMISTRY

Viscosity Dependence of Unimolecular Conversion from the Triplet State

THE dependence of triplet state lifetimes on solvent viscosity noted in the first studies of triplet states in solution[1] is due, in part, to quenching by trace impurities not yet identified[2,3]. Hoffman and Porter[3] showed that, in certain molecules containing heavy atom substituents, the radiationless transition probability is increased to the point where it predominates over these trivial quenching processes and they conclude that, under these conditions, the measured lifetime corresponds to a true unimolecular radiationless conversion to the ground-state.

In the particular case of 9,10-dibromoanthracene, where quenching effects were negligible, the unimolecular decay constant was found to be independent of solvent viscosity in the range 1 (*cyclo*hexane) to 170 (liquid paraffin) centipoise at room temperature. On the other hand there was evidence that the

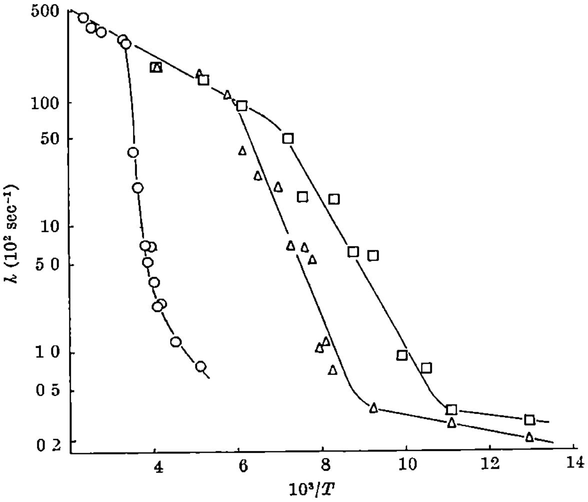

Fig. 1. Plot of logarithm of first-order rate-constant for triplet decay of 9,10-dibromoanthracene against reciprocal of absolute temperature. Solvents: propylene glycol (○), 3-methyl pentane (△), isopentane (□)

rate of decay is considerably reduced in very viscous solvents at low temperatures, and the relative importance of viscosity and temperature on this phenomenon remained unresolved We have now determined the rate constants of first-order triplet decay of 9,10-dibromoanthracene over a wide temperature range (+ 150° to − 196° C) in three solvents of different viscosity Our findings, though rather unexpected, resolve the apparently contradictory observations already referred to

In Fig 1 the logarithm of the rate constant of triplet decay of 9,10-dibromoanthracene in three solvents, propylene glycol, 3-methyl pentane and isopentane, is plotted against the reciprocal of the absolute temperature with which the viscosity increases, though not necessarily in a linear manner Each of the curves exhibits three characteristic regions

(1) A low-viscosity region where the rate constant, at a given temperature, is the same in all three solvents as well as in other solvents investigated, and is therefore independent of viscosity The temperature dependence of rate in this region corresponds to an activation energy of 0 8 kcal/mole in all three solvents

(2) A high-viscosity region where the rate constant is again nearly independent of solvent viscosity and has a zero or very small temperature coefficient (It was not possible to extend this region to lower viscosities in propylene glycol owing to cracking of the glass)

(3) An intermediate region where the rate decreases rapidly with increasing viscosity from limiting value (1) to (2) This region appears in a different temperature range for the three solvents and lies close to the melting point (It should be pointed out that below the melting point all these experiments are made in supercooled liquids and not in crystalline media) The plots are approximately linear in this region and correspond to activation energies of 16 2, 3 8 and 2 7 kcal/mole in propylene glycol, 3 methyl pentane and isopentane respectively

We have measured the viscosity of propylene glycol over the same temperature-range and find that log viscosity is a nearly linear function of the reciprocal absolute temperature with no discontinuities corresponding to those in Fig 1 The activation energy for viscous flow is 15 9 kcal and the viscosity at which the rate constant begins to fall from region 1 is approximately 50 cP

Investigations of other solutes in isopentane solution by Hilpern and Porter[4] also show two distinct regions of triplet decay-rate at high and low viscosity as well as the viscosity dependent quenching processes which are present in some cases In a recent publication[5] Jackson and Livingston mentioned some preliminary measurements on anthracene in glycerol which might be interpreted in a similar way

In seeking an explanation of the two processes of triplet decay, it is necessary to consider carefully the possibility that a further quenching process is operative This would require a quencher the reaction of which became kinetically encounter (diffusion) controlled at a viscosity of about 50 centipoise in propylene glycol This implies a quenching rate constant of 1 3 × 10^8 l $mole^{-1}$ sec^{-1} and a quencher concentration of 2 × 10^{-4} mole/l We have found that the first-order rate-constant is independent of solute concentration in region 1 from 2 × 10^{-4} to 2 × 10^{-6} mole/l and since it is also accurately independent of solvent in six different solvents investigated, quenching processes of this kind seem to be definitely eliminated We conclude that there are two distinct mechanisms of unimolecular decay of the triplet state which predominate in regions of high and low viscosity respectively

Possible ways in which viscosity might affect the rate of radiationless conversion from the triplet state were recently discussed[6] and will not be repeated here Our results show that the influence of viscosity is to eliminate one process completely over a relatively small range of viscosities and that outside the region viscosity effects are negligible The only molecular parameter which is known to change drastically in this region is the relaxation time of Brownian rotation If this is indeed the significant parameter the low-viscosity rate-constant may correspond to a radiationless transition in which coupling of molecular rotation with electronic motion is of prime importance in determining the transition moment

One of us (L J S) thanks the North Atlantic Treaty Organization for the award of a NATO postdoctoral fellowship

G PORTER
L J STIEF

Department of Chemistry,
University of Sheffield

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Reaction of Elementary Carbon and Hydrogen in High-Frequency Discharge

IT has been recently reported[1] that hydrogen atoms produced by the action of microwave discharge on hydrogen gas can react with solid carbon only when the latter is in the plasma Similar observations have been made independently in this laboratory. The results of these and subsequent experiments have led to a possible mechanism for the participation of carbon in such a system

When a spectroscopically pure graphite rod, 3 mm long, 3 mm diam , was placed in a silica tube at the centre of the plasma of purified hydrogen gas, methane, acetylene, and to a much smaller extent, ethane and ethylene, were found among the gaseous hydrocarbon products, as analysed by gas chromatography The results of a number of experiments in which the hydrogen flow has been varied are given in Table 1

Table 1

H_2 flow µmole/min	Pressure mm Hg	Rate of production of hydrocarbon gas µmole/min				
		CH_4	C_2H_2	C_2H_4	C_2H_6	CH_4/C_2H_2
210	0 47	1 50	0 16	0 02	0 02	9 4
125	0 47	1 24	0 21	0 02	0 01	5 9
35	0 47	0 67	0 45	0 02	0 02	1 5

Microwave power, 85 W (frequency, 2,540 Mc/s), length of graphite rod, 1 cm

The overall temperature of carbon in this system was about 700° C as indicated from its faint glow in the discharge When the location of the carbon source was systematically varied, it was found that the total amount of products decreased rapidly as the carbon was moved away from the centre of the plasma This is in contrast to the reactivity of oxygen atoms toward elementary carbon, since the latter is attacked even when far removed from the plasma region

When hydrogen was replaced by a stream of helium gas which had previously been purified by repeated passage through a series of charcoal and molecular sieve traps maintained at − 196° C, it was found that in certain conditions (pressure, 0 7 mm mercury, flow, 10 ml /min, MW power, 42 W) with the graphite rod still at the centre of the field, large deposits of carbon were formed on the silica tube in the shape of rings in the direction of pumping These deposits could be removed when the pressure of the gas was increased and the field moved so as to cover them Similar ring deposits could also be found when a stream of air replaced helium under the same experimental conditions However, no such deposits were noted when hydrogen gas was flowing through the tube (Operation of the discharge with hydrogen gas for long periods resulted in the formation of yellow deposits on the walls of the tube These were presumably high molecular weight hydrocarbon products) These experiments indicate that a mechanism for evaporating carbon from the graphite rod at about 700° C is operative in this system and that the reaction with hydrogen may be wholly due to gaseous carbon which is absent when the graphite is outside the plasma The appearance of methane as the major product indicates that a significant portion of the gaseous carbon is monatomic Acetylene could arise either from the reaction of C_2-species with hydrogen or by the attack of atomic carbon on the newly formed methane That the latter reaction is indeed significant is borne out by the fact that the relative abundance of acetylene is increased when hydrogen gas flow is reduced (Table 1) This is in conformity with the presence of carbon atoms as they are expected to undergo C—H bond insertion reaction with methane to form acetylene[2]

Two possible mechanisms for the production of gaseous carbon in this system appear plausible The first involves collisions of energetic positive ions (for example, He^+) with the surface of the carbon to produce sputtering[3] This process is well known in the production of d -c arcs where the cathode carbon rod is consumed Positive ions in the bulk of the plasma in my system are not expected to acquire the necessary energy for sputtering from the relatively weak and rapidly oscillating fields which are used However, it is expected that the ions which lie within one mean free-path of the surface of the carbon be accelerated through high fields which must appear at pointed regions on the surface of the conductor The second mechanism involves inelastic collisions of energetic electrons with the surface of the carbon, imparting sufficient energy to produce localized heating and evaporation

Contributions of triplet atoms and molecules for sputtering collisions in the case of helium gas must be small, since these species are relatively long-lived[4] and therefore are expected to be available for such a process outside the plasma They could also be formed outside the discharge zone by resonance light absorption of the helium gas However, no sputtering is observed for any of the above gases when the graphite rod is outside the microwave field

Baun and Fischer[5] have recently reported the formation of positive ions of carbon up to C_{28}^+ from a high-frequency spark between graphite electrodes The relative abundance of these species, measured by mass spectrometer, drops sharply as the mass increases. In the electrodeless discharge used in my system, there is no such direct evidence concerning the nature of the carbon species, though no doubt they, too, will be in the ionic form

This work was supported by U S National Aeronautics and Space Administration, grant No *NsG* 192–62

M M SHAHIN

Department of Internal Medicine,
Yale University,
New Haven, Conn

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Isomerism of Disubstituted Manganese Pentacarbonyl Bromide

THE manganese pentacarbonyl halides react with a variety of ligands of the type PR_3 and $P(OR)_3$ to give mono- and di-substituted products, $Mn(CO)_4LX$ and $Mn(CO)_3L_2X$ [1,2] The reaction of 0 2 g $Mn(CO)_5Br$ with 1 1 g $P(OC_6H_5)_3$ in chloroform at 37° for 12 h yields what is believed to be 1,2-*bis*(triphenylphosphite)-4,5,6-tricarbonyl-3-bromo-manganese(I)[3], in which the $P(OC_6H_5)_3$ groups are *cis* to the Br as well as to each other (the '*cis*-isomer'). However, reaction at 55° produces 1,6-*bis*(triphenylphosphite)-2,4,5-tricarbonyl-3-bromomanganese(I), in which the $P(OC_6H_5)_3$ groups are *cis* to the Br but *trans* to each other (the '*trans*-isomer') Both isomers can be precipitated from chloroform with *n*-hexane and analyse correctly for the above compounds The dipole moments obtained in benzene by an approximate method[4] were 4 8*D* for the *cis*-isomer and 3 5*D* for the *trans*, while $Mn(CO)_5Br$ itself had a moment of 3 4*D* These measurements confirm the assignment of the *trans* structure, but do not distinguish between the possible isomers in which the phosphine ligands are (*a*) both *cis* to the bromide, or (*b*) one *cis* and one *trans* to the bromide An attempt has been made to distinguish these by means of the nuclear magnetic resonance of phosphorus which should show splittings in case (*b*) owing to the different environments of the two ligands Although resonance was observed, the resolution was poor and no conclusion could be reached on these grounds (We thank Dr J Verkade and Dr R King, Iowa State University, for making these measurements) However, in view of the known inertness[5] of the CO *trans* to the bromide in $Mn(CO)_5Br$, we feel that alternative (*b*) is unlikely

The molecular weight of the *trans*-isomer in benzene was 761 (calculated value = 839) The infra-red spectrum of the *cis*-isomer had absorptions in the carbonyl stretching region at 2053(*s*), 2000(*s*), 1949(*s*) cm^{-1}, compared with 2070(*w*), 2000(*vs*), and 1949(*s*) for the *trans*-isomer The infra-red spectrum of the previously reported[1] *cis*-$Mn(CO)_3py_2Br$ was very similar to that of our *cis*-isomer, but that of $Mn(CO)_3[P(C_6H_5)_3]_2Br$, prepared in the manner of Abel and Wilkinson[1], was the same as that of our *trans*-isomer, in disagreement with their assignment of a *cis* configuration It seems, therefore, that the *cis*-isomer is produced at low temperatures as a result of a kinetic effect, whereas the *trans*-isomer is formed at higher temperatures owing to its greater thermodynamic stability The number of absorptions and their intensities are consistent with the C_{2v} and C*s* symmetries characteristic of the *trans*- and *cis*-isomers, respectively

Analogous isomers where $L = P(C_6H_5)Cl_2$, $P(C_4H_9)_3$, and $P(OC_4H_9)_3$ have been prepared and identified by their infra-red spectra The *cis*-$Mn(CO)_3py_2Br$ was found not to isomerize to the *trans* form, nor did the aniline complexes On the other hand, $P(C_6H_5)_3$ formed no disubstituted *cis* product, but at higher temperatures yielded the *trans* compound The ligand, $P(OCH_2)_3CCH_3$ (ref 6), forms the disubstituted *cis* compound which isomerizes at 50° only to the extent of 20 per cent, whereas the above isomerizations go essentially to completion This suggests the importance of steric factors in the isomerization

The kinetics of the *cis* to *trans* isomerizations have been studied in *s*-tetrachloroethane from 50° to 70° The rates are first order in complex and independent of added ligand, L The activation energies increase with changing L in the following order

$$P(C_6H_5)Cl_2 < P(C_4H_9)_3 < P(OC_6H_5)_3 < P(OC_4H_9)_3$$

The $P(C_4H_9)_3$ isomerization was measured in $P(C_4H_9)_3$ solvent due to decomposition in tetrachloroethane , however, solvent effects are very small The *cis*-isomer, $Mn(CO)_3[P(OC_6H_5)_3]_2Br$, in tetrachloroethane at 60° reacts with $P(OC_4H_9)_3$ at about ten times the rate of its isomerization to give *cis*-$Mn(CO)_3$-$[P(OC_4H_9)_3]_2Br$ probably via the mixed *cis*-$Mn(CO)_3$-$[P(OC_6H_5)_3][P(PC_4H_9)_3]Br$ This suggests a dissociative mechanism for the isomerization involving the formation of a five co-ordinated intermediate which reacts with free phosphine to reform the *cis*-isomer with an activation energy roughly 1 kcal/mole less than that for formation of the *trans*-isomer

We acknowledge the award of a National Science Foundation fellowship (to R J A) and the granting of leave of absence from the Imperial College of Science and Technology, London (to A J P)

R J Angelici
F Basolo
A J Poe

Northwestern University,
Evanston, Illinois

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Hydrogen Bonding and Charge-Transfer Complexes

If equal amounts of trinitrobenzene and a polycyclic aromatic hydrocarbon are dissolved in chloroform and the solution is dried off on to filter paper, a colour often appears due to the formation of a charge-transfer complex The new absorption bears no relation to that of either of the components separately, and may be almost anywhere in the spectrum depending on the hydrocarbon It is generally believed that negative charge is transferred from the hydrocarbon, acting as donor, to the trinitrobenzene, acting as acceptor Other kinds of donor and acceptor are known[1], and a theory of such complexes, base on molecular orbitals, has been given by Mulliken[2]

Interaction under similar conditions but of a different kind was reported by Szent-Gyorgyi between donor-type compounds and acridine[3] In this case a yellow colour was observed, particularly with the carcinogens As he pointed out, there could have been no charge-transfer complex since the colour was always the same, and in any event acridine has negligible acceptor properties in this context There may have been a weak chemical link between the acridine nitrogen and some unsaturated centre in the other molecule, giving the yellow acridinium cation on one side of the associated system A possible bond of this kind should be contrasted with the delocalized electron interaction found in charge-transfer complexes

We have now found that if a weak acid such as acetic acid is applied to the acridine/donor system a new colour appears which is dependent on the nature of the second component Mineral acid, on the other hand, merely intensifies the yellow colour It seems that a charge-transfer complex can be formed between 'acridine acetate' and donor-type compounds, and tests with each of seventy such compounds showed that the depth of colour produced was similar to that which they produced with 2-methyl naphthoquinone This means that 'acridine acetate' has an electron affinity similar to that of the strongest known biological acceptors With mineral acid there is presumably complete ionization of the acridine, and the absence of any charge-transfer colour under these conditions indicates that the interaction is still essentially non-ionic and would take place in the lipid rather than the aqueous phase

One-electron transfer is believed to play an essential part in metabolism, and some water-soluble Krebs-cycle intermediates have recently been shown to have a high affinity for thermal electrons[4] Lipid-soluble acceptors important in metabolism are not so easy to find Some of the ubiquinones were tested for charge-transfer complex formation and found to be rather weak acceptors compared with 2-methyl naphthoquinone The present observations suggest that suitable compounds can become strong electron acceptors by forming a weak reversible chemical link, such as a hydrogen bond, with a carboxylic acid or other compound containing active hydrogen In support of this view, it was found that hexyl resorcinol was able to induce what appeared to be a charge-transfer complex between acridine and the strong donor N,N' tetramethyl-*p*-phenylenediamine Trichloroacetic acid was likewise able to induce a colour change in a mixture of dibenzylidene-acetone and the above-mentioned amine Indolyl-3-acetic acid gave a brown colour with acridine alone, as did indole and acetic acid applied as separate compounds One may speculate that the plant-growth hormone is not only a good electron donor but also carries, in the acetic acid moiety, a means of inducing certain molecules to accept an electron

Szent-Gyorgyi's aqueous FMN-serotonin system[5] also seems to be activated by acid, though it is not known yet whether the mechanisms are related

T Nash
Medical Research Council
Air Pollution Research Unit,
St Bartholomew's Hospital,
London, E C 1

A C Allison
National Institute for Medical Research,
Mill Hill,
London, N W 7

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Chemical Preparation and Electron Spin Resonance Spectra of Specific Trapped Hydrocarbon Radicals

IN recent years there have been many reports of radicals trapped in solid matrices[1] The radicals have been formed by radiolysis, photolysis, or electrical discharge We have now succeeded in developing a general method of preparing specific trapped hydrocarbon radicals by simple chemical reaction, using a rotating cryostat previously described by one of us[2]

The method is to deposit a thin discontinuous film of sodium atoms (far less than a monolayer) on to a freshly prepared moving surface of halogenated hydrocarbon, preferably an iodide, held in a high vacuum at the temperature of liquid nitrogen The atoms react to form the sodium halide and the hydrocarbon radical A fresh layer of the halogenated hydrocarbon is then deposited on top of the products of reaction, which thus become embedded and preserved in a matrix The process is repeated many times to build up an appreciable amount of material A sample of the deposit is then transferred, still cold and under high vacuum, to an electron spin resonance spectrometer, where the electron spin resonance spectrum of the trapped radical is recorded A feature of the experimental method is that only a single free radical reaction step $RX + \text{Na} \longrightarrow R + \text{Na}X$ (X = halogen) is allowed to take place

Table 1 summarizes our results listing the radicals formed, the material of preparation (which was also the matrix in which radicals were embedded), some salient features of the electron spin resonance spectrum and, in some instances, the molar concentration of radicals in the samples transferred to the spectrometer After being warmed to room temperature, some of the samples were analysed by gas liquid chromatography (G L C), with results which are also given in Table 1 The spectra, supported by the analytical results, demonstrate that the radicals formed from the halides were the expected ones

The electron spin resonance spectra of the radicals *n*-propyl, *n*-butyl, *s*-butyl, isobutyl, *t*-butyl, *cyclo*pentyl and *cyclo*hexyl at 77° K are in good agreement with those reported by Ayscough and Thomson[3] while that for allyl is similar to the one reported by Smaller and Matheson[4] In these other investigations the radicals were produced by γ-irradiation of parent molecules, so that the preparation described here, by an entirely different method involving simple chemical reaction, is strong confirmation of the identities claimed The spectra of both phenyl and benzyl radicals exhibit hyperfine structure Detailed discussion of these and the other spectra will be published later

We thank Mr W N Sailes for assistance with the experimental work

J E BENNETT
A THOMAS

'Shell' Research Ltd ,
Thornton Research Centre,
P O Box 1, Chester

Table 1
Salient features of E S R spectrum at 77° K

Radical	Material of preparation (also matrix)	No of lines	Relative intensities (approx)	Hyperfine splitting oersted ± 5% Ha	Hb	Concentration of radicals in sample (% molar)	Approximate yield of radicals based on sodium deposited	Substances detected by G L C in warmed-up products
n-propyl	1-iodopropane	6	1 3 4 4 3 1	23 3	46 6	—	—	—
n-butyl	1-iodobutane	6	1 3 4 4 3 1	21 9	43 8	—	—	*n*-butane *n*-butene *n*-octane
s-butyl	2-iodobutane	8	1 5 11 15 15 11 5 1*	23 7	41 3	—	—	—
isobutyl	1-iodo-2-methylpropane	5	1 2 2 2 1	20 5	40 0	0 08	10–20%	—
t-butvl	2-iodo-2-methylpropane	10	1 9 36 84 126 126 84 36 9 1	22 2	—	—	—	—
n-heptyl	1-iodoheptane	6	1 3 4 4 3 1	22 0	44 7	0 13	10–20%	—
cyclopentyl	cyclopentyl bromide	8†	1 3 5 7 7 5 3 1	24 6	46 1	—	—	cyclopentane + cyclopentene
cyclohexyl	cyclohexyl bromide	6	1 1 2 2 1 1	20 0	40 6	0 11	30–40%	cyclohexane + cyclohexene
allyl	allyl bromide	5	1 4 6 4 1	14 4	—	0 02	1–2%	1,5-hexadiene
phenyl	iodo benzene	1		63 (half width)		—	—	—
benzyl	benzyl chloride	3		16 9 (–CH_2 protons)		about 0 36	60–90%	dibenzyl

* Outermost lines not observed

† At about 110° K the spectrum changes to one of at least twelve lines, when the sample is cooled back to 77° K the spectrum reverts to the original

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New Evidence for Free AlH

RECENT work in this laboratory on the vapour density of the compound, $AlH_3\ 2N(CH_3)_3$, has indicated that this material dissociates reversibly in two steps In Table 1, the pressures at various temperatures are presented for a sample of known weight in a volume of approximately 1 litre The pressures, measured with a mercury manometer, have been corrected to 0°

Table 1 DISSOCIATION PRESSURES OF $AlH_3\ 2N(CH_3)_3$

(*a*) (0 1477 gm sample) *T*(° abs)	*V* (1)	*P* (mm mercury)	(*b*) (0 07173 gm sample) *T* (° abs)	*V* (1)	*P* (mm mercury)
333 02	0 9752	21 00	337 48	0 9777	17 39
338 06	0 9754	24 69	343 41	0 9777	18 71
342 68	0 9756	27 84	347 13	0 9778	19 37
348 07	0 9759	33 81	353 18	0 9778	20 38
353 15	0 9761	37 01	358 26	0 9779	21 08
358 14	0 9763	40 27	362 84	0 9779	21 86
363 04	0 9765	43 30	367 78	0 9780	22 87
368 16	0 9766	45 03	373 44	0 9780	23 19
373 16	0 9767	46 47	377 74	0 9780	24 10
409 0	0 9769	54 34	381 10	0 9780	23 98
			389 36	0 9781	25 36
			391 22	0 9781	25 44
			398 19	0 9781	26 18
			402 79	0 9781	26 40

The molecular weights calculated from the ideal gas law and the data in Table 1 are plotted in Fig 1 The data for the large (0 1477 gm) sample (curve *a*)

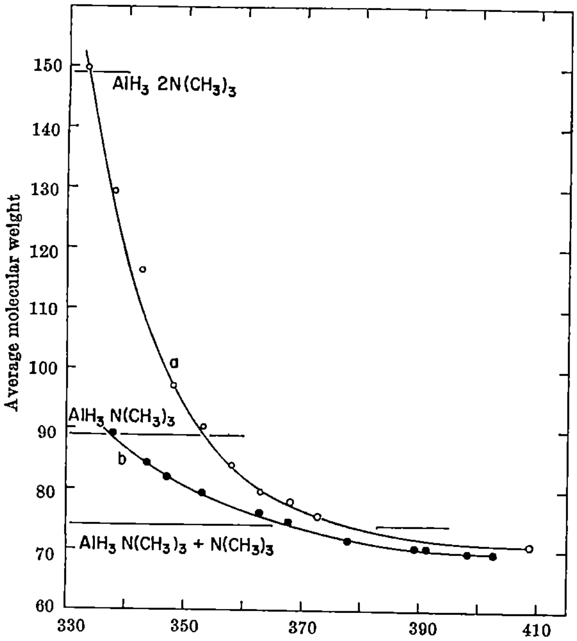

Fig. 1. Molecular weight of $AlH_3\ 2N(CH_3)_3$

obviously indicate the presence of a condensed phase of variable composition and it is not obvious at what point complete vaporization has occurred. However, it is evident that the curve represents an upper limit for the average molecular weight of the system. Curve (*b*) represents the data relative to the smaller (0.07173 gm.) sample and suggests complete vaporization at a lower temperature. For both samples, every other data point was recorded in order of ascending temperature, the remaining points, in order of descending temperature. On cooling to room temperature, the pressure in the system was reduced to 1.80 mm., the original value at the start of the experiment. The pressure was reduced to zero at −196° C. Thus the dissociation at higher temperatures appears to be reversible with no irreversible pyrolysis of the AlH_3 moiety.

Assuming that the second dissociation step is negligible at lower temperatures, equilibrium constants can be evaluated from the data of set (*b*) below 360° abs. for the reaction $AlH_3\ 2N(CH_3)_3 = AlH_3\ N(CH_3)_3 + N(CH_3)_3$. These can be represented by the following equation:

$$\log_{10} K_{p1\,(\text{mm.})} = 11.971 - 3937.0/T$$
$$\Delta H_1 = -18.0 \pm 0.1 \text{ kcal/mole}$$

These values are felt to be quite reasonable, since ΔH_1 indicates that $AlH_3\ N(CH_3)_3$ is Lewis acid of strength comparable with $B(CH_3)_3$ towards $N(CH_3)_3$. The enthalpy increment in the case of $B(CH_3)_3$ is −17.6 kcal/mole[1].

It can be seen that, at room temperature, the pressure of $AlH_3\ N(CH_3)_3$ in equilibrium with a similar pressure of $N(CH_3)_3$ and solid $AlH_3\ 2N(CH_3)_3$ will amount to nearly 0.3 mm. This will account for the fact that crystals of the compound seem to emit smoke even in a relatively dry atmosphere while the crystals themselves remain undecomposed. Evaporation from the surface of the crystals is followed by dissociation and, in the presence of very slight traces of moisture, the $AlH_3\ N(CH_3)_3$ would be expected to co-ordinate a water molecule:

$$H_2O + AlH_3\ N(CH_3)_3 = H_2O\ AlH_3\ N(CH_3)_3$$

The resulting complex, containing both acidic and hydridic hydrogen, would decompose rapidly, perhaps through several steps and further addition of water, to yield Al_2O_3, H_2 and $N(CH_3)_3$.

It is apparent from Table 1 and Fig. 1 that the concentration of any dimeric species such as the structure proposed by Wartik[2] for $AlH_3\ 2N(CH_3)_3$ must be very low indeed. Further evidence from crystallographic studies and gas phase absorption spectra for a monomeric structure will be presented later.

It is also apparent from the data presented here that a second reversible reaction occurs at higher temperatures, to give products lighter than $AlH_3 \cdot N(CH_3)_3$. Such a reaction might be

$$2AlH_3\ N(CH_3)_3 = [HAl\ N(CH_3)_3]_2 + 2H_2$$

However, it is doubtful that such a reaction would be reversible and it is felt that a simple dissociation into AlH_3 and $N(CH_3)_3$ is more likely. The state of polymerization of AlH_3 is not evident from the present data, nor is the possibility of a species such as $(AlH_3)_x\ N(CH_3)_3$ eliminated. Attempts to evaluate equilibrium constants for the second step in the dissociation $AlH_3\ 2N(CH_3)_3$ were inconclusive. Work is at present in progress to obtain more accurate data on this system.

This work was performed in the Ames Laboratory of the U.S. Atomic Energy Commission.

C. W. HEITSCH

Institute for Atomic Research and
Department of Chemistry,
Iowa State University,
Ames, Iowa.

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Zwitterion Ratios of *N*-(Substituted Phenyl)-Glycines: the Non-Validity of the Ebert–Wegscheider Assumption

DURING the course of our investigation of acid-base equilibria in *N*-(substituted phenyl)glycines it has become apparent that the well-known Ebert–Wegscheider[1] assumption equating the inductive effect of a carboxy group with that of a carboethoxy or carbomethoxy group is invalid when applied to these acids. This assumption has been widely used to evaluate the microscopic or individual constants for the following reactions[2]:

$$R\overset{+}{N}H_2\,CH_2\,COOH \overset{K_A}{\rightleftarrows} R\overset{+}{N}H_2\,CH_2\,COO^- + H^+ \quad (1)$$

$$R\overset{+}{N}H_2\,CH_2\,COOH \overset{K_B}{\rightleftarrows} R\,NH\,CH_2\,COOH + H^+ \quad (2)$$

$$R\overset{+}{N}H_2\,CH_2\,COO^- \overset{K_C}{\rightleftarrows} R\,NH\,CH_2\,COO^- + H^+ \quad (3)$$

$$R\,NH\,CH_2COOH \overset{K_D}{\rightleftarrows} R\,NH\,CH_2\,COO^- + H^+ \quad (4)$$

from the experimentally measured ionization exponents pK_1 and pK_2, K_B being assumed equal to the ionization constant, K_E, for the reaction

$$R\overset{+}{N}H_2\,CH_2\,COOEt \rightleftarrows R\,NH\,CH_2\,COOEt + H^+ \quad (5)$$

The zwitterion constant K_Z for the equilibrium

$$R\,NH\,CH_2\,COOH \rightleftarrows R\overset{+}{N}H_2\,CH_2COO^-$$

is readily calculated from the relation $K_Z = \dfrac{K_A}{K_B} = \dfrac{K_D}{K_C}$.

Table 1 SOLVENT WATER TEMPERATURE, 25.0°

Substituent	K_A	pK_1	pK_B	pK_E	pK_B-pK_E
m-nitro	very small	0.06 ± 0.05	0.06	−0.17 ± 0.05	0.23
m-cyano	very small	0.28 ± 0.05	0.28	0.15 ± 0.05	0.13
m-chloro	0.12	1.05 ± 0.03	1.10	0.91 ± 0.01	0.19
m-aceto	0.14	1.14 ± 0.02	1.20	1.02 ± 0.03	0.18
p-chloro	0.42	1.46 ± 0.02	1.61	1.39 ± 0.03	0.22
m-methoxy	0.62	1.68 ± 0.03	1.89	1.67 ± 0.03	0.22
unsubstituted	1.82	1.83 ± 0.02	2.29	2.08 ± 0.02	0.21
m-methyl	2.10	1.89 ± 0.02	2.38	2.19 ± 0.03	0.19
p-methyl	5.04	1.97 ± 0.01	2.75	2.57 ± 0.03	0.18
p-methoxy	9.80	2.08 ± 0.02	3.11	2.85 ± 0.02	0.26
					Mean 0.20 ± 0.03_5

When an attempt was made to apply this method to the evaluation of the constants for the *N*-(substituted phenyl)glycines, anomalies soon became apparent. Thus in the predominately non-zwitterionic *N*-(*m*-nitrophenyl)glycine, the first dissociation constant K_1 was found to be smaller than that of the ester K_E whereas the lower limit for K_1 is equal to K_E if the Ebert–Wegscheider assumption is correct, this occurring when $K_A = 0$. An alternative method for the determination of the zwitterion equilibrium constants based on spectrophotometric measurements was therefore used from which independent estimates of pK_B were obtained. The cationic, molecular and anionic spectra of the acids, R Ph NH CH_2 COOH, have been compared with those of the molecular and cationic species of the correspondingly substituted ethyl esters, the substituent R being varied through the series *m*-NO_2, *m*-CN, *m*-Cl, *m*-$COCH_3$, *p*-Cl, *m*-OMe, H, *m*-Me, *p*-Me, *p*-OMe. We find that for the non-zwitterionic *m*-nitro-phenylglycine the spectra of the anionic and molecular species of the acids are virtually identical with those of the molecular species of the ester. However, as the substituent changes in the order stated, there is an increasing divergence from this state through the above series and this is coupled with an increasing similarity between the molecular form of the acid and the cations of both acid and ester. Thus in other cases in which an equilibrium is present, the relative concentrations of zwitterion and non-zwitterion may be determined spectrophotometrically, the optical density at any wave-length being intermediate between that of the cation, which gives the optical density which would be observed if the equilibrium were virtually all zwitterion, and that of the molecular form of the ester, the absorption of which corresponds to that of the non-zwitterionic molecular form of the amino-acid.

These results are shown in Table 1.

The values in the last column show a mean of 0.20 ± 0.03_5 and appear to bear no relation to the σ_m values of the substituents. Thus the inductive effect of a —COOEt group in these compounds is greater than that of a —COOH group by 0.20 units. It is impossible to extend this conclusion to α-amino aliphatic acids since these are almost entirely zwitterionic and no estimate of pK_B can be drawn from the experimental data. There are, however, numerous systems in which zwitterion estimates have been based on the Ebert–Wegscheider assumption and these may require to be checked by an independent criterion. Details of this work including discussions os the Hammett constants for reactions, 1, 2, 3, 4 and 5 (where R = X Ph) will be published elsewhere.

A. BRYSON
N. R. DAVIES
E. P. SERJEANT

University of New South Wales,
Sydney

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BIOPHYSICS

Reversible Reaction of Double-Stranded Polynucleotides and Hg^{II}: Separation of the Strands

THE reaction of double-stranded deoxyribonucleic acids (DNA) and mercuric compounds[1] proceeds by two principal steps[2–4]. In the DNA's occurring naturally, these steps can be demonstrated and defined by the presence of isobestic points in the ultraviolet spectra of the equilibrium mixture. The first reaction occurs in the region $0 \leqslant r \leqslant 0.5 - 0.6$, the second, in the region $r \geqslant 0.5 - 0.6$, where, as usual, the quantity r is defined as the number of moles of Hg^{II} added per mole of nucleotide.

I have recently shown[5,6] that in the range $0 \leqslant r \leqslant 0.5$ the reaction can be largely understood as the formation of 2 : 1 base-Hg^{II} complexes which form base–*N*–Hg^{II}–*N*–base 'bridges' between the two strands of the DNA double helix.

The preference, at *p*H 5.6, for the 2 : 1 bridge complexes of thymidine deduced earlier[5,6] has since received indirect confirmation especially applicable to the reaction of Hg^{II} and the highly ordered, synthetic polydeoxyribonucleotide, poly (A—T). Ferreira *et al.*[7] have shown that for the isolated nucleosides one can write

$$Hg^{++} + 2T \rightleftharpoons HgT_2 + 2H^+ \qquad K = 10^{2.0} \qquad (1)$$

$$Hg^{++} + 2A \rightleftharpoons HgA_2^{++} \qquad K = 10^{8.5} \qquad (2)$$

where T and A represent neutral thymidine and neutral adenosine.

According to the theory of the reaction[5,6] and the theory of site binding in polyfunctional reactants[8] the equilibrium constants in (1) and (2) are directly applicable to the case of DNA, so the preference of Hg^{II} for thymidine, at *p*H 5.6, may be considered confirmed.

There is therefore an accumulation of evidence to support the view[5,6] that at $r = 0.5$ the reaction product of DNA and Hg^{II} may be described chemically as a two-stranded polynucleotide in which nearly all the bases on a given strand are connected to a base on the opposite strand by a Hg^{II} bridge.

What, then, is the nature of the reaction for $r > 0.5$? In view of the mechanism, briefly described here and discussed in greater detail elsewhere[5,6], to ask the question is virtually to answer it: the chemical mechanism for $r \leqslant 0.5$ strongly suggests that the principal reaction occurring when $r > 0.5$ is

$$\text{chain–base–Hg}^{II}\text{–base–chain} + \text{Hg}^{II} \rightleftharpoons \text{chain–base–Hg}^{II} \quad \text{Hg}^{II}\text{–base–chain} \qquad (3)$$

In equation (3), 'chain' represents one of the two strands of DNA helix. Reaction (3) represents the separation of the strands at a given point by the formation of the 1 : 1 base–Hg^{II} complexes.

This kind of strand separation is readily distinguishable from that produced by thermal means in that the totality of evidence very strongly suggests that no irreversible chemical damage of any sort—release of purines, degradation of the phosphate ester backbone or whatever—has occurred[1–4,9,10]. It differs also in specificity: clearly, by manipulating the *p*H and the Hg^{II} concentration one can select the reactive sites reasonably quantitatively within limits imposed by the various equilibria involved.

The best evidence for strand separation of the type described is the chemical mechanism of the reaction[5,6] Additional evidence of a more fragmentary nature but nonetheless consistent with the basic idea is that mercurated DNA at $r = 1\ 0$ shows[4] a small decrease in 'melting point' relative to mercurated DNA at $r = 0\ 5$ The simplest interpretation of this fact is that some of the Hg^{II} bridges have become disrupted by reaction (3)

Applied to poly(A–T), the mechanism outlined above readily allows calculation of the minimum number of broken bridges at $r = 1\ 3$ (the upper limit of the available experimental data[4]) In the Hg^{II} excess region, the reactions would be those in the polynucleotide analogous to

$$HgT_2 + Hg^{++} \rightleftharpoons 2HgT^+ \qquad K = 3\ 6 \qquad (4)$$

$$HgA_2^{++} + Hg^{++} \rightleftharpoons 2HgA^+ + 2H^+ \quad K = 4 \times 10^{-5}\ ^{5}\ (5)$$

in the isolated nucleosides (The equilibrium constants in equations (4) and (5) were derived from data in ref 7)

Noting that only reaction (5) releases hydrogen ion and assuming only reactions (4) and (5) for $r > 0\ 5$, it is easy to calculate that 32 per cent of all the A–Hg–A bridges are broken at $r = 1\ 3$ The calculation was made not on the basis of the equilibrium constants given above but on the average hydrogen ion release[4] by mercurated poly(A–T) for $0\ 5 \leqslant r \leqslant 1\ 3$ The value 32 per cent represents, minimally, 16 per cent of all the Hg^{II} bridges in poly(A–T) since the polymer contains equal moles of A and T and, according to (4), a small fraction of the T–Hg–T bridges will also be disrupted Obviously, these will not be apparent in pH-stat studies[4]

It is (now) equally obvious, but no doubt worth emphasizing, that in addition to its suitability as a reagent in the study of other aspects of polynucleotides[5,6], Hg^{II} may also serve as a reagent of intrinsically desirable and unique characteristics for the separation of the strands of double-stranded polynucleotides Studies of this aspect of the reaction will be reported in due course

This work was supported in part by a Research Career Development Award, GM–K–3–17, 953–A of the U S Public Health Service

SIDNEY KATZ

Department of Biophysics,
University of Buffalo,
School of Medicine,
Buffalo, New York

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Deoxyribonucleic Acid Code

IN the past few months very important advances have been made concerning how the sequence of nucleotides in DNA corresponds to amino-acid sequences in protein synthesis The work of Crick *et al* [1] of Matthaei and Niremberg[2–4] and of Ochoa *et al* [5–7] gave definite answers to particular questions concerning the code in which information is written in DNA or RNA It seems that such findings are even too abundant and precise, and at first sight it seems to be not an easy task to put all results together

Table 1

Group	Triplets			
	a		*b*	
1	*AAA*		*TTT*	
2	*AAC*	*CAA*	*TTG*	*GTT*
3	*AAG*	*GAA*	*TTC*	*CTT*
4	*AAT*	*TAA*	*TTA*	*ATT*
5	*ACA*		*TGT*	
6	*ACG*	*GCA*	*TGC*	*CGT*
7	*AGA*		*TCT*	
8	*AGC*	*CGA*	*TCG*	*GCT*
9	*AGG*	*GGA*	*TCC*	*CCT*
10	*AGT*	*TGA*	*TCA*	*ACT*
11	*ATA*		*TAT*	
12	*ATG*	*GTA*	*TAC*	*CAT*
13	*CAG*	*GAC*	*GTC*	*CTG*
14	*CGG*	*GGC*	*GCC*	*CCG*
15	*GAG*		*CTC*	
16	*GAT*	*TAG*	*CTA*	*ATC*
17	*GCG*		*CGC*	
18	*GGG*		*CCC*	
19	*GGT*	*TGG*	*CCA*	*ACC*
20	*GTG*		*CAC*	

For that reason it appears worth while to consider once more the code problem from the abstract point of view, starting from a very small number of reasonable postulates, suggested partly by experiments, without expecting the final solution from experiments themselves

A conclusion of Crick's work is that a triplet of nucleotides incorporates a particular amino-acid in proteins All triplets are significant so that the code is degenerate (and it is also comma-less)

We can take this as a starting point (postulate No 1) In that case it seems to me almost necessary to admit that complementary triplets such as *TAC* and *ATG* correspond to the same amino-acid (postulate No. 2) We avoid in this way the difficulty of establishing which of the two strands of DNA must be read

Furthermore, it seems very reasonable to admit that each strand can be read upward and downward without changing the sense of the message (postulate No 3) So the two triplets *TAC* and *CAT* should not be considered different triplets

With these three postulates, the 64 triplets are grouped in exactly 20 groups as is shown in Table 1 Each group must correspond to a different amino-acid and the triplets of the same group must correspond to the same amino-acid

Referring to the code in Table 1, it is not necessary to admit that all the triplets of the same group do work exactly in the same way in incorporating amino-acid The most probable thing we can suppose is that the reaction-rates are different from triplet to triplet in the same group, so that we can find just one triplet in each group to which corresponds the maximum reaction-rate at first sight the code will then appear as non-degenerate and only one strand of the DNA will work in practice Furthermore, we can suppose that every cystron must have the most reacting triplets all on one strand, but different cystrons must not have them always on the same strand So for certain cystrons one strand of the DNA will work and for other cystrons the other

Secondly, if we examine Table 1 from the point of view of combination, we see that each particular base is present in seventeen groups out of twenty

This result is in striking agreement with Ochoa's work if we suppose that the triplets that contain a particular base say uracil in RNA, do have a particularly high reaction-rate

In general, we can suppose that triplets with two or more purines (column *a*, Table 1) differ in stability and reactivity from complementary triplets with two or more pyrimidines (column *b*, Table 1) In that case, when we certainly have all the cystrons on the same strand as in Ø*X*174, the complementary strand must have different mean base composition, as it is observed[8] The same situation holds for some RNA viruses[9], as is well-known, and perhaps also for the α-phage of *B megatherium*, on which Graziosi *et al* are working[10]

Furthermore, if we make fragments of DNA sufficiently short so that each fragment is of the order of one cystron only, we must observe similar irregularities in base composition of the two strands

Several experiments can be devised to test the code of Table 1, but these are not discussed here On the contrary, it is necessary to say that some of Ochoa's results cannot be foreseen by this code If confirmed, they would disprove postulate No 2 and then the code must necessarily be rejected Nevertheless, it seems to me very difficult to admit that complementary triplets may have different meanings, and to reconcile the experimental percentage of *U* in RNA with Ochoa's triplets, other than with a mechanism of degeneracy of the type considered here. Obviously, a more detailed discussion of all the experimental results is necessary

Note After this communication was written I found that the same code was considered by the Russian physicist V V Chavchanidze[11] I would direct attention to his paper, for it appears even more suggestive in view of the new experimental results

MARIO AGENO

Istituto Superiore di Sanità,
Laboratori di Fisica,
Roma

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Experimental Evidence for the Degeneracy of the Nucleotide Triplet Code

EXTENSIVE investigations have been reported recently of the incorporation of labelled amino-acids in cell-free protein-synthesizing systems to which synthetic polynucleotides of known compositions were added The results have led to the assignment of coding triplets to each of the 20 naturally occurring amino-acids[1,2] The peculiar feature of these codes is the presence of at least one uracil residue in each of the 20 triplets

Although the composition of RNA isolated from various sources bears no relationship to the composition predicted by this code[3], many attempts have been made to explain naturally occurring or nitrous acid induced mutations, that is, amino-acid replacements[4,5], on the basis of a single nucleotide change in one triplet of the coding RNA In view of this, we would like to offer experimental evidence of the impossibility of an *in vivo* coding system of the composition as proposed so far In the following argument only one assumption has been made, namely. that the RNA of the plant viruses codes for viral protein

We investigated the structure and composition of the satellite component of tobacco necrosis virus, the smallest virus so far discovered[6] From sedimentation-diffusion measurements we found its molecular weight to be 1 97 × 10^6 The RNA content of the virus we found to be 20 per cent by weight, which indicates a maximum molecular weight for the RNA of 394,000 The sedimentation coefficient of the isolated RNA was 13 5*S*, suggesting that it occurs as a single molecular species From its base composition (U = 24 9 per cent, C = 22 1 per cent, A = 28 0 per cent, G = 25 0 per cent) and molecular weight, the RNA chain would consist of 1,160 nucleotides or 387 triplets From the amino-acid composition of the viral protein, the minimum molecular weight of the protein was about 25,000, corresponding to 240 amino-acid residues The coding of this protein would therefore require about 720 nucleotides, or 60 per cent of the total RNA chain From the coding triplets published in the literature and the amino-acid composition of the viral protein it can be readily calculated that all the uracil residues of the viral RNA must be in the coding part while the remaining 40 per cent of the RNA chain would contain no such residues Moreover, the maximum number of nucleotide residues between two uracils cannot exceed four in the coding part of the RNA

To check the nucleotide distribution along the RNA chain, the isolated RNA was digested with pancreatic ribonuclease, a highly specific enzyme which only splits the 3′-phosphodiester bonds of pyrimidine nucleotides The nucleotides in the digest were separated on a diethylaminoethyl cellulose column, and the amounts of the various oligonucleotide fractions determined[7] The last fraction eluted from the column contained 13 2 per cent (molar) of the total digest and had an average chain-length of 6 3 nucleotides The C U ratio in this fraction was 0 92 and was closely similar to that found in the whole digest (0 89) From the above considerations one would expect this fraction to contain no uracil at all

The considerations in the last paragraph apply to the code derived from studies on synthetic polynucleotides regardless of sequence within the individual triplets If a comparison between the suggested sequences[4,5] and our data is made, the discrepancy is even more pronounced Taking the 240 base triplets from any one of the six sequences arrived at by E L Smith[4], the maximum amount of uracil in the fractions containing tetra- and higher nucleotides could not exceed 0 36 per cent (molar) However, it was found that these fractions amounted to 28 per cent of the total digest and contained 11 per cent of the total uracil content of the viral RNA

In view of these results one must conclude that either the sets of nucleotides coding individual amino-acids are not unique (degeneracy), or that the code is not a universal one

Another consequence of this work is that the ratio of nucleotides to amino-acids (coding ratio) must be less than 5 in this virus Together with the evidence of Crick *et al* [8], this strongly suggests that the coding ratio is 3

Part of this work was supported by the U S Public Health Service We wish to thank Dr B Kassanis for the inoculum of the virus and for his interest in this work

M E REICHMANN*
M W REES
R H SYMONS
R MARKHAM

Agricultural Research Council,
Virus Research Unit,
Huntingdon Road,
Cambridge

* Permanent address Canada Department of Agriculture, Research Station, Vancouver, B C , Canada

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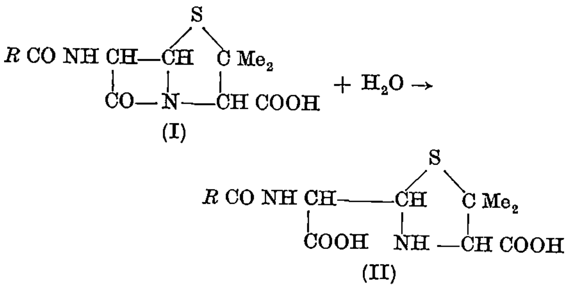
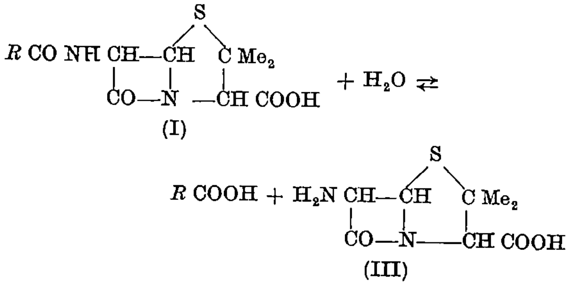
BIOCHEMISTRY

Formation of 6-Aminopenicillanic Acid by Alkaline Hydrolysis of Penicillins

Two groups of enzymes hydrolyse penicillins (I) The earlier of the two to be reported was penicillinase[1], which opens the β-lactam ring to form the corresponding penicilloic acid (II)



More recently a number of workers have reported the enzymatic removal of the side-chain[2-5] to give 6-aminopenicillanic acid (6-APA) (III)



Alkaline hydrolysis of penicillins gives virtually quantitative conversion to the corresponding penicilloic acid This quantitative conversion is the basis of some chemical methods of estimating penicillin The iodometric method depends on the iodine uptake of the penicilloic acid formed It was interesting to find that with certain penicillins alkaline hydrolysis can also remove the side-chain and under certain conditions as much as 1 per cent of the penicillin can be converted to 6-APA

When phenoxymethylpenicillin was stored between *p*H 2 and *p*H 7 no 6-APA formation could be detected, but above *p*H 7 the amount of 6-APA increased with the *p*H, until above *p*H 11, when both 6-APA and phenoxymethylpenicillin were rapidly destroyed The amount of 6-APA formed increased considerably at higher temperatures, and when the penicillin was stored at *p*H 8 5 the Q_{10} was about 2 over the range 25°–70° C

Table 1 EFFECT OF TEMPERATURE
5 mg/ml phenoxymethylpenicillin stored in 0 05 M phosphate *p*H 8 5 for 3 h

Temperature (° C)	μg/ml 6-APA formed	Temperature (° C)	μg/ml 6-APA formed
25	0 5	55	7 5
37	2 0	70	16 0
45	4 5		

The presence of phosphate seemed to have a definite effect on the amount of 6-APA formed, the yield in the presence of phosphate buffer being several times higher than when a borate buffer was used or when the *p*H was kept constant by the automatic addition of sodium hydroxide

The possibility of contamination by micro-organisms was unlikely in view of the high *p*H and temperature at which the phenoxymethylpenicillin was incubated, and when aseptic precautions were taken the yield of 6-APA was completely unaffected Similarly, the possibility of any amidase enzyme being present on the glassware was eliminated by the use of new and specially cleaned equipment

A number of penicillins were found to give rise to small amounts of 6-APA under alkaline conditions but phenoxymethylpenicillin was the most labile

Table 2 RELATIVE 6-APA FORMATION FROM DIFFERENT PENICILLINS
1 per cent solution of penicillin in 0 05 M phosphate *p*H 8 5 for 5 h at 70° C

Penicillin	μg/ml / 6-APA formed
Phenoxymethyl	30
Phenoxybenzyl	17
Phenoxyethyl	2 6
2 6-Dimethoxyphenyl	2 0
Phenoxypropyl	<2 0
Benzyl	<2 0

It is possible that detailed examination of the alkaline hydrolysate would reveal that a greater percentage of the side-chain was removed because, though somewhat more stable than phenoxymethylpenicillin, 6-APA is itself readily hydrolysed

F R BATCHELOR
J CAMERON WOOD

Beecham Research Laboratories, Ltd ,
Brockham Park,
Betchworth, Surrey

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Variations in the α_s-Casein Fraction of Individual Cow's Milk

We have investigated variations in the α_s-casein fraction[1], the calcium-sensitive component of α-casein and a major protein fraction of the casein family. Recently, Aschaffenburg[2] demonstrated the heterogeneity of β-casein and also noted the presence of γ-casein variants. The existence of 5 out of 6 possible phenotypes for β-casein was shown by paper electrophoresis in a citrate–phosphate–urea buffer at *p*H 7·15. The evidence presented suggested that β-casein polymorphism may be restricted to Channel Island breeds. Heterogeneity of α_s-casein fraction was not noted.

The components of the α-casein complex are not conveniently observed by conventional electrophoretic methods. However, the use of starch-gel-urea electrophoresis at *p*H 8·6 has proved to be an extraordinarily good method for separation of casein components. Using this technique, we investigated the homogeneity of the α_s-casein fraction. For determination of molecular parameters and structural features of this protein, assurance of its homogeneity is imperative. In addition, the detection of heterogeneity in α_s-casein would be important since the heterogeneity could possibly be related to the physical instability of the calcium caseinate complex in milk.

Individual bovine milks (Holstein, Brown Swiss × Holstein, and Ayrshire × Holstein) were selected for examination. They were obtained from the U.S. Department of Agriculture herd at Beltsville, Maryland. To prepare the samples, gravity-separated milk was precipitated at *p*H 4·6 and 22°–24° C. The precipitate was washed four times with water and ethanol, twice with acetone and twice with ethyl ether. Starch-gel-urea electrophoresis, as described by Wake and Baldwin[3], was run on casein solutions containing 7 mg casein per millilitre of buffer-urea solution. Approximately 5 V cm^{-1} was applied to the gel for 17 h, after which it was developed with amido black dye.

On examination of caseins from 93 individual cows by starch-gel-urea electrophoresis, patterns identical to pooled-milk caseins were usually obtained. However, in six cases an additional band appeared in the region ascribed to calcium-sensitive α-casein (α_s). The six individuals were all daughters of one sire, an observation which suggested a genetic basis for the heterogeneity.

Fig. 1 shows starch-gel-urea electrophoresis patterns of individual caseins, the larger numbers (3451, 3865, etc.) referring to the animal number. Patterns 1 and 7 are like patterns obtained with pooled whole casein whereas the remainder of the patterns show a double α_s band referred to as α_s-A and α_s-B in order of decreasing mobility. The use of A and B further denotes genetic nomenclature of the α_s-caseins. All other caseins studied show α_s-B casein, but α_s-A has not been observed alone to date. On mixing equal quantities of α_s-B and α_s-A/B caseins and examining the pattern on starch-gel-urea electrophoresis, a pattern typical of α_s-A/B is obtained except that α_s-A is diminished in concentration. Little question exists that α_s-B from α_s-A/B and homozygous α_s-B are identical, but isolation and characterization will be the only unequivocal proof.

In regard to the occurrence of the α_s-A/B split over the course of lactation, we have observed with cow number 4001 that the heterogeneity occurred throughout a complete single lactation and into the next. Other animals (both α_s-A/B and α_s-B) have been examined within the same lactation with identical results.

The α_s-A/B heterogeneity is detectable in veronal buffer at *p*H 8·6, $\Gamma/2 = 0{\cdot}10$, in free boundary electrophoresis, and a bimodal schlieren pattern was observable in the ultracentrifuge at *p*H 7·0, $\Gamma/2 = 0{\cdot}20$ in phosphate buffer. Urea fractionation[4] yields both α_s-A/B in the 4·7 M insoluble fraction. Subsequent treatment of α_s-A/B with calcium chloride reveals that both are calcium-sensitive. In addition, α_s-A/B caseins are eluted together from DEAE-cellulose-urea chromatographic columns[5].

As already mentioned, the double α_s band has been observed only in caseins from daughters of a single sire (*P*-17). Of 14 daughters of this sire in the Beltsville herd, six showed the heterogeneity. These daughters are coded as 3865, 3866, 3869, 3870, 3889 and 4001 (all Holstein). At this point of the investigation little can be said of the genetic nature of the occurrence of α_s-A/B casein. However, the examination at this time is centring around studying daughter–dam pairs of the *P*-17 sire. In addition, we are seeking the possible occurrence of cows producing only α_s-A casein.

Note added in proof. Since the preparation of this manuscript an additional variant (α_s-C) has been

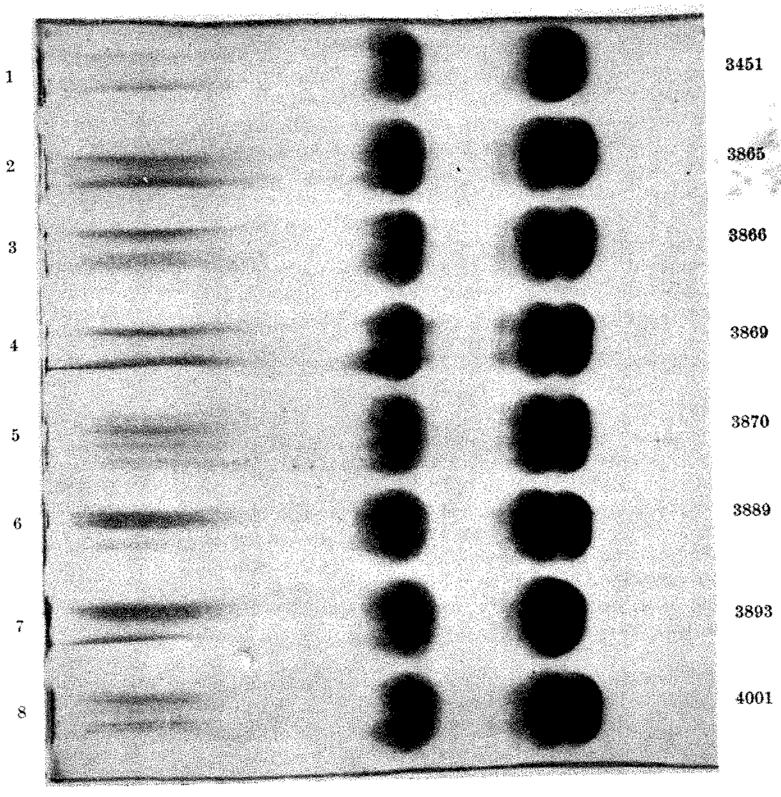

Fig. 1. Starch-gel-urea electrophoresis patterns of casein from individual cows

discovered with the co-operation of Dr. R. Aschaffenburg, Reading, England. This discovery does not alter the acceptable nomenclature of α_s-*A* and α_s-*B*, and a detailed report of its occurrence is in preparation.

M. P. Thompson*
C. A. Kiddy†
L. Pepper*
C. A. Zittle*

Eastern Regional Research Laboratory,
Philadelphia 18.

* Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

† Dairy Cattle Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

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Poly-α-amino-acids containing L-Glutamyl Residues as Substrates for Pepsin

In a study of the action of pepsin on various poly-α-amino-acids we have found that poly-L-glutamic acid as well as some amino-acid copolymers containing L-glutamic acid residues are readily hydrolysed by pepsin. Digestion was tested at 35° in 3 ml. solutions containing 12 mg of polyamino-acid[1] and 0·8 mg crystalline pepsin, adjusted to *p*H 2·3. Since most of the polypeptides investigated do not dissolve directly at acid *p*H, they were dissolved in the minimal amount of 0·1 N sodium hydroxide, and the required *p*H was obtained by adding 0·1 N hydrochloric acid. Hydrolysis was followed by measuring : (*a*) the uptake of acid in the *p*H-stat ; (*b*) the increase in the colour produced with ninhydrin[2] ; (*c*) the increase in Van-Slyke amino-nitrogen. Fair agreement was found in the results obtained by the foregoing three methods. The peptic digests were analysed by descending paper chromatography in *n*-propanol/water/concentrated aqueous ammonia (100 : 50 : 1 v/v). The chromatograms were run for 48 h, and were revealed with ninhydrin.

The following poly-α-amino-acids were resistant to peptic action : poly-L-aspartic acid, poly-L-lysine, poly-DL-alanine, poly-L-serine, poly-L-proline, poly-hydroxy-L-proline and the water insoluble poly-L-phenylalanine and poly-L-tyrosine.

Approximately 16 per cent of the peptide links of a poly-L-glutamic acid preparation, with an average degree of polymerization $n = 125$, were hydrolysed within 30 min. No marked increase in the extent of hydrolysis occurred on additional incubation for 24 h. From the uptake of hydrochloric acid at *p*H 2·3 an initial rate of hydrolysis of 3·0 μmoles peptide bond per min per mg of pepsin was calculated, assuming a negligible extent of ionization of the newly formed carboxyl groups. The rate of hydrolysis of poly-L-glutamic acid is thus very markedly greater than that of low molecular weight synthetic pepsin substrates[3].

Oligopeptides containing 2–9 glutamic acid residues could readily be detected on paper chromatograms of the peptic digests of poly-L-glutamic acid. High oligopeptides Glu$_4$ to Glu$_9$ appear in the incubation mixture at the initial stages of the enzymatic reaction (10–30 min of incubation). Further incubation results in their conversion into lower oligopeptides, triglutamic acid being the major product of exhaustive enzymatic hydrolysis. Synthetic α,L-glutamyl oligopeptides (Glu$_2$ to Glu$_8$)[4] were used as markers in these experiments.

Chromatographic analysis revealed that under the experimental conditions used, synthetic α- di-, tri-, and tetra-L-glutamic acids are essentially resistant to hydrolysis by pepsin. Penta-, hexa-, hepta- and octa-L-glutamic acids, however, are readily hydrolysed, mainly to triglutamic acid.

The rate of enzymatic hydrolysis of poly-L-glutamic ($n = 125$) at 35° decreased approximately 100-fold, on increasing the *p*H from a value of 2·3 to 4·5. The relatively low rate of peptic digestion of poly-L-glutamic acid at *p*H 4–4·5 is in accord with the recent findings of Simons *et al.*[5].

Copolymers of L-glutamic acid with D-glutamic acid, L-aspartic acid, L-lysine, L-alanine, L-tyrosine or L-phenylalanine were also tested for susceptibility to peptic digestion. Copolymers with relatively high L-glutamic acid content, such as L-Glu : D-Glu (molar residue ratio 11 : 1), L-Glu : L-Asp (9 : 1) or (5 : 1) and L-Glu : L-Tyr (9 : 1) were hydrolysed at a rate and to an extent similar to those of poly-L-glutamic acid. Copolymers with relatively low contents of L-glutamic acid were hydrolysed at slower rates and to lesser extents than poly-L-glutamic acid. The copolymers L-Glu : D-Glu (1 : 1), L-Glu : L-Asp (1 : 5) and L-Glu : L-Lys (1 : 5) were resistant to peptic digestion.

Since the high-molecular-weight poly-L-glutamic acid is readily hydrolysed by pepsin at *p*H values (*p*H 2·3–4·0) at which the substrate molecules possess a helical conformation[6], it seems that pepsin might act on polypeptides possessing a specific macromolecular stereochemical structure. A similar conclusion was reached recently by Simons *et al.*[5] from their investigation of the action of pepsin on poly-L-glutamic acid at *p*H 4·18.

The formation of the high oligopeptides at the initial stages of the enzymatic hydrolysis of poly-L-glutamic acid suggests that pepsin acts on the high-molecular-weight synthetic substrates by an 'intermediate' mechanism[7]. Our experimental findings rule out for pepsin a 'one by one' mechanism[7], in accord with the findings of Ginsburg and Schachman[8].

We thank Mr. I. Jacobson for the preparation of the polyamino-acids and Dr. Y. Levin for the synthesis of the glutamic acid oligopeptides.

This investigation was supported in part by grant No. *A*-3083 of the National Institutes of Health, U.S. Public Health Service.

Hava Neumann
Nathan Sharon
Ephraim Katchalski

Department of Biophysics,
Weizmann Institute of Science,
Rehovoth,
Israel.

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Comparison of Bovine and Human Orosomucoids

THE existence of acidic glycoproteins in human[1-3] and bovine[4,5] sera has been well established A bovine M-2 glycoprotein has been isolated in an apparently pure state as described by us earlier[5], and bovine orosomucoid has been purified by Weimer and Winzler[4] This communication describes the isolation of reasonably homogeneous bovine orosomucoid and discusses its properties in relation to the corresponding human material

Human orosomucoid was prepared from fraction VI as previously described[3] Bovine orosomucoid was prepared from a bovine plasma fraction by the carboxymethyl cellulose method[5] Orosomucoid was the only protein not retained by the carboxymethyl cellulose exchanger, equilibrated with pH 4 1 acetate buffer, ionic strength 0 033, when plasma ammonium sulphate fraction P-4 (ref 4) was applied to it at pH 4 1 (acetate) Apparent purity of the bovine orosomucoid preparation was determined by filter paper- and cellulose acetate paper-electrophoresis at pH 4 5, 7 0 and 8 6 In all cases the orosomucoid preparation showed only one band when paper strips were stained for protein (bromphenol blue and Ponceau S) and for carbohydrate (periodate-permanganate[6]) The preparation also appeared homogeneous in the ultracentrifuge at concentrations 3 2 mg/ml –10 mg/ml giving a $S_{w,20}$ of 3 2 at infinite dilution Intrinsic viscosity, determined by plotting relative viscosities versus concentration, was 0 076 Partial specific volume was 0 714, calculated from composition data Molecular weight of bovine orosomucoid, calculated from the above data, was 49,000 Amino-acid composition, except tryptophan, was determined by the method of Moore *et al* [7], tryptophan was determined according to Bencze and Schmid[12], and carbohydrates were estimated as described by Winzler[8] and Dische[9] Sialic acid was determined by the Ehrlich reaction[10] and by the orcinol method[11] Table 1 summarizes properties of bovine and human orosomucoids

Only minor differences in the amino-acid, hexose, and hexosamine content of the two proteins are apparent The sialic acid content in bovine orosomucoid was somewhat higher than in the human preparation, although by paper- and moving boundary-electrophoresis methods bovine orosomucoid has a mobility towards the anode of only 75 per cent that of human orosomucoid at pH 4 5 The sialic acid content of bovine orosomucoid, prepared in our laboratories, was also considerably higher than that of purified bovine orosomucoid as reported by Weimer and Winzler[4] Minor differences in amino-acid composition also exist between human orosomucoid reported herein and orosomucoid isolated by Weimer *et al* [1] In the latter case, amino-acids were determined microbiologically

Table 1 COMPOSITION AND PROPERTIFS OF BOVINE AND HUMAN OROSOMUCOIDS

	Bovine (per cent)	Human (per cent)
Nitrogen	11 1	11 1
Lysine	6 10	4 72
Histidine	1 57	1 13
Ammonia	1 51	1 95
Arginine	3 39	3 33
Aspartic acid	5 84	6 08
Threonine	3 37	4 02
Serine	2 87	1 62
Glutamic acid	10 50	10 10
Proline	1 57	2 06
Glycine	1 44	1 22
Alanine	3 11	1 87
½ cystine	1 64	1 08
Valine	2 22	2 55
Methionine	0 74	0 50
Isoleucine	3 30	2 65
Leucine	3 91	4 27
Tyrosine	3 36	4 52
Phenylalanine	8 85	7 12
Tryptophan	0 75	1 70
Hexose	12 0	14 4[3]
Hexosamine	11 8	10 2[3]
Sialic acid		
Ehrlich	16 2	
Orcinol	19 2	12 5[3]
Fucose	0 4	1 5[3]
Uronic acid	0 9	none
$S_{w\,20}$	3 2	3 1[13]
Intrinsic viscosity	0 076	0 069[13]
Partial specific vol	0 714	0 675[13]
Molecular weight	49,000	44,100[13]
$E_{1\mathrm{cm}}$, 1%	6 2	8 0[3]

In conclusion, relatively large amounts of apparently homogeneous bovine orosomucoid were conveniently prepared by the ammonium sulphate–carboxymethyl cellulose method Amino-acid, carbohydrate compositions and the molecular weights of bovine and human orosomucoids were very similar

We thank Dr R Becker, Oak Ridge National Laboratory, for the amino-acid analysis A portion of this work was done while A B was a research associate at the Biology Division, Oak Ridge National Laboratory

ANATOLY BEZKOROVAINY

National Animal Disease Laboratory,
Ames, Iowa

DAVID G DOHERTY

Biology Division,
Oak Ridge National Laboratory,
Oak Ridge, Tennessee

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Detection and Metabolism of Hydroxychloroquinsulphate and Hydroxychloroquindiphosphate in Human Beings

FOR the long-term treatment of chronic polyarthritis and collagenosis with hydroxychloroquinsulphate (HS) and hydroxychloroquindiphosphate (HP), accurate adjustment to a definite blood-level would be desirable Unfortunately, at the present time, there are no specific methods of quantitative determination which can be used by the clinician for both substances We therefore endeavoured to achieve satisfactory detection of HS and HP by paper chromatography and arrived at the following technique of investigation

(1) *Qualitative detection of HS and HP* Schleicher and Schull filter paper No 2043 B, isopropanol/glacial acetic acid/water (70 15 15), descending paper chromatography After drying at room temperature, tint with platinum iodide reagent (1 1 per cent potassium iodide (Merck) and 0 14 per cent platinum iodide (Merck) added fresh and mixed well in equal parts) After drying at room temperature, HS and HP are visible on a wine-red background as blue-black specks R_F values (mean values of 20

chromatograms of each) for HS, 0 75, for HP, 0 71 Minimum sensitivity of the qualitative detection by means of paper chromatography 5γ for both substances Both substances fluoresce markedly in ultra-violet light even before being dyed with a platinum iodide reagent Attempted elution of the blue-black colouring with acetone, ethanol, isopropanol, chloroform or distilled water was unsuccessful However, by parallel testing of both substances at different concentrations, it was possible to arrive at a semi-quantitative estimation

(2) *Metabolism* With oral administration of 200–800 mgm HS and HP to two separate groups, each of five adults suffering from chronic polyarthritis, all the subjects investigated revealed 1–2 further blue-black specks in their serum with R_F values of 0 16 and 0 36 respectively, which were not detectable before administration Even in thousands of other chromatograms tinted with platinum iodide reagent, I was unable to detect these substances spontaneously Allowance must therefore be made for decomposition products of HS and HP Their quantity in the serum is some 5–10 times greater than that of HS and HP 2 hr after oral administration the serum-levels of HS and HP were at their highest It was, roughly, 2–3 mgm per cent, while it was 10–20 mgm per cent in the case of the two decomposition products in question 48 hr after a single oral administration, HS and HP and their decomposition products could still be detected in the serum In the 24-hr urine I found some 1–10 mgm HS and HP, and 30–60 mgm of the decomposition products mentioned

For comparative evaluation of the rate of resorption of HS and HP, I gave two adults with chronic polyarthritis each 400 mgm HS and HP respectively, at intervals of two weeks, and determined the concentration of both substances in the serum 30 min , 2, 24 and 48 hr after oral administration Within 2 hr , the serum concentration of HS was, roughly, three times as great as that of HP

DIETER MUTING

Clinic of Medicine
University of the Saar,
Homburg/Saar

PHYSIOLOGY

Active Transport of Sodium and Chloride by the Isolated Skin of the South American Frog *Leptodactylus ocelatus*

WE have investigated the origin of the short-circuit current[1] in the isolated skin of the local frog *Leptodactylus ocelatus*

It was found that the short-circuit current was invariably and considerably smaller than the net transport of sodium determined with radioisotopes

To explain this difference from previous evidence obtained in other species of frogs, a series of experiments were performed (Table 1) which led to the conclusion that chloride ions are actively transported in the same direction as sodium ions, from the outside to the inside of the skin

If all the chlorides of Ringer solution bathing the skin are replaced by sulphates, a very good agreement between the short-circuit current and sodium influx is found (Table 1) , the current was 94 4–100 8 per cent of sodium influx

The determination of chloride unidirectional fluxes with chlorine-36 in short-circuited skins with normal Ringer solutions in both sides shows that the influx of this ion is considerably higher than the outflux The mean value for the net transport of chloride was 1 16 μequiv /cm^2

Table 1 ION FLUXES IN SKIN OF *Leptodactylus ocelatus* MEASURED WITH SODIUM-24 AND CHLORINE-36 OVER SUCCESSIVE PERIODS OF 30 MIN

	No of experiments	No of sampling periods	μequiv / h/cm^2 (± *S E*)	μamp/cm^2 computed from flux	μamp/cm^2 from short-circuit current
Na influx	8	53	2 44 ±0 35	65 5	42 0*
Na outflux	4	28	0 25 ±0 05	6 7	49 0
Na influx (in sulphate Ringer)	4	35	2 98 ±0 32	79 8	78 0
Cl influx	6	44	1 90 ±0 22	51 0	51 8
Cl outflux	4	29	0 74 ±0 25	19 9	44 0

* The mean difference, for paired data, between μamp computed and measured is statistically significant ($P<0\ 01$)

Furthermore, when sodium ions are substituted by choline in both sides, a reversal of the potential is observed and a short-circuit current due only to chloride transport could be determined If sodium is again added to the outside solution, the short-circuit current rapidly attains its normal direction and magnitude

In the skin of *Leptodactylus ocelatus*, the short-circuit current is equal to the algebraic sum of the current from active transport of sodium and chloride

If the values of the net transports are expressed in μamp and referred to the short-circuit current (I) then, $I = I_{Na\ net} - I_{Cl\ net}$ In four experiments in which sodium and chloride net transports were determined simultaneously a mean short-circuit current of 49 5 μamp/cm^2 compares with a difference of 41 2 μamp/cm^2 between the net transports of sodium and chloride

Active chloride transport in animal tissues has been described in intact Anurans[2], gastric mucosa[3] and the ileum of the rat[4] The isolated skin of European frogs shows chloride transport after the application of adrenaline, but this transport is in the outward direction and caused by the stimulation of skin glands[5]

This demonstration of a contribution to the short-circuit current by chloride transport in a frog skin clearly suggests that a ‘chloride pump is present in animal systems other than the digestive-tract

J A ZADUNAISKY
O A CANDIA

Cátedra de Física Biológica,
Facultad de Ciencias Médicas,
Buenos Aires

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Brain and Muscle Potentials from the Intra-Uterine Fœtus in Unnarcotized, Conscious Animals

IN a continuation on investigations of the development of muscular activity and brain potentials in fœtal guinea pigs[1] it was observed that punctures made with thin needles of the pregnant uterus and the fœtal membranes did not cause an appreciable outflow of the amniotic fluid In cases where such an outflow

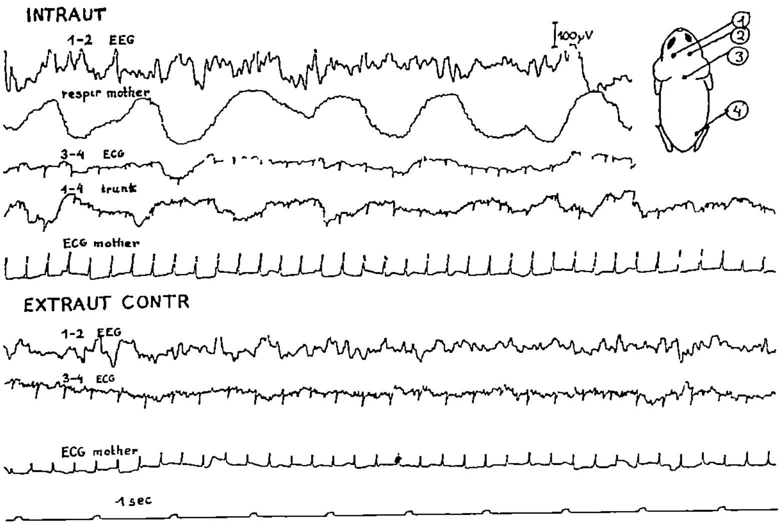

Fig 1 Recordings of the EEG and the ECG of the intra-uterine fœtus in a conscious, unnarcotized pregnant guinea pig (age of the fœtus, 50 days) The insert shows the position of the electrodes The control recording made on the same fœtus under extra-uterine conditions The μV calibration refers to EEG tracing only

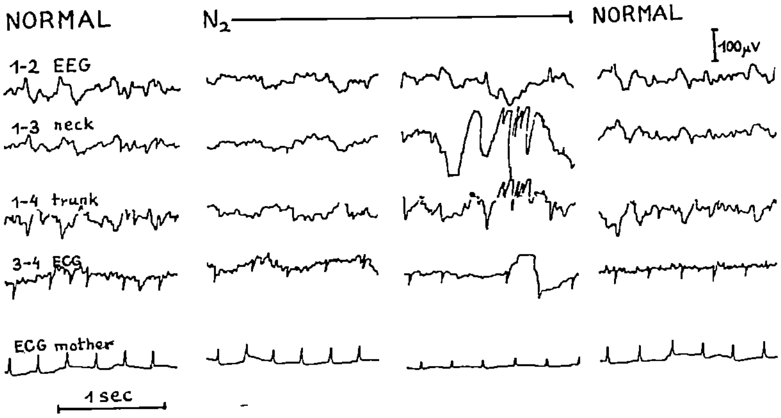

Fig 2 The effect of nitrogen breathing of the pregnant, conscious guinea pig on the EEG, ECG and muscular activity of the intra-uterine fœtus (age 60 days) The different lead numbers refer to the insert of Fig 1 The μV calibration refers to the EEG tracing only

appeared, it was soon stopped, possibly by a reaction of the membranes The experience gained in this respect led to the development of a technique which allowed of a registration of bioelectric potentials from the intra-uterine fœtus in chronic experiments with unnarcotized, conscious, pregnant animals

Pregnant guinea pigs were narcotized with 'Thiogenal' (methylthioethyl-2-pentyl-thiobarbituric acid, sodium salt, 100–150 mgm /kgm , intraperitoneal) and the uterus was exposed under sterile conditions The hook-formed steel electrodes (insulated to the bare tip) were introduced through the uterine wall, and the membranes in the desired parts of the fœtus and the flexible electrode leads were fixed by means of sutures in the abdominal wall and connected to a 6-channel electroencephalograph (Kaiser) After the experiments (observation time up to 24 hr) the recordings were controlled with the fœtus in extra-uterine conditions but in placental connexion with the mother animal

Fig 1 shows a recording made on an intra-uterine (above) guinea pig fœtus in a conscious, pregnant animal The control recording, made later on under extra-uterine conditions on the same fœtus, is seen below In this case, the fœtus was 50 days old (gestation age 65 days) as estimated from its weight[2] The electrode positions are seen in the insert with reference to the different tracings From the top can be seen the EEG of the fœtus (record 1–2), the respiration movements of the pregnant animal (electrodes in the abdominal wall near the diaphragm), the ECG of the fœtus (record 3–4), the muscular activity of the trunk of the fœtus (record 1–4, here relatively silent) and the ECG of the pregnant animal (recorded between the forelegs) In the control tracings there can be seen the EEG of the extra-uterine fœtus, the ECG of the fœtus and that of the mother The brain potentials obtained in this manner correspond to earlier findings on guinea pig fœtuses, in which the recording of the EEG followed directly on the exposure of the cortex[1]

Fig. 2 shows the result of an experiment where the mother animal was allowed to breathe nitrogen gas In this instance the fœtus was 60 days old The electrode lead numbers refer to the inset of Fig 1 The first recording on the left shows the normal state The two following recordings were taken during the period in which the mother was breathing nitrogen (3 min) The recording to the right shows the normal state of the mother and the fœtus about 5 min after the experiment It can be seen that the hypoxia caused a decrease in the amplitude of the EEG potentials of the fœtus The neck and trunk tracings show that the fœtus was in a hyperactive state at the same time as the EEG was depressed and as the ECG of the fœtus showed arrhythmia and a slowing of the heart-rate In common, it was observed that the muscular activity caused by hypoxia appeared first in the neck region of the fœtus

The results obtained by the technique reported show that it is possible to register brain and other bioelectric potentials from the intra-uterine fœtus in an unnarcotized, conscious pregnant animal The method makes possible the investigation of the development of the motor functions and certain electrical functions of the central nervous system of the animal fœtus under physiological, intra-uterine conditions It may also help in the examination of certain problems involved in obstetrics

This work was supported by a grant from the Finnish State Committee for Medical Sciences

R A M Bergstrom*

Institute of Physiology,
University of Helsinki

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* Present address Institute of Physiology, University of Marburg

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Role of the Liver in Enteral Insufflation of Oxygen during Hypoxia in Infant Rats

IN 1916 Yllpo suggested insufflation of the intestine with oxygen as a means of resuscitation of asphyxic new-born infants Since that time this method has been used clinically, but opinions as to its value are not undivided In recent years the method has been revised experimentally Again results are not equivocal[1]

It has been shown that the oxygen saturation of the blood in the portal vein increases after enteral insufflation with oxygen in both adult and new-born animals (rabbit, rat)[2,3] Oxygen saturation in the blood of the caudal vena cava centrally to the orifice of the hepatic vein, however, is not raised[3,4] It was concluded that oxygen absorbed from the intestine is utilized by the liver It could further be demonstrated that previous administration of oxygen into the intestine increased the survival-time of new-born mammals suffering different types of hypoxia (altitude 0 1 atm , nitrogen, potassium cyanide) significantly as compared with control animals not so treated After enteral oxygen insufflation in new-born animals a resuscitating effect in the stage of clinical death due to nitrogen hypoxia was also noted

The idea was put forward that this beneficial effect of enteral oxygen insufflation is mediated by a humoral action of the liver as suggested earlier by Rein[5] Hence a rough aqueous extract of minced liver tissue was prepared from normal rats and from rats insufflated with oxygen and also from animals that died in hypoxia These extracts were given intraperitoneally to infant rats and the survival-time of these animals suffering different types of hypoxia was then tested The results (Fig 1) show that a normal liver extract slightly prolongs survival-time during hypoxia This effect is increased with an extract from livers of rats which had first been given oxygen into the intestine The extract is ineffective if obtained from infant rats which died in hypoxia The basis of this humoral effect is being analysed

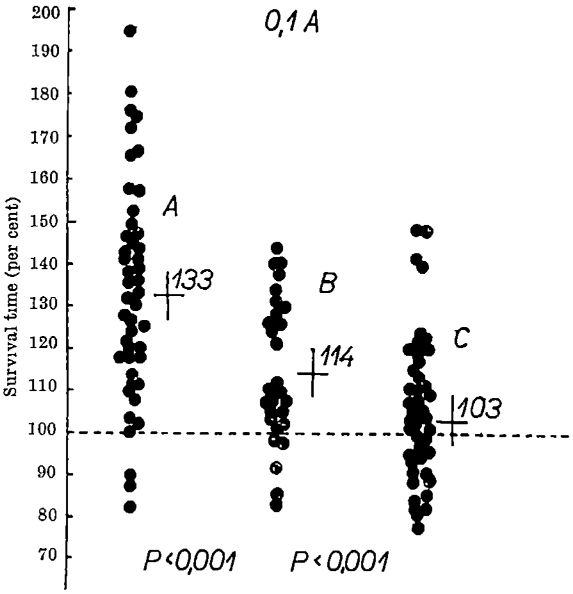

Fig 1 Changes in the survival-time in hypoxia (0 1 atm) as percentage after intraperitoneal application of a rough aqueous extract prepared from livers of rats from the same litter 100 per cent is the average survival-time of control infant rats *A*, extract from liver of animals insufflated with oxygen , *B*, the same from normal infant rats *C*, the same from infant rats that died from hypoxia

B KORECKÝ

Department of Pathological Physiology,
Faculty of Paediatrics,
Prague, 2

O POUPA

Department of Pathological Physiology,
Faculty of Paediatrics,
Prague, 2

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Sex and Age Differences in Post-tetanic Potentiation

POST-TETANIC potentiation has been investigated by a large number of authors in animal muscles[1], but only rarely in other structures and in man According to Peregrin[2], post-tetanic potentiation in man is in close relation to the action-rest phenomenon of Sechenov We have examined in 319 children aged 10–16 the activation of dynamic (phasic) work of the hand following static strain of 15–20 sec, the same hand working thus without rest A mercury dynamometer was used, the frequency of work being 1 contraction in 1 sec Five contractions after the static strain were statistically correlated with the last five contractions before it A statistically significant rise was observed in only half my 1,188 experiments (51 86 per cent girls, and 48 40 boys), which is markedly less than in the group of higher age studied by Peregrin

There was a clear difference between the group of 13–14 years old girls and the group of boys of the same age, thus

	Distribution in the group (per cent)	Limits for *P* 0 05 (per cent)
girls	62 83	57 29–68 37
boys	54 30	47 08–61 58

In our groups of older children only the tendency of the described direction is present A possible explanation is perhaps in connexion with earlier onset of fatigue in girls , fatigue for its part is necessary for successful activation[3,4]

JAROSLAV TEJMAR

Department of Physiology,
Institute for Care of Mother and Child,
Prague, 4

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Lowered Resistance to Iron in Vitamin-E Deficient Piglets and Mice

ADMINISTRATION of iron preparations to piglets is a routine procedure for prevention of anæmia It is observed that some or most of the piglets in an occasional litter may develop symptoms of acute disease and die within some hours or days after the administration The post-mortem picture is character-

Table 1 FATTY ACIDS AND VITAMIN E IN FOODS AND SOW'S MILK (MEANS OF THREE OR MORE ANALYSES)

	Fatty acids*	PFA*	Vitamin-E†
Food *A*	7 8	3 92	10 4
Food *B*	2 4	1 26	37 4
Mice food, commercial	3 9	1 87	33 5
Milk, sow 17	8 6	1 64	10 8
Milk, sow 147	11 0	1 89	13 2
Milk, sow 148	8 0	1 32	11 1
Milk, sow 52	6 7	0 88	42 2
Milk, sow 54	8 3	0 84	52 2

* Percentage w/w of dry food substance and of milk, respectively
† μgm /gm dry food substance and of milk fat, respectively

ized by pericardial and pleural effusion[1], and by waxy degeneration of the skeletal musculature[2]

In our experiments, pigs of the Swedish Land Breed were used Food *A* consisted of ground grain (oats and barley, 1 1), 76 3 per cent, skim milk powder 16 8 per cent, cotton seed oil 4 6 per cent, and minerals ($CaHPO_4$ and Na_2HPO_4, 3 1) 2 3 per cent The grain and the oil were heated to 100° C for 30 hr under continuous flow of air Food *A* produced muscular dystrophy in weaned, growing pigs within 2–3 weeks Food *B* was a commercial food for sows (Table 1)

Methods for determination of vitamin E (ref 3), polyunsaturated fatty acids (PFA)[4,5], and glutamic oxaloacetic transaminase (GOT) in pigs[6] are found elsewhere The iron preparation used was an organic iron complex (IC) containing 75 mgm of Fe^{+++} per ml The dose to a piglet was 2 ml given intramuscularly.

Sows 17, 147 and 148 were given food *A* before parturition, for 6 days at the shortest and 27 days at the longest, and onwards during the lactation Their piglets showed normal growth When treated with organic iron complex at 10–27 days of age deaths occurred in all 3 litters with a mortality-rate of 9/17 (0/5 in non-treated litter mates) (One piglet was killed on the 6th day in a paralytic condition) Apathy, dyspnœa, and staggering gait were seen some hours or a day after the injection The mean survival-time in these piglets was 2 5 days

In 2 litters of sows 52 and 54, given food *B*, 7 of 11 piglets were similarly treated without any deaths or symptoms of disease In addition, we have treated 31 litters of sows given food *B* once or twice during the first 3–4 weeks of life, without any fatal results

Plasma GOT was determined before the organic iron complex injection (day 0) and afterwards at an interval of 1 or 2 days Piglets from sows 17, 147 and 148 showed a significant elevation (mean value day 0 21 7 ± 3 13, day 1 3,330 00 ± 465 31, mean difference 3,308 30 ± 465 32, $P < 0\ 001$) In surviving piglets GOT returned to normal within a week No significant rise of plasma GOT occurred in litters 52 and 54 (mean value day 0 33 40 ± 1 77, day 1 40 30 ± 5 62, mean difference 6 90 ± 5 89, $P < 0\ 3$)

Post-mortem examination showed accumulation of clear fluid in the pericardial sac and in the pleural sacs, and waxy degeneration in the body musculature

Table 2 INTRAPERITONEAL LD_{50} DOSE OF THE ORGANIC IRON COMPLEX IN MICE EACH SERIES COMPRISED 32 MICE (8 GROUPS OF 4)

Series	Diet	LD_{50}*
I	Mice, food commercial	1,800
II	Food *A*†	810
III	Food *A*†‡	> 4,000

* Mgm /kgm body-weight Calculated from summarized mortality on seventh day after injection of organic iron complex

† From 21 days before injection of organic iron complex and onwards

‡ α-Tocopheryl acetate intramuscularly twice a week, corresponding to 7 mgm /kgm body-weight per day, beginning 21 days before injection of organic iron complex

In litter 17, 2 of 4 pigs died when given the organic iron complex at 10–14 days of age (see col 1) The two surviving pigs had plasma-GOT levels of 64 and 2,500 units, respectively, on day 1 Four pigs of the litter were given α-tocopheryl acetate intramuscularly once daily for 4 consecutive days, 6 mgm /kgm body-weight, and organic iron complex on the 5th day (age 19 days) No deaths and no elevation of plasma GOT occurred (mean value day 0 31 25 ± 4 48, day 1 37 25 ± 3 97, mean difference 6 00 ± 5 92, $P < 0\ 5$)

The LD_{50} dose of the organic iron complex was determined in 3 series of albino mice, weighing about 17 gm at the time of injection of the complex (Table 2) Repeated determinations have given corresponding values

As reviewed by Golberg *et al* [7], vitamin-E destroying activity of iron in food has long been known They showed that iron overloading in rats produced changes indicating vitamin-E deficiency

Traces of iron (Fe^{+++}) accelerate the formation of lipoperoxides and the destruction of tocopherols in autoxidizing fats[8] Formation of highly reactive hydroperoxide–metal complexes was postulated The results presented here support the assumption that large amounts of iron destroy vitamin E *in vivo*

It is generally considered that vitamin E exerts an anti-oxidant effect within the cells The introduction of iron into tissues containing high amounts of PFA and low amounts of vitamin E will possibly speed up the oxidation of PFA and enhance the reactivity of the oxidation products

This work was supported by the Jordbrukets Forskningsråd

NILS LANNEK
P LINDBERG
G TOLLERZ

Department of Medicine,
Royal Veterinary College,
Stockholm

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PHARMACOLOGY

Antibiotic Activity of Various Types of Cannabis Resin

SEVERAL methods of characterizing cannabis resin have been previously reported Thus, methods have been developed based on indophenol reaction with 2,6-dichloroquinonechlorimide[1], on a test with peroxide and sulphuric acid[2], on determining the content of the acid fraction[3] as well as on direct ultra-violet spectrophotometry[4] It has been shown in the course of this work that the differences in chemical composition shown by various types of cannabis resin may be explained by the stage of development of a phytochemical process by which cannabidiolic acid is gradually converted to cannabidiol, tetrahydrocannabinols and finally to cannabinol In previous work in this Institute this conversion process was referred to as 'ripening' of the resin Thus, cannabis in which cannabidiolic acid (the initial substance in the conversion of cannabinols) predominated was classified as 'unripe'. The resin containing mostly physiologically active tetrahydrocannabinols was referred to as 'ripe'. An 'intermediate' type of the drug contains predominantly cannabidiol, while the resin, including mostly cannabinol (the final conversion product), corresponds to the 'overripe' cannabis. An alterated type of resin, supposed to contain mostly disintegration products of cannabinols, has also been encountered. The progress of the ripening process in cannabis resin and consequently the formation of a given type was supposed to be affected by climatic, genetic and other ecological factors.

According to Krejči *et al.*[5,6] and Schultz and Haffner[7] cannabidiolic acid seems to represent the main antibiotic agent in cannabis resin. We have therefore assumed that the unripe type of cannabis, containing the largest amount of cannabidiolic acid, might exhibit the strongest antibiotic activity. Consequently it was supposed that the ripeness and the type of a drug might be roughly indicated by its antibacterial potency. No comparative data are available in the literature dealing with the antibiotic activity of various types of cannabis resin.

The purpose of the work recorded here was to examine the differences between the antibacterial potency of various types of cannabis resin and to compare the results obtained with chemical properties of corresponding samples. Eleven different samples were examined by using the diffusion method with filter paper disks, on nutrient agar medium, pH 6.0, with *B. subtilis* strain NCTC No. 8236 as test organism. The disk diameter was 12 mm and the incubation time 18 h at 37° C. A solution in penicillin G sodium in phosphate buffer of pH 6.0, in concentrations 0.5–6.0 I.U. in 1 ml., was used as standard. The dosage–response curves for ethanolic solutions of various samples were worked out in preliminary assays and accordingly the concentration of 60 mg in 1 ml was adopted for the procedure applied. The result of each assay corresponds to the calculated mean value of 10 zone diameter readings, which have been expressed in corresponding concentrations of penicillin units according to the dosage–response curve obtained simultaneously for standard preparation. As the scope of this work was not to determine the real activity of cannabis as related to penicillin, in the simplified technique adopted only one single set of tests was used, without changing the dilution in case when the readings of the inhibition zone were taken they might have been out of the reliable range. Consequently, the numerical expression of results for various samples does not necessarily correspond to a strictly quantitative relationship.

The assays were repeated on different days and the data presented correspond to the average values of several experiments. The reproducibility of results was very good. The results obtained for 11 samples examined are given in Table 1. For comparison, the type (ripeness) of the resin, as estimated by chemical methods[1–4] is also recorded.

Table 1. ANTIBIOTIC ACTIVITY AND RIPENESS OF VARIOUS SAMPLES OF CANNABIS RESIN

Sample	Antibiotic activity (I.U. of penicillin corresponding in diameter to 60 mg of resin)	Type (ripeness) by chemical methods
Germany *UN* 26	10.0	Unripe
Cyprus *UN* 32	5.60	Unripe to intermediate
Spain *UN* 45	4.12	Unripe to intermediate
Greece *UN* 1*E*	1.54	Intermediate to ripe
Greece *UN* 1*C*	0.85	Intermediate to ripe
Greece *UN* 1*F*	0.91	Ripe
Yugoslavia *KZ* 110	0.81	Ripe
Brazil *KZ* 101	0.56	Ripe
Brazil *KZ* 102	0.68	Ripe
Brazil *KZ* 103	0.54	Ripe
Canada *UN* 41	1.00	Alterated

It can be seen that the lowest values were exhibited by ripe samples from Brazil. A low potency was also shown by samples from Greece, Yugoslavia and Canada. Samples from Cyprus and Spain were much stronger, while the highest antibiotic activity was exhibited by the unripe sample from Germany, known to contain a large amount of cannabidiolic acid[3,8].

The results obtained are in good agreement with those obtained by chemical methods. To demonstrate the relationship between antibiotic activity and the content of cannabidiolic acid in cannabis resin, in Fig. 1 the results of microbiological assays have been plotted against E_{300}/E_{310} ratio as obtained by direct ultra-violet spectrophotometry. As previously reported[4], this ratio is in reverse relation to the approximate content of cannabidiolic acid in the resin examined. As seen in Fig. 1, both values appear to be correlated to each other. Only in the alterated Canadian sample, exhibiting both a low antibiotic activity and a low E_{300}/E_{310} value, may a lack of this correlation be observed. Consequently, a position on the diagram far from the line drawn might be supposed to indicate a certain alteration of phytochemical processes in the sample examined.

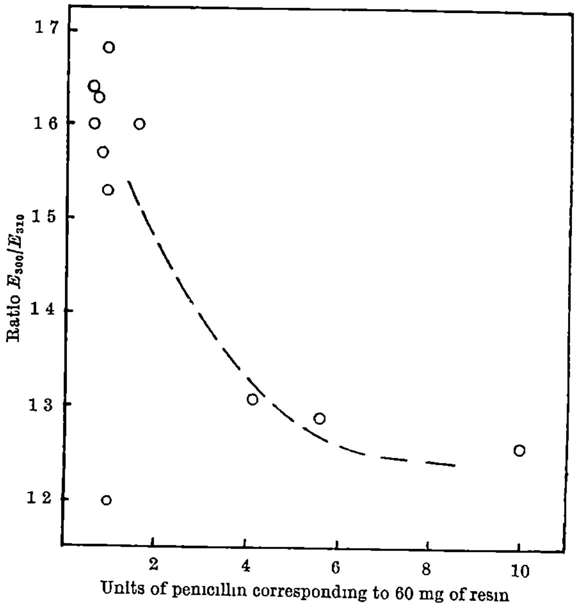

Fig. 1. Relationship of antibiotic potency and E_{300}/E_{310} value in 11 samples of cannabis resin

According to the results obtained, antibiotic activity decreases together with the progress of phytochemical conversion of cannabinols, that is, together with the increase of hashish activity. Antibacterial agent (cannabidiolic acid) is by the ripening process obviously converted into hashish-active constituents (tetrahydrocannabinols). The antibiotically active unripe cannabis seems to be more common in regions having unfavourable climate, whereas tropical samples more often correspond to the ripe, hashish-active drug. The determination of antibiotic activity may be usefully applied for the characterization of cannabis resin.

We thank the Division of Narcotic Drugs of the United Nations and Prof Décio Parreiras, Faculty of Medicine, Rio de Janeiro, for the supply of cannabis samples

A RADOŠEVIĆ
M KUPINIĆ
LJ GRLIĆ

Institute for the Control of Drugs,
Zagreb, Yugoslavia

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Biliary Excretion of some Azo Dyes related to Tartrazine

THE excretion of a number of water-soluble azo dyes in rat bile, following intravenous injection, has been previously examined by us[1] as a means of estimating the likely extent of uptake and metabolism of these compounds Among the dyes examined, two phenylazopyrazolones provided highly contrasting results Tartrazine (1*a*, R^1 = COONa, R^2 = H) was virtually not excreted at all, whereas lissamine fast yellow 2*G* (1*b*, R^1 = CH_3, R^2 = Cl) was excreted quantitatively (Table 1) We have now obtained evidence showing that the carboxyl group of tartrazine is responsible for the difference in excretion

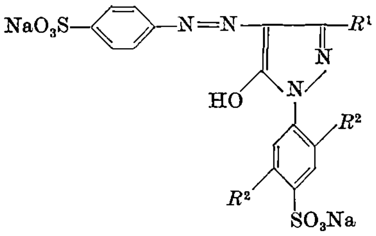

Fig 1

The ethyl ester of tartrazine (1*c*, R^1 = $COOC_2H_5$, R^2 = H) and 1-(*p*-sulphophenyl)-3-methyl-4-(*p*-sulphophenylazo-)-5-pyrazolone (1*d*, R^1 = CH_3, R^2 = H) were prepared by standard methods[2] and isolated as their disodium salts Dichlorotartrazine (1*e*, R^1 = COONa, R^2 = Cl) was similarly prepared, but could only be isolated by preparative chromatography of the reaction mixture using ethyl acetate / pyridine / water (3 1 1 by vol) as the developer The major band was eluted from the paper with hot methanol and recrystallized from the same solvent The compound possessed the expected ultra-violet spectrum and satisfactory analytical figures were obtained

The biliary excretion of these dyes was determined by our earlier method[1] The results are set out in Table 1

Table 1

Dye	Percentage excreted unchanged in bile (mean of four determinations)
Tartrazine	1
Dichlorotartrazine	25
Tartrazine ethyl ester	77
Lissamine fast yellow 2*G*	96
1-(*p*-sulphophenyl-)-3-methyl-4-(*p*-sulphophenylazo-)-5-pyrazalone	80

It is clear that in this series of compounds low excretion in the bile is correlated with the presence of a free carboxyl group in the 3-position of the pyrazolone ring Tartrazine (1*a*) and dichlorotartrazine (1*e*) are poorly excreted, while the remaining compounds all show high excretions Of these the high excretion of tartrazine ester (1*c*) is particularly significant since it rules out any possibility that the steric constraints placed upon (1*b*) and (1*e*) by the chlorine atoms make any more than minor contributions to their biliary excretions

The reasons for these differences are not yet clear Azo dyes can be reductively cleaved *in vivo*[3] and we have found amines derived from injected azo dyes in rat bile in several cases[4] However, following injection of doses of tartrazine sufficient to bring about some excretion of the dye in bile (about 2 mg) only the unchanged dye could be detected by paper chromatography No amines corresponding to reductive fission could be detected even after acid hydrolysis

Preliminary work on the urinary excretion of tartrazine has provided some additional information After intraperitoneal injection, dye is rapidly excreted in the urine This dye is a conjugate since it travels more slowly than tartrazine when chromatographed on paper The nature and amount of the conjugate are at present being investigated, but it appears likely that the carboxyl group is involved Again no amines corresponding to reductive cleavage could be found in the urine

The high resistance of these dyes to reductive fission *in vivo* is of great practical and theoretical interest It is possible that these dyes are not true azo compounds but substituted phenylhydrazones and we are at present attempting to define their fine structure by physical methods

A J RYAN
S E WRIGHT

Department of Pharmacy,
University of Sydney

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PATHOLOGY

Serological Comparisons among Tissue Culture Cell Lines

IT has long been a question whether cells derived from normal tissues acquire some malignant attributes after long-term tissue culture, especially those cells which have become established *in vitro* as lines This has been a particularly vexing problem to pharmaceutical houses, necessitating the use of primary cell cultures for vaccine production Some cells of supposed normal derivation have been shown to produce tumours after inoculation into suitable recipients[1-4] and to assume the heteroploid pattern of malignant cells[5] It is the purpose of this preliminary report to present evidence that cells of known malignant origin contain antigenic material which is not found in cells of normal origin Suggestive evidence that this antigenic material is shared by cells of malignant human and murine tissues, thus transcending species barriers, will also be given

Table 1 MICROGRAMS OF CELL-LINE ANTIGENS SHOWING COMPLETE FIXATION OF COMPLEMENT WITH FOUR UNITS OF ANTISERUM

Cell line	Species	Tissue of origin	Malignant	Antiserum HeLa	Antiserum *J*111
HeLa	Human	Cervix	+	25 (40)‡	> 100 (< 10)
*J*111	,,	Blood	+	100 (10)	25 (40)
*HEP*₂	,,	Larynx	+	50 (20)	100 (10)
KB	,,	Nasopharynx	+	12 5 (80)	50 (20)
*D*116*P*	,,	Pleural fluid	+	100 (10)	50 (20)
*D*6	,,	Marrow	+	50 (20)	100 (10)
*D*189	,,	Foreskin	+ *	50 (20)	50 (20)
Wish	,,	Amnion	−	> 100 (< 10)	> 100 (< 10)
*D*98	,,	Marrow	−	> 100 (< 10)	> 100 (< 10)
Chang	,,	Liver	−	> 100 (< 10)	> 100 (< 10)
Hene	,,	Intestine	−	> 100 (< 10)	> 100 (< 10)
Henle	,,	Intestine	−	> 100 (< 10)	> 100 (< 10)
Chang	,,	Conjunctiva	−	> 100 (< 10)	> 100 (< 10)
Girardi	,,	Heart	−	> 100 (< 10)	100 (10)
FL	,,	Amnion	−	> 100 (< 10)	> 100 (< 10)
Salk	Monkey	Heart	−	100 (10)	> 100 (< 10)
*P*1534	Mouse	Leukæmia	+	50 (20)	50 (20)
*Ca*755	,,	Breast	+	12 5 (80)	50 (20)
*G*99	,,	*Ca*755	− †	> 100 (< 10)	> 100 (< 10)

* 'Malignant transformation' of normal tissue

† Non-tumour producing clone of *Ca*755

‡ Figures in parentheses indicate reciprocal of dilution of antigen showing complete fixation

The growth and extraction of HeLa and *J*111 cells have been described recently by us[6] The final aqueous supernates of the HeLa and *J*111 cells after fluorocarbon homogenization were adjusted to contain 2 mg of protein per ml and emulsified with equal volumes of complete Freund's adjuvant Rabbits received a total of 6 mg of either antigen in adjuvant as a series of six weekly intramuscular injections The rabbits were bled by cardiac puncture 10 days after the final injection and the sera stored at −20° C until used

All cells used for the extraction of test antigens were purchased as frozen pellets of spinner cultures from Microbiological Associates, Inc, Bethesda, Maryland, and were treated with fluorocarbon and assayed for protein, as described[6] Where no protein was detectable in a final supernate, this supernate was treated as if it contained 0 5 mg of protein per ml Either on this basis (for cells of normal origin) or from actual protein determinations, the antigens were adjusted to contain 100γ protein/ml The hæmolytic system used was as described[6], except that falling dilutions of each antigen were tested against an optimal concentration of both anti-HeLa and anti-*J*111 rabbit sera

The results of duplicate complement fixation tests using the 19 cell lines tested thus far against anti-HeLa and anti-*J*111 rabbit sera are given in Table 1 The results are expressed as the γ protein/ml required to cause complete fixation of 4 H_{100} units of complement in the presence of four units of antiserum A value of > 100 indicates that no fixation occurred, and the effective complement finding capacity of each antigen can thus be estimated in terms of γ of protein All the antigens extracted from cell lines of reported human malignant origin reacted with both antisera to varying degrees Of the eight cells of normal human origin, only the Girardi human heart showed any fixation of complement and that only at 100 γ/ml The same applies to the Salk cynomolgus monkey heart line It is not possible at the present time to state that these two reactions are *de facto* characteristic of malignancy as little is known of the antigenic purity of the extracts used to produce the antisera

An unexpected finding reported here was the observation that both the antigens extracted from murine lines *P*1534 and *Ca*755 fixed complement in the presence of antisera to both HeLa and *J*111 antigens Of further interest was the finding that no fixation was found using antigen of *G*99 This cell was reported[7] as a non-tumour producing clone of *Ca*755 Investigations are at present being extended to a variety of other cell lines of different animal origin in addition to studies to determine the true species of origin of the cells

J M McKENNA
W S BLAKEMORE

Harrison Department of Surgical Research,
University of Pennsylvania

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Mono- and Di-amine Oxidase Activities of Human Tumours and Host Tissues

THE available information on the decarboxylation pathways of histidine and aromatic amino-acids in human cancer is incomplete

Histidine decarboxylase activity was found to be present in the basophils from chronic myelogenous leukæmic patients[1], and their blood histamine is greatly increased[2,3] No change of the histamine content was observed in fibroid growth as compared with normal human skin[4] Carcinoma of the lung were found to contain less histamine than the surrounding tissues of the patients[5] Histamine is abundant in human prostate adenomas[6]

Carcinoid tumours have large amounts of 5-hydroxytryptamine[7,8] Mono-amine oxidase activity was found to be low in human carcinoma[9], and in

Table 1 MONO- AND DI-AMINE OXIDASE ACTIVITIES OF HUMAN TUMOURS

	µl O_2/mg tissue nitrogen/h Monoamine oxidase	Diamine oxidase
Chronic myeloid leukæmic cells	−1 65	−0 35
Melanoma	−1 77	−0 30
Ovary metastases of breast adenocarcinoma	−0 60	0 7
Enlarged lymph node generalized lymphosarcoma	1 70	−0 19
Non-pigmented melanoma	2 62	0 37
Adrenal metastasis of scirrhous carcinoma of breast	3 58	0 21
Squamous cell carcinoma of renal pelvis	4 8	−0 09
Lymph node metastasis of gastric adenocarcinoma	11 73	7 88
Grawitz's tumour of kidney	13 98	−0 03

Table 2 AMINE OXIDASE ACTIVITY OF REGENERATING RAT LIVER

	µl O_2/mg tissue nitrogen/h
Normal rat liver	10 64
Rat liver (removed 20 h after partial hepatectomy)	2 04

Table 3 MONO- AND DI-AMINE OXIDASE ACTIVITIES OF HUMAN HOST TISSUES

	µl O_2/mg tissue nitrogen/h Monoamine oxidase	Diamine oxidase
Plasma from chronic myeloid leukæmia	−0 42	—
Liver (from leiomyosarcoma	11 76	−0 13
Adrenals (from scirrhous carcinoma of breast)	12 38	−0 89
Adrenals (from squamous cell carcinoma of vulva)	19 0	−0 75
Adrenals (metastatic anaplastic carcinoma of lymph node primary in breast	26 03	−1 32

lung and liver metastases[10] In carcinoid metastases and phæochromocytoma the low mono-amine oxidase activity was associated with increased amino-acid decarboxylation[11,12] Mono-amine oxidase activity was found normal in glia tumours[13] and argentaffin carcinoma[14]

The activity of mono- and di-amine oxidase of different human tumours and host tissues was determined as described elsewhere[15,16]

Table 1 shows that the mono-amine oxidase activity varies with the kind of tumour A tendency towards low activity is observed as in fast-growing tissues From Table 2 it can be seen that mono-amine oxidase activity is strongly decreased in the regenerating rat liver as compared with normal liver

Diamine oxidase was absent in all the tumours studied excepting the lymph node metastases of gastric adenocarcinoma As can be seen from Table 3, host tissues appear to have unchanged mono-amine oxidase activity and no histaminase activity was observed in them

K W STARR
J GIBBONS
J C CLAUDATUS

New South Wales State Cancer Council,
Special Unit for Investigation
and Treatment,
Randwick, New South Wales,
Australia

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RADIOBIOLOGY

Changes in Density of Blood Serum with Irradiation Dose and Post-irradiation Period

WE have shown that after the whole-body irradiation of rats using a 600 r dose the increase of density of their blood serum was statistically significant[1] This increase was not observed after some stress impulses as, for example, scorching and thirsting

This communication shows that these changes are dependent on time and on the whole-body irradiation dose

The method used was the same as before[1] The density was determined by weighing the serum which was measured by means of a microburette screw placed in the thermostat The measured volume was calibrated by redistilled water The required quantity of serum for one determination amounted to 0 25 ml Each sample was determined twice, and the average of the two values was taken Rats of the Wistar strain, weighing 150–200 g, were used Irradiation was carried out with the Makrophos' X-ray apparatus at 150 kV, 15 m amp, 0 5 copper filter, 100 cm focus distance, dosing 6 7 r /min

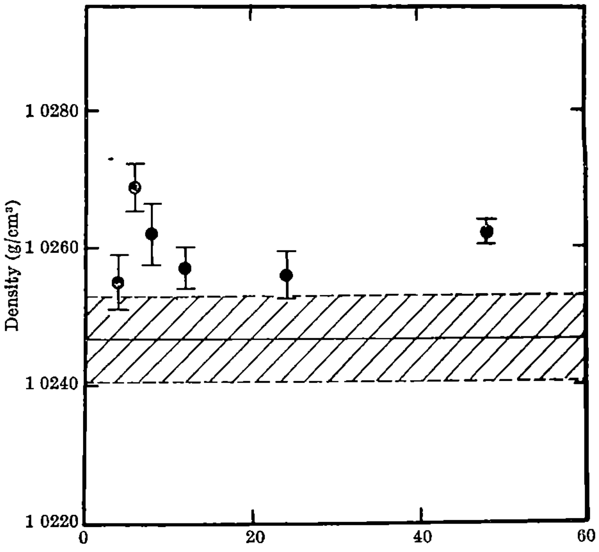

Fig 1

As illustrated in Fig 1, the maximum changes after an irradiation dose of 600 r were observed 6 h after the irradiation The abscissa indicates the density values in g/cm³, the ordinate represents the time period in hours The number of animals in the control group was 18, in the group observed 6 h after irradiation 12, the other group consisted of 6 rats The average values and the standard errors are also indicated The dashed line indicates the average and the standard errors in the controls

Fig 2 illustrates the dependence of the changes in the blood serum density 6 h after irradiation on the whole-body irradiation dose The abscissa gives the density values in g/cm³, the ordinate shows the

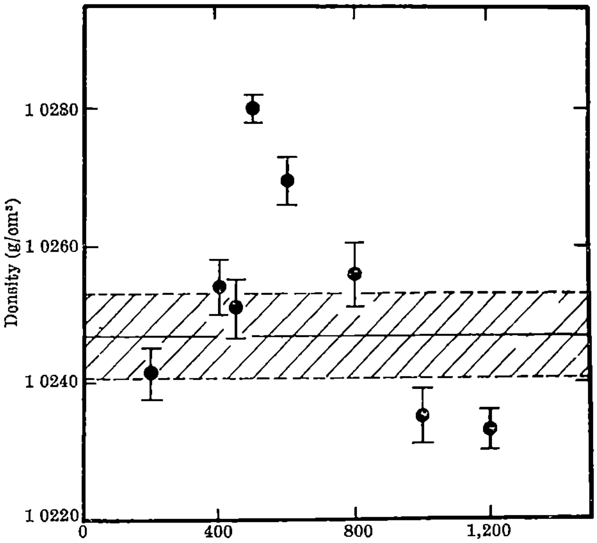

Fig 2

dose in r Here again the average values and the standard errors are given The dashed line indicates the average values and the standard errors in the control animals

A significant increase in the density of the blood serum was observed 6 h after the application of 0 2 mg *tris*-(β-chlorethyl amine hydrochloride) and 24 h after intravenous application of colloidal chromium phosphate labelled with phosphorus-32 in a quantity corresponding to an absorbed dose of 7,000 rads in the liver

From the results achieved, it follows that the changes in the density of the blood serum depend on the extent of the whole-body irradiation dose and on the period of irradiation Maximum changes were observed 6 h after the application of a 500 r irradiation dose Knowing that the changes in the blood serum density are subjected to a series of factors, we presume, however, that the disturbance in proteosynthesis and the disturbance in DNA creation leads to the accumulation of catabolites in the blood These catabolites, in the same way as the decay products, especially those of the white blood elements can take part in the changes affecting the serum density It is also possible that a change in the space configuration of the protein macromolecules and the altered conditions in the water quantity bound on proteins may play here an important part In doses greater than LD_{100} it is necessary to take into consideration the disturbed renal function These factors are being examined at present, and we are investigating the correlation between the changes in blood serum density and other changes in the physico-chemical properties of blood

Z Dienstbier
M Rakovič

Biophysical Institute,
Charles University,
Prague

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Complex Amine Oxides with Radioprotectant Properties

Previous reports[1–3] have shown that certain amine oxides can increase total survival after lethal whole-body X-irradiation Recently it was found that the complex compounds in Table 1 were active radioprotectants

Groups of 20 *CF*-1 male mice were given orally 250 mg/kg of the compounds 24 h prior to exposure to 600 r X-irradiation under our usual conditions[3] The degree of radioprotection based on total survival varied from 15 to 40 per cent, although the —N=C— structure required for activity was present in all compounds These differences in activity may be related to substituent groups exerting directing influences on the molecules and forcing the radiation-induced oxidizing radicals to attack substituent groups which are less readily oxidizable Both isoimidazole derivatives present two methyl groups where such oxidations could occur, but directing influences could cause the oxidation to occur in the 4 position of the benzene ring Under such conditions one would expect the compound containing the phenyl group to be more potent because hydroxylation is a one-step process whereas the tolyl group usually is converted to the corresponding acid by a three-step process The results in Table 1 support this explanation

Table 1 Effect of Complex Amine Oxides on Survival after X-Irradiation

Medication	ST_{50} and range	Slope and range	Mortality Final (%)	Day
Saline control	13 1 (10 8–5 9)	1 54 (1 34–1 78)	90	30
4,5-dimethyl-2-(*p*-tolyl)-1,3-isoimidazole-1,3-dioxide	15 5*(12 8–18 8)	1 52 (1 30–1 78)	75	30
4,5-dimethyl-2-phenyl-2-isoimidazole-1,3-dioxide	—	—	65	30
3,4-diphenyl furazan-1-oxide	—	—	60	30
Saline control	8 0 (6 6–9 7)	1 55 (1 35–1 78)	95	17
5-Quinazolinol,3-oxide	—	—	55	30

ST_{50}, day on which 50 per cent of animals are expected to be still alive Confidence limits are calculated at $P=0\ 05$ (ref 4) All drugs 250 mg/kg orally 24 h pre-irradiation

*Not significant at $P=0\ 05$

Similarly, radiation-induced hydroxylation of the phenyl group could explain its radioprotectant activity The quinazolinol compound can exist *in vivo* in the keto or lactam form and should behave analogously to a hydroxyquinoline This would form the —N=C— structure, and the substituent group in position 4 could direct the oxidation to the 2 position Previous work[1,2], as well as these results, would support the foregoing radical trapping mechanism as an explanation for the radioprotectant activity of the compounds in Table 1 Furthermore, such results indicate a direction for further research in the development of new and better radioprotectant drugs

We thank Parke-Davis and Co for the quinazolinol compound and Lilly Research Laboratories for the other compounds used in this investigation, which was supported by Contract *AT*(04-1)*GEN*-12 between the Atomic Energy Commission and the University of California

Thomas J Haley
A M Flesher
L Mavis

Laboratory of Nuclear Medicine and Radiation Biology,
Department of Biophysics and Nuclear Medicine,
School of Medicine,
University of California at Los Angeles

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Indirect Action of X-Radiation on Mammalian Cells

Ionizing radiations may impair the reproductive capacity of cells either by directly altering specific biochemical sites or indirectly by giving rise to free radicals in water which then react chemically with specific sites In the frozen state the mobility of free radicals is hindered, and consequently the radiosensitivity of cells reduced to an extent that depends on the degree to which the indirect mechanism is involved

Although the demonstration that radiosensitivity of a system is reduced in the frozen state does not by itself constitute proof that the indirect mechanism is involved, since a number of physico-chemical factors (oxygen sensitization[1], temperature dependence of radiosensitivity independently of change in state[2,3])

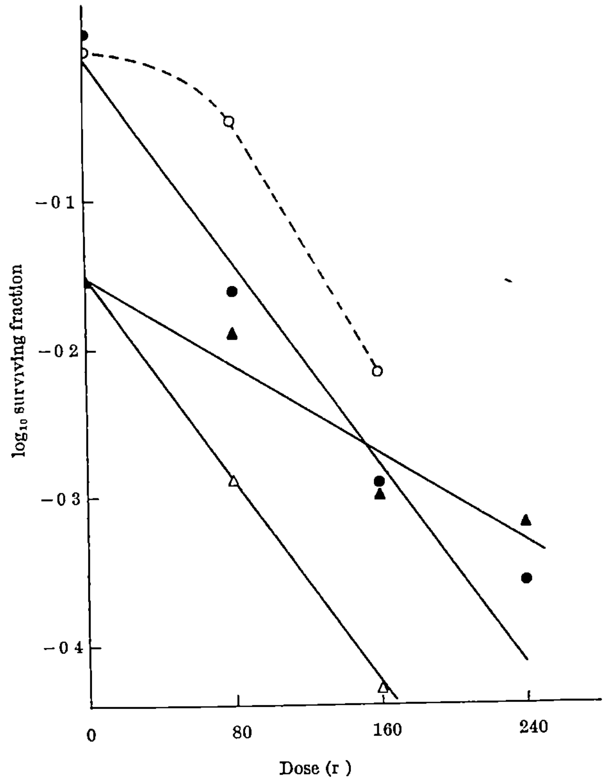

Fig 1 Dose-survival curves for *S*3 HeLa cells irradiated at room temperature without added dimethyl sulphoxide (*A*, O), with 10 per cent dimethyl sulphoxide at room temperature (*B*, ●), at −79° (*C*, ▲), and at room temperature after thawing (*D*, Δ) Each point plotted represents the mean for up to five replicate determinations Data at zero dose were included in calculating all regression lines except curve *A*

may also contribute to the phenomenon, nevertheless, for single cells, and especially for mammalian cells which, unlike spores or seeds, cannot exist in the desiccated state, this may constitute the strongest type of evidence at present available[4]

Bacteria[5] and seeds[6] have been shown to be more radioresistant when frozen, but surprisingly the opposite effect has been reported for mammalian cells In a series of carefully controlled experiments, Lasnitzki[7] showed that mouse ascites cells when irradiated *in vivo* were considerably more sensitive at − 79° C than at room temperature, both to γ- and to X-rays

We have explored this phenomenon in another mammalian cell line—HeLa *S*3 strain derived from a human cervical carcinoma—by using the single-cell plating technique described by Puck[8] The cells were cultured under an atmosphere of 5 per cent carbon dioxide in a medium consisting of 20 per cent pooled human AB serum, 10 per cent horse serum and 70 per cent Eagle's medium (Difco, Detroit) Reproductive capacity was demonstrated by the ability of single cells to form colonies Immediately before freezing, dimethyl sulphoxide was added to give a final concentration of 10 per cent[9,10] Freezing was carried out at the rate of 1° C per min down to −15° C and then as rapidly as possible down to −79° C [11] One hour after freezing cells were irradiated with 140 kVp X-rays at a dose-rate of 240 r /min, with doses of 80, 160 and 240 r respectively, and were allowed to remain at −79° C for a further 24 h, when they were rapidly thawed by immersing in a water-bath at 38° C Three control series were investigated at the same time In the first two, cells were subjected to the same irradiation procedure but without subsequent freezing and thawing, with and without the addition of dimethyl sulphoxide respectively In the third series irradiation was carried out immediately after thawing

67 per cent of the cells survived freezing alone Dimethyl sulphoxide at a concentration of 10 per cent did not reduce their viability, but appeared to eliminate the 'shoulder' of the dose-survival curve usually exhibited by this strain of cell (Fig 1 compare curve *A* with curves *B*, *C* and *D*) In the frozen state there was considerable reduction in radiosensitivity, the inactivation dose, D_{37}, being 573 ± 113 r (*S E*), compared with 252 ± 36 r for cells irradiated at room temperature (curves *C* and *B*, respectively) The possibility arose that cells which had survived freezing were also more resistant to irradiation or that freezing may have altered cells in a manner to confer radioresistance However, D_{37} for cells irradiated immediately after thawing was 252 ± 72 r (curve *D*) This difference in the value of D_{37} for cells irradiated in the frozen state from that for cells irradiated at room temperature or on thawing was significant by Student's *t* test ($P \leqslant 0\ 01$ and $0\ 02 < P < 0\ 05$, respectively)

Our results therefore provide evidence for the indirect action of X-radiation on mammalian cells The difference between our results and the paradoxical finding of Lasnitzki may reside in the difference in experimental conditions Principal among these was that we used an entire *in vitro* system, whereas Lasnitzki adopted an *in vivo* test of viability, in which tumour–host immunological relationships may have played a prominent part Moreover, we examined radiosensitivity over a comparatively low dose-range in which the effects on reproductive capacity conform to the predictions of classical target theory A single, comparatively high, dose of 1,500 r was used in the *in vivo* studies and it may be difficult to interpret such results without more knowledge of the shape of the dose-survival curve for the cell system concerned

We thank Dr J H L Playfair for his help in freezing the cells, and Miss P M Scully for technical assistance

K B DAWSON
E O FIELD
H MADOC JONES

Radiobiological Research Unit,
Radiotherapy Department,
Institute of Cancer Research,
Royal Cancer Hospital,
London, S W 3

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BIOLOGY

Connexion between Protein Synthesis and Salt Absorption in Plant Cells

From an examination of the effects of various factors, for example, temperature, external *p*H, and provision of a suitable nitrogen source on salt absorption and protein synthesis in tissue slices, Steward and Millar[1] concluded that the two processes are closely related The possibility that the relationship is an indirect one through a common dependence on respiratory energy was not at that time excluded New evidence for a more direct connexion came from the observation that chloramphenicol, which is known to be a specific inhibitor of protein synthesis in micro-organisms[2] and in higher plants[3], also inhibits salt absorption in storage tissue slices[4]

More recent experiments have shown that chloramphenicol interferes both with the uptake of amino-acids labelled with carbon-14 by slices of carrot root and with the incorporation of the label into newly synthesized soluble proteins of the tissue It thus appears to be confirmed that chloramphenicol inhibits protein synthesis in the tissue under conditions in which salt absorption is also prevented The details of these experiments and a discussion of the results are presented elsewhere[5]

MacDonald *et al* [6] have demonstrated that net protein synthesis occurs in tissue slices cut from the storage organs of various plants and transferred to aerated tap water They have suggested that the rate of synthesis depends on the concentration of nitrate ions in the solution In the present investigation, the effects of washing carrot tissue in various solutions on the amount of protein synthesized and potassium taken up were examined in an attempt to discover whether any quantitative relationship between the two processes could be demonstrated The results presented in Table 1 show that whereas the amount of protein synthesized in the presence of potassium chloride did not differ significantly from that synthesized by tissues suspended in distilled water, potassium nitrate increased the rate of synthesis by about 3 times The potassium content of slices incubated in potassium nitrate solution on the other hand was only about 1 2 times as great as that of slices aerated in potassium chloride solutions It is evident that nitrate has a much greater effect on protein synthesis than it has on potassium accumulation When chloramphenicol was supplied in the presence of potassium nitrate, both the synthesis of protein and potassium uptake were inhibited by about 50 per cent

The present results may be most simply explained as follows (Fig 1) Various kinds of protein (P_1, P_2, P_3, P_4, etc) are synthesized from common precursors (X) which may be the amino-acids, in a series of reactions, and one of the steps in each case is inhibited by chloramphenicol The accumulation of potassium ions is perhaps related to the synthesis of a particular kind of protein (P_1) which may be located in the surface membrane of the cell and act as an ion carrier through a cycle of breakdown and resynthesis The remainder of the proteins in the cell may be synthesized without participating in ion absorption and it is possible that they are broken down and resynthesized less rapidly than are the surface membrane proteins Because the latter represent only a small fraction of the total proteins in the cell, it may be difficult to determine their rates of turnover and to correlate these with rates of salt accumulation Nevertheless an attempt to accomplish this is being made

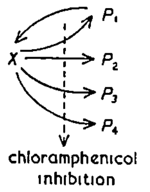

Fig 1. Cycle of synthesis and breakdown during which P_1 acts as an ion-carrier

Table 1 Protein Synthesis and Potassium Uptake by 10 Carrot Root Slices (1 mm Thick, 7 5 mm Diam) aerated for 96 hr at 25° C in (*a*) Water , (*b*) Potassium Chloride (10^{-2} *M*), (*c*) Potassium Nitrate (10^{-2} *M*), (*d*) Potassium Nitrate (10^{-2} *M*) + Chloramphenicol (2 μgm /ml.)

		Treatments *a* H$_2$O	*b* KCl	*c* KNO$_3$	*d* KNO$_3$ + CAM	*L S D** †*P*= 95%	*P*= 99 9%	Ratios $\frac{c}{b}$	$\frac{c}{d}$
Protein N content (μgm)	66	90	92	143	109	13	24		
Increase in protein N (μgm)	—	24	26	77	43	13	24	3 0	1 8
K$^+$ Content (μmol)	7 6	5 8	27 2	31 6	19 7	4 6	8 6		
K$^+$ Uptake (μmol)	—	−1 8	19 6	24 0	12 1	4 6	8 6	1 2	2 0

*Least significant difference

† Probability of significance

B Jacoby

Hebrew University of Jerusalem,
Rehovot

J F Sutcliffe

Department of Botany,
King's College,
University of London

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Freeze-Drying of Plant Tissues

Freeze-drying has proved a valuable technique in cytological investigations of animal cells and tissues, but it has not met with the same success when applied to plants[1] Frozen-dried animal tissues are usually embedded in de-gassed paraffin wax which is later removed from the sections with xylol Unfortunately, infiltration of frozen-dried plant tissue with molten paraffin or ester wax has proved extremely difficult, if not impossible Previous failures may have been due to the large vacuoles and intercellular spaces which make plant cells particularly liable to damage during infiltration These capillary spaces fill with air when frozen-dried tissues are brought from the vacuum chamber into the atmosphere and, even when subsequent infiltration is done *in vacuo*, the trapped air probably prevents complete impregna-

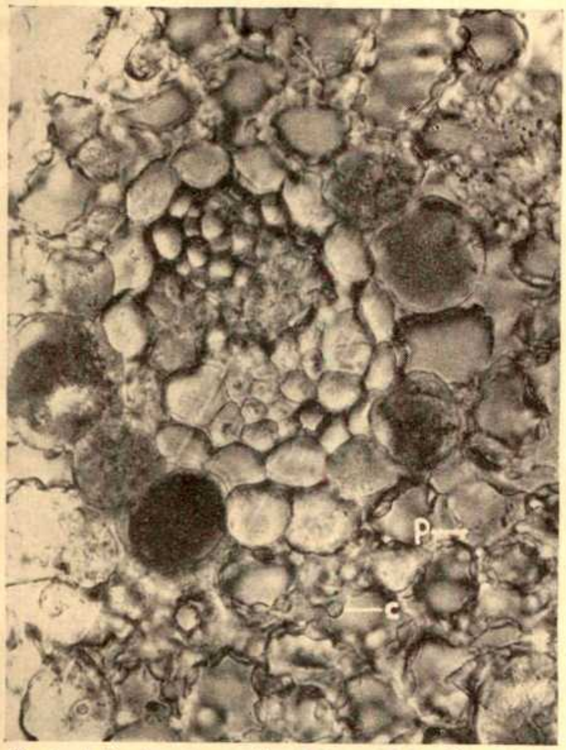

Fig. 1. A 15-μ transverse section of frozen-dried seedling oat leaf embedded in 'Epon 812', showing a vascular bundle surrounded by mesophyll cells in which chloroplasts (*c*) and cytoplasmic particles (*p*) are preserved. (× *c*. 950)

tion. Another likely cause of damage is the removal of lipoidal substances from the membranes in plant protoplasm by molten paraffin wax and fat solvents.

A new freeze-drying apparatus has been developed which incorporates improvements in operation and tissue treatment. The vacuum chamber design is based on a theoretical analysis of the physical requirements for efficient low-temperature drying. A description of the apparatus is to be published elsewhere. The purpose of this communication is to report the preservation of cell contents in frozen-dried plant tissues. Under the light microscope, cellular and protoplasmic morphology compares favourably with the structures seen in living cells.

Two methods of post-drying treatment were used: (*a*) direct embedding with epoxy resin 'Epon 812'; (*b*) post-drying fixation with osmium tetroxide in benzene followed by embedding in ester wax. 'Epon 812' is an embedding medium used in electron microscopy, and a detailed procedure for embedding chemically fixed specimens has been published by Luft[2]. The new freeze-drier incorporates an embedding side-arm which forms part of the glass vacuum chamber and allows the isolation of the embedding medium during the drying period. Such an arrangement is essential when embedding with synthetic resins because the fluid monomer would otherwise sublime on to the 'cold finger'. The complete monomer, including the catalyst, is placed in the bulb of the embedding side-arm and degassed prior to the start of drying. When the specimens are dry the glass isolation tap is opened and the monomer is poured over the specimens. After 1 hr., air is admitted and the specimens are transferred to resin-filled capsules. As Luft explains, final hardness can be adjusted by varying the proportion of resin constituents. To obtain sections for observation under the light microscope, a block similar in hardness to butyl methacrylate can be cut with a steel knife. Direct embedding with 'Epon 812' avoids the use of solvents and no air is allowed to enter the dry tissue. Thus liquid/gas interfaces do not pass through the tissue, and diffusible cell compounds are less likely to be displaced. Fig. 1 is a photomicrograph from a 15-μ transverse section of a small (1 × 1 × 0·5 mm.) seedling oat leaf specimen embedded in 'Epon 812' after 4 hr. vacuum drying at − 30° C. The section was observed and photographed without removing the 'Epon' matrix. There is very little distortion in the tissue and the preservation of chloroplasts and cytoplasmic particles is clearly illustrated.

If the specimens are not required for cytochemistry or microscopic autoradiography, an effective method of post-drying fixation is to introduce 0·1 gm. of osmium tetroxide in 5 ml. of benzene, again using the embedding side-arm to deliver the fluid on to the specimens. Post-drying fixation is judged visually and leaf pieces usually require 1–2 hr. Sometimes specimens do not sink in benzene and they have to be passed through ethyl alcohol to xylol, with the usual intermediate baths. Once the specimens have sunk they can be transferred directly to capsules of molten ester wax. Fig. 2 is a 5-μ transverse section of frozen-dried soybean leaf fixed with osmium tetroxide in benzene and embedded in ester wax. The section was flattened on an aqueous solution of Mayer's albumin, and the ester wax was removed with xylol. Fig. 2 shows that the structural arrangement of the tissue was preserved in freeze-drying. Within cells the parietal position of chloroplasts and well-defined vacuoles indicate that the tonoplast and cell contents were not disturbed. A microscopic system of thread-

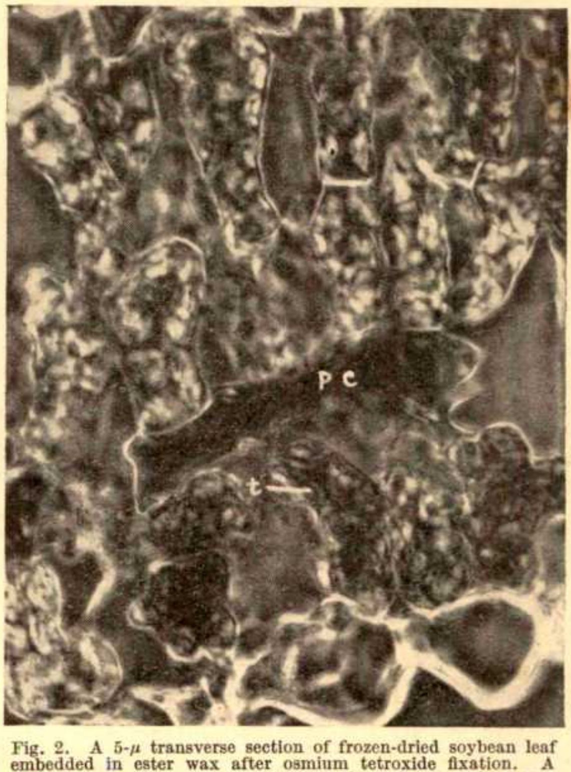

Fig. 2. A 5-μ transverse section of frozen-dried soybean leaf embedded in ester wax after osmium tetroxide fixation. A parenchyma cell (*pc*) occurs between the palisade and spongy mesophyll cells, and thread-like structures (*t*) are apparently associated with chloroplasts. (× *c*. 950 phase contrast)

like structures is revealed within mesophyll cells between chloroplasts and overlying chloroplast membranes. The paradermally oriented parenchyma cell which occurs between the palisade and spongy mesophyll cells is a structural feature not previously revealed in leaves.

I thank Prof. R. D. Preston for help.

R. THAINE

Astbury Department of Biophysics,
University of Leeds.

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Fatty Acid Control of Ethane Production by Sub-cellular Particles from Apples and its Possible Relationship to Ethylene Biosynthesis

Burg and Burg[1] have pointed out that the gas evolved in incubation mixtures of thiomalic acid and sub-cellular particles from apples and tomatoes was in fact not ethylene, as previously reported[2]. However, the appearance of an 'ethylene-like' gas which chromatographed very close to ethylene was confirmed. The purpose of this communication is to identify the 'ethylene-like' gas, to report that linolenic acid may be an important component of the enzymatic system, and to indicate the possible pertinence of this system to the pathway for ethylene biosynthesis in plant cells.

The 'ethylene' evolved in the enzymatic system of Lieberman and Craft[2] and the 'ethylene-like' gas of Burg and Burg[1] is, in fact, ethane. This was established by co-chromatography of authentic samples of ethane and the 'ethylene-like' gas evolved in the enzymatic systems. A typical gas chromatogram of authentic ethylene, authentic ethane, the 'ethylene-like' gas, and a co-chromatogram of ethane and the 'ethylene-like' gas is shown in Fig. 1. The co-chromatograph yields one peak which chromatographs exactly like authentic ethane. D. F. Meigh, of the Ditton Laboratory (personal communication), utilizing some of our freeze-dried particulate preparations earlier identified ethane as the 'ethylene' gas of Lieberman and Craft[2]. Therefore the enzymatic particulate system from apples and tomatoes, when incubated with thiomalic or thioglycolic acid and co-factors, produced ethane.

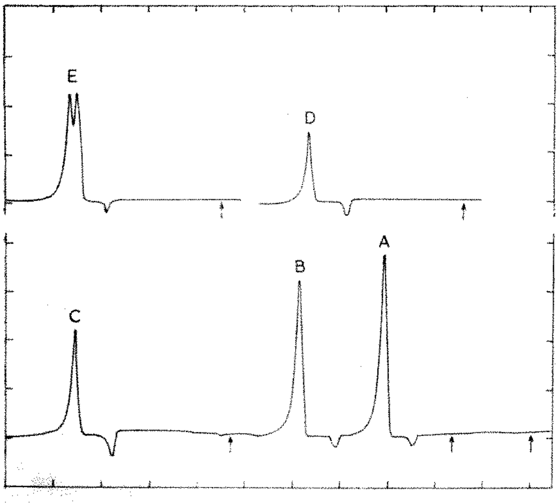

Fig. 1. Gas chromatogram of (*A*) authentic sample of ethylene, (*B*) authentic sample of ethane, (*C*) volatiles evolved by apple particles, thiomalic acid and co-factors, (*D*) co-chromatograph of *B* and *C*, (*E*) co-chromatograph of authentic ethylene and ethane. Samples were inserted at times indicated by arrows. The graph reads from right to left. Each horizontal division corresponds to 1 min. The gas chromatographic system was similar to that previously described (ref. 4)

Table 1. DEPENDENCE OF ETHANE-PRODUCING SYSTEM ON A LIPID CONSTITUENT AND ITS SUBSTITUTION BY UNSATURATED C-18 FATTY ACIDS

Flask contents*	µl. ethane (× 10⁻³) per mgm. protein/hr.
Particles† + co-factors + thiomalic acid (no lipid)*	1·6
,, + ,, + ,, ,, + natural lipid	5·6
,, + ,, + ,, ,, + palmitic acid	1·2
,, + ,, + ,, ,, + stearic acid	1·2
,, + ,, + ,, ,, + methyl stearate	1·4
,, + ,, + ,, ,, + methyl oleate	2·4
,, + ,, + ,, ,, + methyl linoleate	6·0
,, + ,, + ,, ,, + methyl linolenate	20·0

* Each flask contained: 50 µmoles thiomalic acid, 3 µmoles adenosine triphosphate, 40 µmoles PO_4, 200 µmoles acetate buffer *p*H 4·5, and 1·0 ml. enzyme containing about 20 mgm. protein. 25 µmoles fatty acid or ester was added to flasks containing these. The natural lipid was obtained and concentrated by extracting 1 gm. of freeze-dried powder with 100 ml. ethyl ether (0° C.) and evaporating to dryness. The residue was taken up in water containing a drop of 'Triton *X*-100'. A three-times concentrated lipid preparation was added to flask containing natural lipid.

The methyl esters of the fatty acids were pure compounds (99 per cent) obtained from the Hormel Institute.

† Particles were prepared by homogenizing apple pulp in 0·4 *M* sucrose. The homogenate was centrifuged for 5 min. at 1,000*g* followed by 1 hr. at 15,000*g*. The final pellet was suspended in 0·1 *M* acetate buffer *p*H 4·5 and freeze-dried. One gram of powder contained about 300 mgm. of protein.

The ethane-producing particulate system requires the presence of an unsaturated fatty acid. This was demonstrated by the fact that the system was almost inactivated following ether extraction of the freeze-dried particulates but activity was restored by addition of the lipid fraction or by addition of oleic, linoleic, or linolenic acids or their methyl esters. As the results in Table 1 show, saturated fatty acids gave negative results. A slight stimulation was obtained with oleate, but a much greater effect was observed with linoleate and linolenate. Linolenate was about three times as effective as either the natural lipid or methyl linoleate. It is not yet clear whether linolenate is functioning as a co-substrate or as a co-factor in the reaction. One hypothesis visualizes thiomalic acid and linolenate forming an adduct, which breaks down and releases a 2-carbon fragment which may be transformed to either ethane or ethylene. The available evidence, however, appears to favour the thesis that ethane is produced entirely from thiomalic acid.

These findings seem to have some bearing on the problem of ethylene biosynthesis in plants. While ethane and ethylene may be synthesized through very different pathways in the cell, some evidence points to a relationship between them. Usually a small quantity of ethylene is associated with ethane production by the isolated enzymatic system. However, ethylene is sometimes difficult to observe because of the relatively large quantity of ethane produced. When ethane production is limited, either by sampling after only 1 or 2 min. of incubation or after inhibition, the amount of ethylene produced is comparatively large relative to ethane.

Apples normally produce much less ethane than ethylene. Meigh[3] reports a ratio of ethylene to ethane of 14,000 to 1 in whole apples. However, when apples are homogenized and incubated in buffer the amount of ethane found is approximately equal to ethylene. While all these factors tend to suggest a biochemical relationship between ethane and ethylene, it is at present premature to state what this relationship is However, present information suggests either that one may be a precursor of the other or that they may be derived from a common source

The work described in this communication was carried out at the Low Temperature Research Station, Cambridge

M LIEBERMAN
L W MAPSON

Pioneering Research Laboratory,
Agricultural Marketing Service,
U S Department of Agriculture,
Plant Industry Station, Beltsville, Md ,
and Low Temperature Research Station,
Agricultural Research Council,
Cambridge

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Use of an Integrating Stage for Measurement of Wood

IN OUR search for a less time-consuming method for measuring anatomical components and spaces within samples of wood of varying ages and heights in trees, we have found that the use of an integrating stage attached to the stage of a microscope has proved satisfactory and reliable[1] Accumulating knobs are provided to measure units in hundredths of millimetres for six different readings in one traverse, and obtaining percentage values for each component is a simple operation.

We have been able, by the use of cores approximately 5 5 mm in diameter and taken vertically from cross-sections of tree trunks at different heights, to obtain wood specimens of known age from the pith and at different heights in trees After specific gravity determinations these specimens are boiled in water to expel the air and are stored in 70 per cent ethyl alcohol Free-hand sectioning for a complete and precise transverse section of 20–30μ has been possible in eastern cottonwood and black walnut used thus far in our work The sections are then carefully mounted in chloro-iodide of zinc solution[2] on microscope slides and are covered with No 0 or No 1 coverglasses This stain when properly prepared gives a bluish coloration to the inner wall of gelatinous fibres and aids in their identification

In our first analysis of wood samples from eastern cottonwood camera lucida drawings were made of cell wall and lumen components along known traverses and the measurements were taken from these In the use of the integrating stage method accumulation of as many as six readings were taken simultaneously and percentage values were computed from the totals Furthermore, it was possible to return to the beginning of the same traverse and repeat for checking purposes, or for re-assigned knobs for additional measurement values We have used magnifications of 500× satisfactorily for sections 30-μ thick

An analysis of variance for wood samples from the same tree of eastern cottonwood showed the two methods did not give significantly different results The measurements expressed in percentages with the integrating stage method are, therefore, shown to be reliable. This, together with the fact that the operator's time is reduced by more than one-half, makes the method one to be highly recommended

One of us (M. K.) acknowledges receipt of grant *G*-15886 from the U S National Science Foundation

MARGARET KAEISER

Department of Botany,
Southern Illinois University,
Carbondale, Illinois

STEPHEN G BOYCE

Central States Forest Experiment Station,
Forest Service,
U.S. Department of Agriculture

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Thyroid Feeding of Salmon Parr

IN the spring of 1961, the Salmon Research Trust of Ireland, at its Research Station at Newport, Co Mayo, conducted an experiment on feeding fresh thyroid to one-year-old salmon parr, on a small commercial scale

The experiment began on February 20 and terminated on May 1—a duration of 69 days This period was chosen as being that in which salmonid fish could be expected to show maximum response to thyroid stimulation[1] The fish were large-grade parr, from the same source, contained in two 15-ft diameter circular rearing ponds, each having approximately the same population density (controls, 3,568 , experimentals, 3,838) Both groups of fish were fed three times daily with raw minced beef liver ; but the experimental fish received 20 per cent of their diet as raw, minced beef thyroid This dosage-rate was selected as the optimum in the light of earlier experiences with rainbow trout[2]

The fish fed and survived well during the period of the experiment, when mortalities were virtually the same, averaging less than one a day in each pond

During May 3–6, all 1+ smolts were graded out of each pond, when it was found that the thyroid-fed group yielded 913 smolts, against 510 in the controls This constitutes an 80 per cent increase in the production of 1+ smolts in comparable populations, which is felt to be economically significant

The percentage smolt transformation in each pond was as follows

Controls,	510 smolts	2,993 parr	14 7 per cent
Experimentals,	913 smolts	2,853 parr	24 2 per cent

During the first month of the experiment, the control fish increased in mean length from 72 to 78 mm, while the experimentals increased from 71 to 88 mm Unfortunately, the mean lengths of the two groups were not measured at the end of the experiment, but the 510 control smolts averaged 130 mm while the 913 experimental smolts averaged 134 mm Clearly, the experimental group was composed of larger fish where 24 2 per cent of the population were 4 mm longer than 14 7 per cent of a comparable group This effect of thyroid treatment on the growth of salmonid fish has been noted for rainbow trout[1]

During the grading-out of the smolts, it was noticeable that those of the thyroid-fed group were markedly more silver than those of the control group, and that there were no fish in the intermediate 'parr-smolt' ('silvery-parr') stage

A large proportion (721) of the thyroid-fed smolts were tagged or fin-clipped and liberated in fresh water, some two miles above a counting fence and trap, situated at the head of tidal water Their survival and speed of migration, as measured at the trap, were exactly comparable with results from normal, hatchery-reared 1+ smolts. There are some grounds for believing that thyroid treatment could improve survival of smolts during the period of osmotic stress when they first enter tidal water[3,4]. This may apply more particularly to hatchery-reared smolts which necessarily have been subjected to some handling.

In this small experiment there was an increase of 80 per cent in the production of 1+ smolts for an increase of not more than 25 per cent in the feeding costs for the first year. However, had these 400 fish not become 1+ smolts, the cost of keeping them for a further full year would have been considerably more than the cost of thyroid material used in transforming them into 1+ smolts. The comparison in other districts may be even more favourable than in County Mayo, where the cost of thyroid material is high, relative to that of low-grade liver. Experimental work on lowered dosage-rates is planned for the coming season.

D. J. PIGGINS

Farran Laboratory, Newport,
Co. Mayo, Eire.

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Culture of *Spinellus*

MOULDS of the genus *Spinellus* of the Mucorales are generally regarded as being obligate parasites of agarics, particularly those belonging to the genus *Mycena*. Recently, Ellis and Hesseltine[1] reported that they had grown a species of *Spinellus* in pure culture; they noted the difficulty of making the spores germinate, and demonstrated the necessity for a relatively low temperature (below 20° C.) for growth.

Last autumn, a culture of *Spinellus* sp. was obtained from a parasitized fruit-body of *Mycena galopus*. The isolation was made by placing spores on a medium, later referred to as the 'basal medium', containing glucose, salts and yeast extract. A rapidly growing mycelium developed, producing numerous sporangia resembling those on the parasitized agaric. The mould has since been maintained in culture, using mycelium for inoculation as it proved to be very difficult to make the spores germinate. Various natural media have been tried, as well as the basal medium, and 5 per cent corn-meal agar also gave good growth, although others seemed less suitable.

This isolate, like that obtained by Ellis and Hesseltine, was extremely sensitive to temperature. No growth was obtained with cultures kept above 23° C and the optimum seemed to be about 20° C. At this and lower temperatures numerous sporangia were produced, with sporangiophores up to about 1.5 cm tall. Cultures incubated at 22°–23° C produced fewer sporangia, containing fewer spores, and with much shorter sporangiophores.

The spores from cultures grown at a low temperature (13° C. was used in these experiments) were elongated in form, with a ratio of length/breadth of about 3 : 1. Cultures grown at 22.5° C. produced relatively shorter and broader spores with a length/breadth ratio of about 2 : 1. The actual size of the spores varied somewhat according to the medium but the relative shapes were the same on different media and seemed to depend on the temperature at which the culture had been grown.

Various attempts have been made to determine the conditions necessary for spore germination and some relevant observations have been made. Spores germinate readily in contact with the fruit-body of a suitable agaric but only rarely on culture media. Some of the broader spores produced by cultures grown at 22.5° C. on corn-meal agar or basal medium germinated in water or nutrient solutions or on agar media, whereas spores produced by cultures grown at 13° C. did not germinate under these conditions.

Mycelium which developed from germinated spores produced, when grown at 13° C., the types of sporangia and spores characteristic for that temperature. There is thus no permanent change induced by growth at 22.5° C., but the spores produced differ in their shape and ability to germinate from those produced at lower temperatures. This presumably explains the successful initial isolation of *Spinellus*, as the spores used came from sporangia which had developed in the laboratory, at a temperature which was probably suitable for the production of the broader type of spore and the isolate could have resulted from the germination of one or more of these. Previous unsuccessful attempts had been made to isolate from sporangia developed in the field, and the spores, presumably of the narrower type, would not germinate on culture media.

Apart from bringing them into contact with a suitable agaric fruit-body, one other way has been found of obtaining some germination of spores at lower temperatures. This was to place them in association with various unidentified bacteria, which were first acquired as contaminants. The substance which stimulates germination appeared to diffuse through 'Cellophane' but has not so far been demonstrated in a Seitz filtrate of liquid cultures of the bacteria.

A more detailed account of these investigations will be published later and it is also hoped to study further the factors necessary for spore germination in *Spinellus* and other aspects of its relationship with its hosts.

PAULINE WATSON

Department of Botany,
Durham Colleges in the
University of Durham.

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ENTOMOLOGY

Identification of Iso-Amyl Acetate as an Active Component in the Sting Pheromone of the Honey Bee

THE sting of the honey bee (*Apis mellifera* L.) carries volatile substances which incite bees to aggression. This was first demonstrated by Huber[1], who elicited attacks by the guard bees when he placed freshly excised stings or the odour of stings near the hive entrance. Free[2] found that cotton balls containing stings were more frequently stung than control balls[3].

When not in action, the sting is retracted entirely within the sting chamber of the bee's abdomen. However, in the event of attack, the sting is quickly protruded and thrust into the enemy. The worker bee is usually unable to withdraw her barbed sting from the skin of vertebrates. Thus, the sting with its motor apparatus and glands is torn from the bee along anatomically predetermined breaking lines

During this investigation it was noticed that stings which were left behind by stinging bees had a sweet scent reminiscent of the odour of banana oil. Although protrusion of the sting by the bee produced the scent, maximum release was observed when the sting was detached. The characteristic odour was present in the stings of guard and foraging bees, but was absent in the stings of queens and of very young workers.

When presented at the hive entrance, stings from newly hatched bees did not agitate the guard bees. However, a cotton ball containing stings from older bees and exhibiting the characteristic banana oil odour aroused the guard bees, who attacked and stung it.

Because of the alarm and attack behaviour which it evidently releases in bees, the sting odour is to be included in the pheromones[3]. Chemical identification of one of the active principles in the sting pheromone of the honey bee is reported here.

Thirty stings were removed from bees, macerated immediately in 20 µl. of carbon disulphide, cooled in dry ice, and centrifuged, yielding approximately 12 µl. of solution. Gas chromatographic separation of 5 µl. of this solution was carried out using a Perkin–Elmer 154C vapour fractometer equipped with a flame ionization detector. The 4½ ft. × ¼ in. columns were packed with 10 per cent of liquid phase on 160–170 mesh 'Anakrom U' support. The carrier gas was nitrogen. Fig. 1 is a typical, although highly attenuated, gas chromatogram which was run at 130° C using a column containing 'Carbowax 1500' as the liquid phase. Peak No. 1 is due to the solvent, and No. 4, coming off at 1·75 min., was investigated further as it was a major component of the volatiles contained in stings of guard and foraging bees. By comparison of retention times of this fraction with known compounds on four different columns (Table 1) it was identified as iso-amyl acetate. Stings of queens and newly hatched workers did not contain iso-amyl acetate as the gas chromatograms showed only the solvent peak.

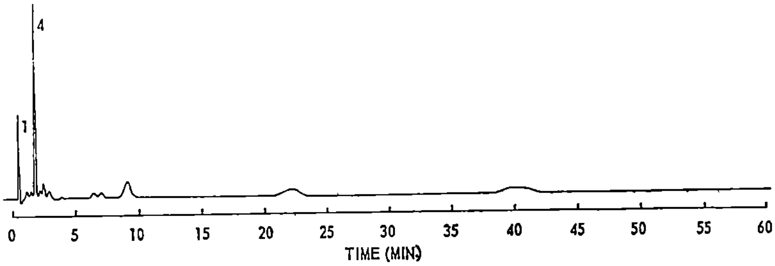

Fig. 1. Gas chromatogram of an extract of bee stings. Conditions: 10 per cent 'Carbowax 1500' on 'Anakrom U' at 130° C

Table 1. COMPARISON OF RETENTION TIMES OF A FRACTION FROM STINGS WITH ISO-AMYL ACETATE

Liquid phase	Column temperature (° C)	Retention time (min.) Fraction 4	Retention time (min.) *iso*-Amyl acetate
'Carbowax 1500'	130	1·75	1·75
	80	4·05	4·05
'Apiezon L + 1 per cent H_3PO_4'	130	1·70	1·70
	90	7·05	7·00
'Silicone 550'	130	2·50	2·45
	90	8·05	8·05
'Diethylene glycol succinate polyester'	110	1·82	1·80
	80	3·20	3·20

For the infra-red analysis an extract from 700 stings was chromatographed and fraction No. 4 was trapped and transferred in carbon tetrachloride solution to a 0·10-mm. ultra-micro-cavity cell. The infra-red spectrum (Fig. 2A) was obtained using a Perkin–Elmer model 21 spectrophotometer equipped with a lens-type beam condenser. This spectrum was identical with that of an authentic sample of iso-amyl acetate obtained under the same conditions (Fig. 2B).

The amount of iso-amyl acetate recovered from stings was estimated to be approximately 1 µgm. per sting. To our knowledge this is the first reported occurrence of iso-amyl acetate in animal tissue.

Authentic iso-amyl acetate was mixed with paraffin oil and the mixture absorbed in cotton balls. When presented at the hive entrance the balls treated in this way did alert and agitate the guard bees; however, cotton balls which contained an equivalent number of odoriferous stings, in addition to agitating the guard bees, incited them to sting. This suggests

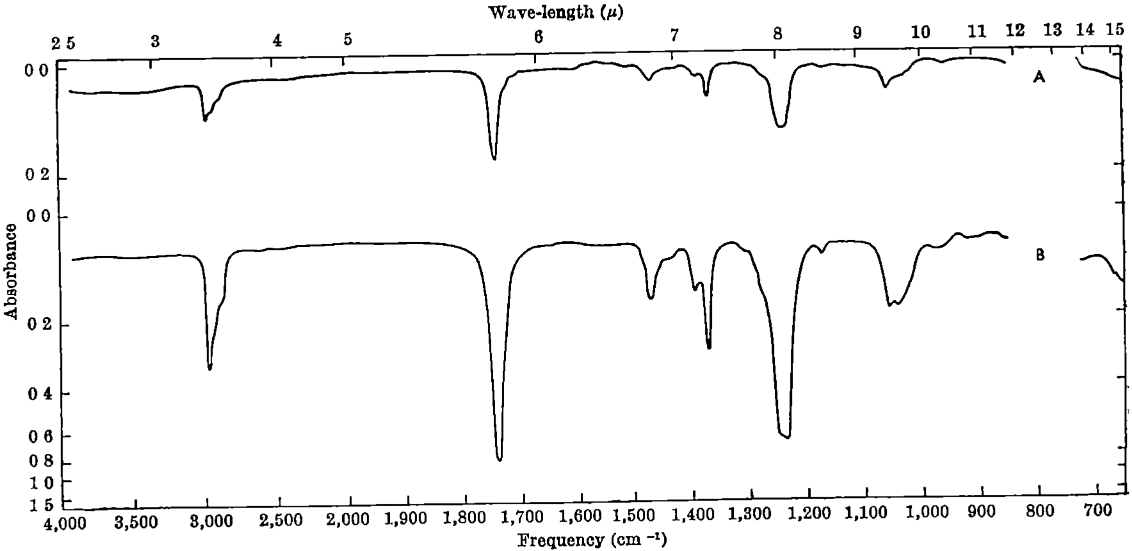

Fig. 2. The infra-red spectra of fraction 4 of bee stings (A) and of iso-amyl acetate (B) in carbon tetrachloride

that iso-amyl acetate is only one of the active components of the sting pheromone

R Boch

Entomology Research Institute,

D A Shearer
B C Stone

Analytical Chemistry Research Service,
Canada Department of Agriculture,
Ottawa

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Host Selection and Number of Eggs per Oviposition in the Egg-Parasite *Trichogramma embryophagum* Htg.

It has been shown by Salt[1,2] and Laing[3] that the behaviour of *Trichogramma* in parasitizing host eggs can be divided in the following elements

(*a*) The parasite makes random movements on the substrate and is visually attracted by small objects which rise above the surface

(*b*) The parasite climbs up the object and spends some time in walking over its surface in a characteristic way, making intensive movements with the antennæ (here called drumming) If the object has already just been parasitized by *Trichogramma*, it will be rejected if the odour of the first female is still present on the chorion

(*c*) After drumming, the parasite starts drilling on the surface of the object, not only on eggs, but also on small seeds and grains of sand Odour, colour and texture of the surface of the object seem not to play a predominant part in stimulating the drilling act The object can be rejected, however, if its measurements are such that the development of the parasite in the inside is an impossibility[1]

(*d*) If the object is a host egg the chorion is eventually perforated and the parasite's eggs are deposited The host is rejected after perforation, however, if it has been parasitized before[2] or the embryo has reached an advanced stage in development[4,5]

In this series of acts the function of drumming is puzzling, because the act of drilling can be released whatever the kind of the object It has been suggested that the function of this curious behaviour is to give the host egg an odour in order to mark it as being parasitized The drumming act is characterized by criss-cross movements over the chorion, the parasites often take some steps backwards when they touch the substrate, turning and walking on in another direction, following the curve of the chorion until another meeting with the substrate causes a new change of direction The antennæ are alternately and quickly moved up and down

We observed just as Quednau did[6] that *Trichogramma* deposits a variable mean number of eggs in hosts of different size during a single act of oviposition (Table 1) Therefore, we deduce from this fact and the typical behaviour during drumming that the function of the latter act might be looked for in the determination of the size of the host egg This idea was supported by the fact that the time spent in drumming proved to be correlated with the size of the egg (Table 1)

Table 1 Duration of the Drumming Act and the Number of Parasite Eggs deposited per Oviposition

Host	Diameter of host egg (mm)	Mean duration of drumming (sec)	No of eggs		No of observations
			Mean	Range	
Ephestia kühniella	0 3	37	1 1	1–2	70
Bupalus piniarius	0 9	62	6 7	4–10	92
Ellopia fasciaria	0 9	71	8 2	6–10	10
Hyloicus pinastri	2 2	172	25	—	2
Dendrolimus pini	2 1*	—	—	24–34†	—

* In Holland † According to Ryvkin (ref 7)

Table 2 No of Eggs deposited in Eggs of *Ephestia kuhniell* after Drumming on a Host of Larger Size

First host Species	Diameter of egg	No of eggs		No of observations
		Mean	Range	
'Egg' of type 1	1 1	3 4	3–4	7
Bupalus piniarius	0 9	2 7	2–3	9
Dendrolimus pini	2 1	8 5	7–10	4
No of Eggs deposited in Eggs of *Bupalus piniarius* after Drumming on Eggs of *Ephestia*				
Ephestia kühniella	0 3	1 5	1–2	4

To prove this we have carried out the following experiments

(1) Artificial eggs were made by clumping together some 4–8, or even more, eggs of *Ephestia* with a mixture of gelatin and agar-agar If this cluster of eggs was dipped in the same mixture two or three times, a small sphere was attained, in which the *Ephestia* eggs in some places were very close to the periphery, being covered only by a very thin layer of the desiccated liquid (type 1) Without this dipping the *Ephestia* eggs protruded and were on most places separated from each other by deep incisions (type 2)

If 'eggs' of type 1 were offered to the parasite female the whole surface of the sphere was covered in the drumming performance and 4 1 eggs on the average (3–5 in 8 experiments) were deposited, if the parasite happened to drill in an *Ephestia* egg

If 'eggs' of type 2 were offered, then the drumming performance was nearly always limited to one protruding *Ephestia* egg and only one parasite egg was deposited in that host After the first oviposition the parasite proceeded to the second *Ephestia* egg of the same cluster, parasitizing this one in the same way, and so on

In the latter series there were two exceptions to the rule, in which the parasite deposited 3 and 2 eggs in one *Ephestia* egg In these cases, however, the parasite covered the whole and half of the cluster in its drumming performance, respectively Thus, the parasite conceived the 'eggs' in these cases wholly or partly as eggs of type 1

(2) Artificial eggs of type 1, *Bupalus* eggs or *Dendrolimus* eggs were offered, on which the drumming act was performed On the moment that the drilling act was started an *Ephestia* egg attached to the top of a hair was placed between the legs of the parasite and it was forced to accept this apparent host for drilling and oviposition This sometimes was successful after a relatively short interim period of drumming on the new host, and in these cases a mean of 3 4 eggs on the average were deposited in *Ephestia* if host 'eggs' of type 1 released the drumming act, 2 7 eggs if *Bupalus* eggs did so, and 8 5 eggs if drumming was released by eggs of *Dendrolimus* (Table 2)

(3) *Ephestia* eggs were offered, on which the drumming act was performed At the moment that the drilling act was started a *Bupalus* egg attached to the top of a hair was placed before the parasite and it was forced to accept it for drilling and oviposition In four successful cases a mean number of 1 5 eggs was deposited in the apparent host (Table 2)

These preliminary experiments show that the drumming act is connected with the determination of the size of the host egg To give a complete analysis more experiments will have to be carried out Moreover, the biological significance of the phenomenon of laying a different mean number of eggs in hosts of various size will be examined Quednau[6] has suggested that in a large egg only a relatively high number of parasites can survive, because the adults cannot emerge when part of the fluid contents of the host are left Our preliminary observations do not indicate this, but suggest that the mean number of eggs deposited in a definite species of host gives rise to the maximum number of the offspring which can be produced in that particular host egg, taking into consideration both the number of parasites emerging and their fecundity

H KLOMP
B J TEERINK

Zoological Laboratory,
Agricultural University,
Wageningen, The Netherlands.

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MICROBIOLOGY

Hybridization of Homothallic Yeasts by Chen's Technique

IN the course of experiments performed in this laboratory it was found that spores of *Saccharomyces cerevisiae* var *ellipsoideus* were unable to germinate unless the medium contained glucose, fructose or mannose[1] Acetate agar (0 5 per cent sodium acetate in 2 per cent agar), or agar without added nutrients, are incapable of promoting germination On the contrary, vegetative cells are able to form microcolonies on 'nutrient-free' agar or on acetate agar, although the number of buds on this last medium is considerably smaller than on agar without additions The formation of microcolonies on solid 'nutrient-free' media is not at all surprising, and it has been studied in considerable detail in bacteria[2,3] The microcolonies formed under those conditions have larger numbers of cells than those formed on acetate agar, an observation which confirms the inhibitory effect of acetate on yeast growth reported in the literature[4,5]

The yeast strain used was isolated from fermenting grape juice and classified as *Saccharomyces cerevisiae* var *ellipsoideus* (strain 20 *SJ*) It is typically homothallic, and diploidization occurs very early in the life of the monosporic cultures The morphological events taking place during germination have been arbitrarily divided into the stages schematically represented in Fig 1 Diploidization usually occurs immediately after stage *F*, and two zygotes are formed In some cases, however, the four-celled stage *F* is followed by another haploid generation, but copulations occur immediately afterwards

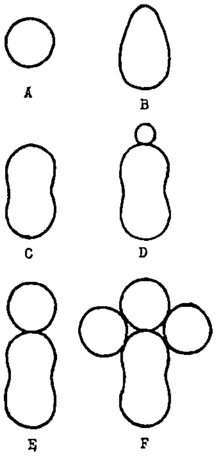

Fig 1

When spores were allowed to germinate in a suitable 'complete' medium (autolysed yeast-agar films in culture chambers) and, as soon as stage *C* was reached, they were transferred to acetate agar or to agar without additions by means of micromanipulation techniques, it was found that the germination process could continue and that the cells of the microcolonies thus obtained did not undergo diploidization through pairing These colonies were very small, particularly in the presence of acetate When spores which had attained stage *F* on 'complete' were transferred to acetate agar or to 'nutrient-free' agar, most of the microcolonies produced were constituted by haploid cells, except for a few colonies on 'nutrient-free' agar, which contained zygotes and diploid growth Acetate appeared, then, as a more efficient agent to prevent diploidization of the monosporic cultures Experiments performed with other strains of the same species in our laboratory seem to indicate that this phenomenon is of a rather general occurrence among the homothallic strains of *S cerevisiae*

The four cells of stage *F* could be used for pairing in genetic experiments However, with the homothallic strains of our laboratory we have found it very difficult to take apart the four cells without damaging them On the contrary, the cells from the microcolonies obtained according to the 'replacement' technique described here are rather loose and no difficulties are encountered during their isolation with the micromanipulator

Fig 2 shows the different stages of copulation between two haploid cells, one of which, the upper, has been picked from a microcolony formed on acetate The lower cell has been taken from a stable haploid strain obtained from *Saccharomyces aceti*[6] by spore isolation in this laboratory The true hybrid

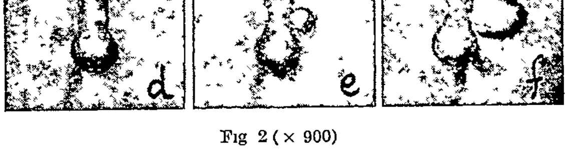

Fig 2 (× 900)

nature of this and other diploids obtained in a similar manner was checked by a study of the segregation of several genetic markers.

The method constitutes a handy extension of Chen's procedure[7] to homothallic strains. It allows the hybridization of those strains where diploidization occurs very soon after spore germination, in the cases when it is of interest to study the characteristics of the cultures derived from the same spores to be crossed. Obviously, since the growth on acetate agar or on agar without added nutrients is very limited in any event, it is necessary to transfer the remaining cells of the microcolonies to complete medium, as soon as the desired pairing has been successful.

This work has been aided by a grant from the Consejo Nacional the Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

NORBERTO J. PALLERONI

Cátedra de Microbiología,
Facultad de Ciencias Agrarias,
Chacras de Coria, Mendoza, Argentina.

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Inhibition by Chloramphenicol of Protein Synthesis in the Cell-free System of a Chloramphenicol-resistant Strain of *Escherichia coli*

IN an investigation of the biochemical mechanism of chloramphenicol (CM) resistance in *Pseudomonas fluorescence*, Kuschner[1] reported that oxidation of succinate by intact cells of a CM-resistant strain was not affected by CM, although this reaction by the wild strain was moderately inhibited. On the other hand, the same reaction in cell-free systems of both strains was found to be equally sensitive to CM inhibition. From this result, he proposed that CM resistance in the resistant strain was due to decreased permeability against CM. Ramsey[2] investigated the action of CM on the formation of inducible enzymes by ultra-socially disrupted cell preparation of *Staphylococcus aureus*, and observed that the preparation from the CM-resistant strain still retained the resistance of intact cells so that changes in permeability was not the main source of resistance in this organism.

We investigated the mechanism of resistance with cell-free systems of protein synthesis from the CM-sensitive and resistant strains of *Escherichia coli B*. It was demonstrated that incorporation of amino-acid into protein by the preparation from resistant cells is almost equally sensitive to CM as in the case of sensitive cells. While this work was in progress, Yokota and Akiba[3], using the lysed protoplast system of CM-resistant *E. coli*, showed that the synthesis of β-galactosidase is sensitive to CM.

The strains used were *E. coli B*, and a CM-resistant strain developed by successive passages through media containing increasing concentration of CM. The concentrations of CM which were required to inhibit 50 per cent of growth in liquid media of the two strains were 0.4 μg per ml. for the wild and 300 μg per ml. for the resistant strain, respectively. Cells were grown on glucose-peptone medium for the wild strain or on that supplemented with 100 μg per ml. of CM for the cultivation of CM-resistant cells, under aeration, and gathered in the early logarithmic phase. The preparation of cell-free extract and amino-acid incorporation into protein was carried out according to Lamborg and Zamecnick[4] and Matsubara and Watanabe[5]. Cells were washed twice with 0.02 M *tris* buffer (*p*H 7.6) containing 0.008 M Mg^{++}, suspended in 2 vol. of the same buffer and disrupted by sonic oscillation (10 kc/s for 10 min). Large cell debris was removed by successive centrifugations (at 10,000*g*, 30,000*g* and 40,000*g*, each for 30 min). The 40,000*g* supernatant was further fractionated by centrifugation at 100,000*g* for 1 h to give supernatant (soluble fraction) and precipitate (ribosome fraction). For the experiment of amino-acid incorporation, ribosome fraction, soluble fraction, ^{14}C-labelled amino-acid mixture (acid hydrolysate of *Chlorella* protein supplied by the Institute of Applied Microbiology University of Tokyo). and co-factors required were mixed as described in the legend of Table 1 and incubated at 37° C for 20 min. The reaction was stopped by the addition of 5 ml. of 5 per cent trichloroacetic acid. The precipitate was washed once with 5 per cent trichloroacetic acid, treated once with hot acid (90° C for 15 min), dissolved and reprecipitated twice with 0.5 N sodium hydroxide and 5 per cent trichloroacetic acid, washed twice with ethanol/ether (3 : 1), and finally dissolved in 0.05 N sodium hydroxide. Radioactivity of aliquots were measured by a windowless, gas-flow counter. For the determination of the grade of inhibition by CM, serially diluted concentration of CM was added to the reaction mixture, and the incorporated radioactivity was compared with that of control without CM.

Table 1. INHIBITION BY CHLORAMPHENICOL ON THE AMINO-ACID INCORPORATION BY CELL FREE PREPARATIONS FROM CM-SENSITIVE AND RESISTANT STRAINS OF *E. coli B*

Strains	Sensitive		CM-resistant	
CM added	Total counts/min	% of inhibition by CM	Total counts/min	% of inhibition by CM
μg/ml				
0	522	0	1193	0
0.63	362	31	962	19
1.25	320	39	863	27
2.5	284	47	638	46
5	190	64	553	54
10	170	67	501	58
20	160	69	384	68
40	116	78	332	72

Each tube contained: supernatant fraction, 1 mg protein; ribosome fraction, 3 mg protein; $MgCl_2$, 8×10^{-3} M; KCl, 6×10^{-2} M; adenosine triphosphate, 0.5 μmoles; creatine phosphate, 6 μmoles; creatine kinase, 0.2 mg protein; *tris*, 2×10^{-2} M, *p*H 7.6; ^{14}C-amino-acid mixture 2×10^5 c.p.m.; with or without CM as indicated; total volume, 1.0 ml. Incubation, 20 min at 37° C.

As shown in Table 1, amino-acid incorporation by the cell-free systems of sensitive and resistant strains are almost equally inhibited by low concentration of CM. Thus, it can be deduced that in this resistant strain the process of protein synthesis is almost as sensitive as that of sensitive strain. Hence, it appears that CM is prevented from reaching its site of action by some sort of permeability barrier. Since, as reported previously[6], spheroplasts of resistant cells retain the most part of resistance, the role of cell wall does not seem to be important. The cell membrane or some inside structures will be responsible for the resistance.

It was consistently observed that the inhibition of amino-acid incorporation was not complete even in the presence of 40 μg per ml. of CM, where 20–30 per cent of incorporation continues to occur, whereas in intact cells the amount of the residual protein synthesis under CM inhibition is about 5 per cent. Since incorporation of amino-acid in this system is not inhibited by penicillin (40 μg per ml.), this may not be related to synthesis of cell wall substance. The nature of this residual incorporation remains to be ascertained.

Our results conflict with Ramsey's observation on CM resistant cells of *Staph. aureus.* Two possibilities may be considered. First, in the CM resistant strain of *Staph. aureus*, there may be a CM resistant pathway of protein synthesis which is not present in *E. coli.* Secondly, in the disrupted cell system used by Ramsey, some sort of permeability barrier may be still present.

This work was aided by a grant to Dr. J. Tomizawa from the Jane Coffin Childs Memorial Fund for Medical Research. We thank Drs. K. Matsubara and J. Tomizawa for their help and advice.

S. OKAMOTO
D. MIZUNO[*]

National Institute of Health, Osaki,
Tokyo.

[*] Present address: Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo.

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A Selective-Diagnostic Medium for the Isolation of Pectinolytic Organisms in the Enterobacteriaceae

IN the family Enterobacteriaceae, the genus *Erwinia* has been divided into those which cause dry necroses, galls or wilts in plants, and those which produce the enzyme protopectinase and cause soft-rots in plants[1]. The latter group has been given the name *Pectobacterium* by Waldee[2].

The ability to liquefy pectate gels is of great importance in characterizing the soft-rot *Erwiniae* (Pectobacteria), and the technique devised by Paton[3] for preparing pectate gel has simplified the use of the test.

The isolation of soft-rot *Erwiniae* where large numbers of other bacteria (*Escherichia, Aerobacter, Pseudomonas, Bacillus,* etc.) are present may be somewhat difficult, and necessitate the examination of many types of colonies from primary plate media. The salicin medium of Noble and Graham[4] relies on the inhibiting action of a bile salt (sodium taurocholate) to prevent the growth of organisms other than those in the Enterobacteriaceae. The fermentation of the salicin is used as a diagnostic characteristic for recognizing possible soft-rot erwiniae. Colonies are slow to develop on this medium, which has only an ammonium salt as a nitrogen source.

It was felt that a solid plating medium combining the inhibitory power of bile salts and the diagnostic characteristic of pectate liquefaction, along with lactose or salicin fermentation, would prove of some value for isolating soft-rot *Erwiniae* from material heavily contaminated with other types of bacteria.

The following medium was devised:

Basal layer

McConkey agar granules ('Oxoid *C.M.*7')	5·2 g
Calcium chloride	0·4 g

These were dissolved with boiling in 75 ml. distilled water. The medium was autoclaved at 10 lb. pressure for 10 min, cooled and pipetted in 15 ml. amounts into sterile Petri plates.

Pectate layer

Sodium polypectate ('Exchange' brand)	2 g

This was suspended in 6 ml. of ethanol. 0·1 g of ethylenediamine tetraacetic acid (disodium salt) was dissolved in 100 ml. of distilled water, and this solution was added to the pectate suspension to a final volume of 100 ml. This medium was adjusted to *p*H 7·4, autoclaved at 10 lb. pressure for 10 min, and after cooling to about 55° C, was pipetted in 5-ml. amounts on to the cold, gelled McConkey agar base in the Petri plates.

The pectate layer set in a few minutes, and was ready for streaking after overnight drying at 37° C.

After 48 h incubation at 25° C, *Erwinia*, fermenting lactose, develops as red colonies in shallow pits formed from the liquefaction of the pectate. *Escherichia, Aerobacter* and other lactose fermenting, non-pectate liquefying bacteria appear as red colonies with no surrounding pit in the medium. Non-lactose fermenting bacteria produce yellow or orange colonies.

Salicin could be used instead of lactose in the basal layer where the type of *Erwinia* and/or the types of contaminating bacteria indicated its use. Its use would, however, involve the preparation of McConkey agar from its basic constituents.

D. J. STEWART

Department of Agricultural Bacteriology,
Ministry of Agriculture for Northern Ireland and
The Queen's University of Belfast.

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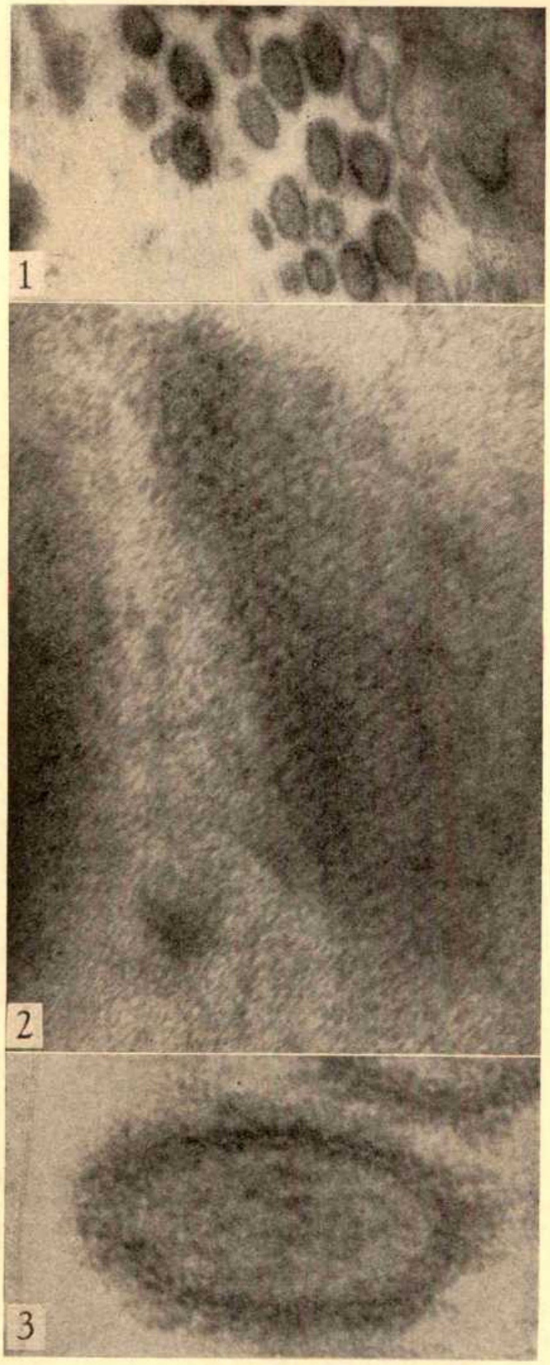
CYTOLOGY

An Electron Microscopic Demonstration of a Surface Pattern on the Plasma Membrane of Sectioned Intestinal Epithelium after Saponin Treatment

A TRANSVERSE section of the plasma membrane, after osmium or permanganate fixation, appears in electron micrographs as a triple-layered structure, about 8 mμ thick, composed of two outer opaque layers separated by a clear layer[1]. This unit membrane appears to be universal for all higher animal and plant cells and has been considered to represent the protein-lipid-protein molecular model as originally proposed for the plasma membrane by Danielli and Davson[2]. Until the recent report by Dourmashkin, Dougherty and Harris[3], little electron microscopic evidence had been produced to suggest heterogeneity on the plane surface of the plasma membrane. These authors demonstrated, however, that *in vitro* saponin treatment produced hexagonally arranged pits or holes in isolated membranes from erythrocytes, liver cells, Rous sarcoma cells and virus particles. The pits were seen in isolated membranes spread on grids after negative staining, and they



Figs. 1–3. 1, Electron micrograph of the brush border from the jejunum of a rat after exposure to 0·1 per cent saponin for 15 min (× 50,000). 2, An enlargement of the tangentially sectioned membrane from Fig. 1 (× 320,000). 3, A transverse section of a single microvillus (× 320,000)

state that they were unable to demonstrate these structures in sections of similarly treated liver cell membranes.

Intestinal epithelium is a convenient system for examining the *in vivo* effect of saponin since it can be injected into the lumen and the brush border presents a large area of regularly arranged membrane. Under 'Nembutal' anæsthesia, rats' jejunum was mildly distended with 0·1 per cent aqueous saponin, the intestine returned to the abdomen and later fixed by filling the lumen with Dalton's chrome-osmium fixative. The intestinal wall was dehydrated in absolute ethanol, embedded in 'Araldite' and the sections stained with 1 per cent potassium permanganate before examination in an A.E.I. *E.M.*6 microscope. After 15-min exposure to the 0·1 per cent saponin, the lumen contains cellular debris and the cells on the apices of the villi are necrotic but the crypts of Lieberkuhn, sub-mucosal and muscle coats appear unaffected. Hence along the sides of the villi a gradation from well-preserved cells to mere remnants of membrane and brush border can be studied. Fig. 1 shows an oblique section of the brush border just below the level of necrosis; in the lumen, on the left, a portion of membrane lies in the plane of section. At higher magnification (Fig. 2) this piece of membrane is seen to be covered with small clear rings; most of these possess dark centres and, in places, they are packed into a hexagonal pattern. The diameters of the rings cannot be measured, but the centre-to-centre spacing of 10–12 mμ is compatible with the 14–16 mμ separation reported in unfixed isolated membranes[3]. The relatively critical concentration of saponin required to produce these pits *in vitro*, coupled with the difficulty in obtaining true tangential sections, presumably account for the infrequent observation of the hexagonal pattern, but isolated rings on oblique membranes are often seen.

Unfortunately, tangential sections do not indicate whether these rings represent holes or pits. In transverse sections of the membrane the spacing of the rings is such that two or three layers will be superimposed even in a thin (20 mμ) section. Nevertheless, in such transverse sections (Fig. 3) definite discontinuities in the triple-unit plasma membrane are seen, these are not observed in control specimens and they have a width similar to the diameter of the rings. This observation could be due to linear discontinuities in a weakened saponin-treated membrane and would not seem sufficient evidence to conclude that the rings represent true holes in the plasma membrane.

A. R. Muir

Department of Anatomy,
University of Edinburgh.

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Cytological Estimation of the Proportion of Proliferating Donor Cells during Graft-versus-Host Disease in F_1 Hybrid Mice infected with Parental Spleen Cells

Previous work has indicated that during graft-versus-host disease in mice, donor elements constitute only a small proportion of the cellular proliferation in the spleen of the hosts[1–3]. The fate of transplanted homologous cells has been examined experimentally by methods involving discriminant spleen assays of immunologically competent cells[1,3], estimation of humoral antibodies following transplantation of spleen cells hyperimmunized against sheep red cells[4] and more directly by chromosome analysis of all dividing cells[2,5].

Although in radiation chimæras the grafted cells react immunologically against the host, the reaction

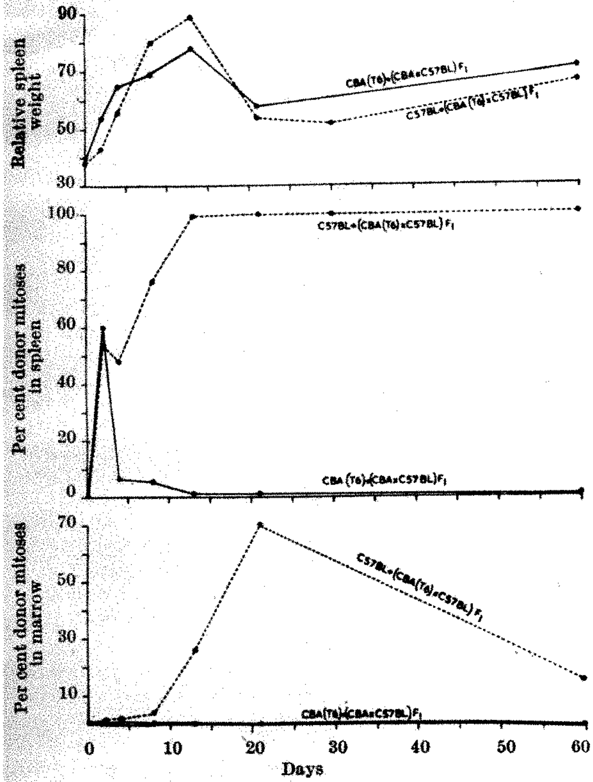

Fig. 1

is complicated by a number of factors including the after-effects of irradiation and some immunological reactivity of the host tissues. In order to obviate any potential host-versus-graft reaction, the response was examined in F_1 hybrid animals injected with cells from one or other of the parent strains against which the recipients are immunologically defenceless. Howard, Michie and Simonsen[6] studied the graft-versus-host reaction of this type in two different strain-combinations of mice by the discriminant spleen assay. They found that the greater part of the cellular proliferation in the spleen was of host origin, the number of immunologically active donor cells diminishing from about 19·5 per cent on the first day after injection. In the strains examined, Gorer and Boyse[7] believed that death of donor cells occurs, while Simonsen's[8] views are that with certain strain combinations a minority of the cells survive and become specifically tolerant to host antigens.

In the present work a cytological analysis of dividing cells in the spleen and marrow was carried out during graft-versus-host disease in (*CBA* × *C57*/*BL*)F_1 hybrid mice injected intravenously with 100 million of one or other of the parental strain spleen cells. By using mice with marker chromosomes[9] and making chromosome preparations by a modification of the drying technique as described by Fox and Zeiss[10] a simple and reliable method of tracing and quantitatively estimating proliferating donor cells was made available when either the host or donor animals were labelled with these markers. *CBA*(*T6*) mice were used; they are isogenic with regard to histocompatibility antigens with *CBA* animals, but their cells can be recognized by the chromosome markers, each nucleus containing two distinct short chromosomes (or one short chromosome in the F_1 hybrid). The proportion of donor to host dividing cells was determined in the spleen and bone marrow and the findings were correlated with the degree of graft-versus-host reaction as judged by the relative spleen weight.

Fig. 1 summarizes the results obtained from chromosome examination of spleens and bone marrow of 61 mice. The animals were killed 2, 4, 8, 13, 21, 30 and 60 days after injection, and approximately 300 mitoses were analysed at each stage in both spleen and bone marrow preparations.

When *CBA*(*T6*) spleen cells were injected intravenously into (*CBA* × *C57 BL*)F_1 hybrids, the response in the spleen could be divided into three consecutive phases: (1) an initial short-lived but tremendous burst of donor cell proliferation when the donor cells formed the majority of the dividing cells; (2) a more protracted rise of host mitotic activity resulting in splenomegaly; (3) a final period when donor mitotic activity was constant but remained in a minority.

There was a similar early burst of donor cell mitotic activity in the spleen following intravenous injection of *C57 BL* spleen cells into the F_1 hybrid (in this case the host cells carried the chromosome markers). No subsequent decline in the proportion of donor cells was observed, as when *CBA* cells were injected. On the contrary, a continued increase occurred so that by the thirteenth day, when spleen enlargement was maximal, all mitoses examined were of donor origin. No host cell mitoses could afterwards be found in the spleen of these animals, examination being carried out up to the sixtieth day after injection. One animal died on the forty-third day from graft-versus-host disease (loss of weight, diarrhœa and splenic atrophy).

The pattern of donor cell mitotic activity in the marrow was unlike that found in the spleen. No initial burst of donor cell activity was found when *CBA* spleen cells were injected. The highest proportion of donor cells occurred on the second day (0·5 per cent), and no donor mitoses could be demonstrated after the fourth day. Following *C57 BL* spleen cell injection, the proportion of donor cells increased gradually to the eighth day and then more rapidly, so that by the twenty-first day 70 per cent were of donor origin. By the sixtieth day dividing donor cells contributed 16 per cent of the mitoses in the bone marrow.

I thank Prof. M. F. A. Woodruff for his help and Dr. J. G. Howard and Dr. Donald Michie for their criticisms. The work was carried out during the tenure of a clinical research fellowship from the Medical Research Council.

MILES FOX*

Department of Surgical Science
and the Medical Research Council
Research Group on Transplantation,
University of Edinburgh.

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* Present address: Renal Research Unit, General Infirmary, Leeds, 1.

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GENETICS

Autosomal Recessive White-eyed Mutation in *Muscina stabulans* (Fall.)

PATERSON[1] has described a sex-linked and sex-limited white-eyed mutant in *Muscina stabulans* (Fall.). This communication reports a white-eyed mutation in the same species which involves a different mode of inheritance.

In the autumn of 1959, a normal female, sole survivor of a second laboratory generation of a strain trapped in Chicago, produced two broods (F_1) containing 33 white-eyed flies and 98 wild-type. The mutation appeared in both sexes (15 ♂, 18 ♀). Mass breeding of the mutants failed initially due to deposition of sterile eggs. After fertile eggs were laid, larval and pupal development were found to be normal with respect to rate and general behaviour. A possible defect in the F_1 mutant males is suggested by the following. The cross, mutant male × normal female produced only sterile eggs, whereas the reverse cross was productive. Appearance of the testes of the male was normal, but no microscopic examination was made. There was also evidence of reduced male longevity in the original broods. For example, 87 per cent of F_1 males were dead after 26 days, compared with 43 per cent of the females; whereas the per cent mortality of the F_2 generation after 26 days was: ♂, 53; ♀, 55. Other effects in the mutants were tremors in adults of both sexes and differences in pigmentation of the testes and Malpighian tubes. To examine these latter features, dissections were made of 2-week-old flies subsisting on meat, sugar and water. The results are summarized in Table 1. White-eyed flies produced unpigmented Malpighian tubes but their testes contained some pigment. Flies with intermediate eye colours, described here, varied from white-eyed to wild-type pigmentation. Paterson mentions no additional pigmental effects in his white-eyed *M. stabulans*; but the same sex-linked, sex-limited mutation is associated with normally pigmented testes and Malpighian tubes in *Calliphora erythrocephala* (= *vicina*)[2]. An autosomal recessive gene for white eyes produces colourless Malpighian tubes in *Lucilia cuprina*[3].

Table 1. PIGMENTATION IN VARIOUS EYE-COLOUR PHENOTYPES OF *Muscina stabulans*

Eye colour	Malpighian tubules	Testes
Wild-type	yellow	rust*
White	white	light orange
Yellow	light yellow or white	rust*
Ruby	yellow*	light orange

*Normal for the species

The F_2 progeny contained individuals with white eyes and various intergrading shades of buff, lemon, peach, cherry and ruby. Flies of the first 3 intergrades (yellow) were mass-bred together for 2 generations, as were those of the last 2 intergrades (ruby). Both groups produced white-, yellow-, ruby-, and wild-type flies. It became increasingly difficult after two generations to distinguish between the two intermediate groups; they were therefore combined and mass-bred. After 4 generations, the numbers of each type produced by the intermediate group were: white—87 ♂, 87 ♀; intermediate—684 ♂, 714 ♀; wild-type—119 ♂, 160 ♀. The totals after 4 generations of wild-type, mass-inbreeding were: white—13 ♂, 11 ♀; intermediate—64 ♂, 85 ♀; wild-type—119 ♂, 132 ♀. Comparison with other species shows that yellow-eyed *Phormia regina*[4] and *Callitroga macellaria*[5] breed true but *Musca domestica* does not[6]. Mass breeding of F_2 white-eyed flies produced the following F_3 progeny: white-eye—53 ♂, 40 ♀; yellow-eye—8 ♂, 5 ♀. Mass inbreeding of white-eye F_3 produced only white eyes in the following and 12 subsequent generations. As these flies aged, the white eye became slightly suffused with pink.

In June 1960, I sent by air pupæ of each type to Rome in order to continue this work at the Istituto Superiore di Sanità. The flies emerged but failed to breed and on my arrival all were dead. In the meantime, the colonies in Chicago began to fail and were lost after a few months. The observations recorded here are necessarily preliminary but the evidence presents a noteworthy difference in the mode of inheritance of the white-eyed mutation from that previously reported for this species. It indicates the existence in this fly of recessive autosomal loci acting independently of the sex-linked, sex-limited factor described by Tate and Paterson. So far as I am aware, this is the first such case reported in muscoid Diptera.

The occurrence of intergradations in eye colour of the intermediates may be a nutritional effect brought about by the limited availability of tryptophan derivatives in the larval diet. Ward and Hammen[7] have shown that some genotypic green-eyed houseflies develop pink eyes if reared in the presence of wild-type larvæ or their products. My intermediates are not true-breeding, and wild-type larvæ develop from the same egg-batches and in the same larval medium as the mutants. Under these conditions intermediate larvæ probably utilize limited amounts of precursors excreted by the wild-type, which the white-eyed larvæ are unable to do.

This work was supported by a grant *G*9934 from the U.S. National Science Foundation.

BERNARD GREENBERG

College of Pharmacy,
University of Illinois,
Chicago 12.

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Inherited Variants in the Transferrins and Conalbumins of the Chicken

PREVIOUS electrophoretic studies of the serum proteins of the chicken have utilized moving boundary[1-5], paper[6,7], or starch-gel[8,9] techniques, the latter with 'continuous' borate buffer. Differences between individual sera were reported dependent variously on sex[1,2,6], onset of maturity and egg-laying[4,7,8], treatment with sex hormones[4,6], or stage of embryonic development[3,5,7,9]; but no inherited differences were found. Using horizontal starch-gel electrophoresis in the Poulik[10] discontinuous buffer system, both Lush[11] and ourselves have obtained considerably improved resolution of the zones. The former[11] confirmed and extended previous knowledge of variation dependent on sex and egg-laying, but did not find inherited variation among the sera of 66

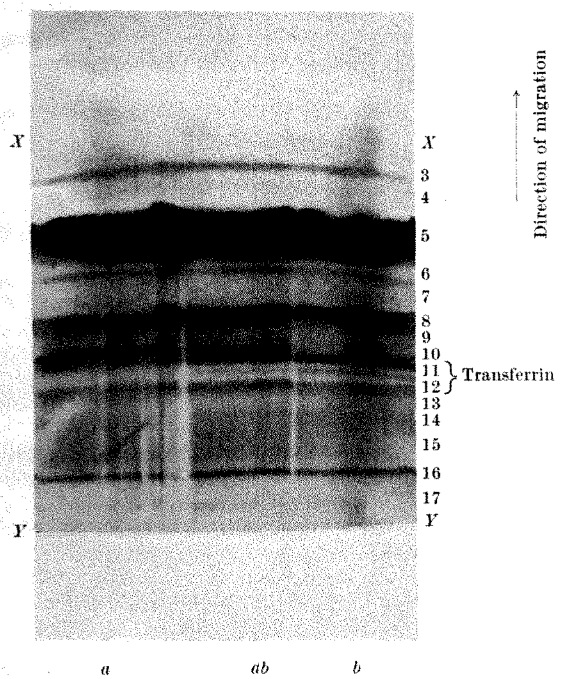

Fig. 1. Starch-gel electrophoresis of cock sera, showing the three transferrin types. Y—Y marks the position of insertion of the samples, and X—X the position of the brown line at the end of the run

chickens of various breeds. We have found inherited variation on one breed out of several studied, and have also found parallel inherited variation in egg-white protein.

In order to avoid possible confusion owing to the known variation dependent on egg-laying, most of our early work was carried out with cock sera. They were all subjected to electrophoresis separately at Stock and at Cambridge, under slightly differing conditions (Stock: 4¼ hr. at a field strength of 7 m.amp./cm.^{2} in a modified Poulik buffer system using lithium hydroxide instead of sodium hydroxide; Cambridge: 4 hr., 4 m.amp./cm.^{2} in the original[10] Poulik system) and our account is based on excellent agreement between the two sets of results.

The most frequent type obtained, with 14 distinguishable bands, is shown as type *b* in Fig. 1. Occasionally, under the most favourable conditions, 15 bands can be distinguished in male sera, and these are numbered 3–17 in type *a*, Fig. 1. (The major difference between types *a* and *b*, an inherited one, is described later.) More often band 9 is obscured by band 8, as in type *b*, Fig. 1. Band 2 occurs only in laying females and band 1 only at the onset of lay[11], while band 8 is absent in females during laying so that band 9 is easily visible. Other differences associated with sex and laying are matters of intensity and not of the presence or absence of whole bands. Type *b* was the only pattern found in the following breeds and strains, with the numbers tested in brackets.

White Leghorn: Reaseheath inbred lines, *C*(20), *I* (20), *R* (11) and *W* (12) at Cambridge; Cambridge *WICOB* outbred (25); Thornber strains, 1 (8) and 2 (12).

Black Leghorn: Thornber strain 3 (14).

Exchequer Leghorn: Thornber strain 4 (9).

Rhode Island Red: Thornber strain 5 (36).

In a Light Sussex strain (Thornber 6) three distinctly different patterns were seen. The differences involved bands 11 and 12. In the typical form (*b*) these consisted of a faster-migrating strong band (No. 11, Fig. 1) and a slower-migrating faint band (No. 12, Fig. 1). In the variant which we have called type *a* a similar pair of bands is seen, each migrating faster than its counterpart in type *b*, such that the slower, fainter band (No. 12) of type *a* coincides with the faster, stronger band (No. 11) of type *b*. In another variant (type *ab*) three bands are seen, corresponding in mobility with Nos. 11 and 12 of types *a* and *b* together. We have presumed this to be a heterozygous form (see later). The bands involved in this polymorphic variation were studied in autoradiographs (in collaboration with Dr. John Williams of the Department of Biochemistry, Cambridge, to whom our thanks are due), and were shown to bind iron, from which we conclude they are transferrins. Difficulty arose in interpreting the results of some of our electrophoretic studies, owing to the variability in position of a component (band 13) migrating close to transferrin. Its position relative to the transferrin bands could be faster, coincident, or slower, according to the conditions of electrophoresis, and in particular was dependent on the time in the whole run when the 'brown line'[10] passed through the transferrin region. The most satisfactory results were obtained by arranging the position of the insert, and the time of the run, such that band 13 consistently appeared well behind the transferrins, as in the gel illustrated in Fig. 1.

The simplest genetic hypothesis is that types *a* and *b* represent the products of alleles at a single locus, and *ab* of the heterozygote. Tests of sera of offspring from matings of tested parents gave results shown in Table 1, which are clearly in accord with the hypothesis. In conformation with a previously suggested system of nomenclature for mammalian transferrins[12], we propose the designations Tf^a and Tf^b for the

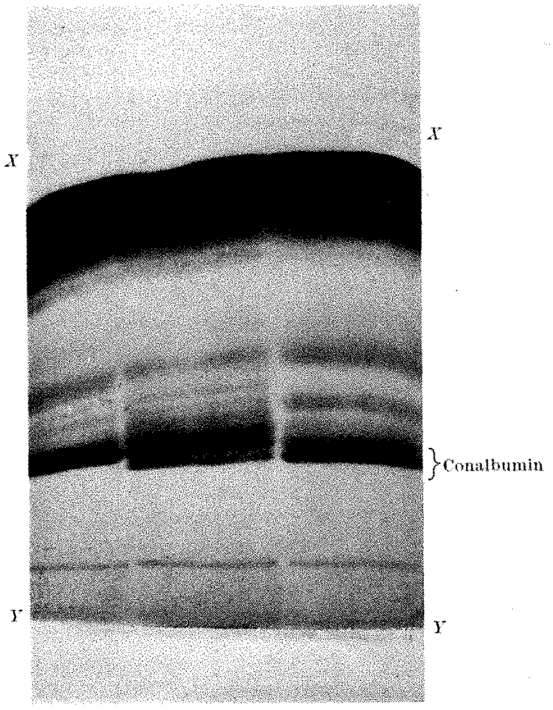

Fig. 2. Starch-gel electrophoresis of egg-white, showing the three conalbumin types. Y—Y marks the position of insertion of the samples, and X—X the position of the brown line at the end of the run

Table 1. SEGREGATION OF TRANSFERRIN TYPES AMONG OFFSPRING OF TESTED MATINGS

Mating type	No. of matings	Offspring *a*	Offspring *ab*	Offspring *b*	Total	Test for single locus d.f.	Test for single locus χ^2
a × *a*	4	38	0	0	38	—	—
a × *ab* *ab* × *a*	4	24	28	0	52	1	0·308
a × *b*	3	0	20	0	20	—	—
ab × *ab*	5	14	20	12	46	2	1·015
ab × *b* *b* × *ab*	5	0	26	34	60	1	0·533
b × *b*	4	0	0	45	45	—	—

alleles, and Tf-*a*, Tf-*ab* and Tf-*b* for the three phenotypes.

Williams[13] obtained evidence that chicken serum transferrin and egg-white conalbumin are glycoproteins, which have the same amino-acid sequence, but which differ in their carbohydrate moiety, causing a difference in their electrophoretic mobilities. We thus anticipated finding genetic variation in conalbumins parallel with that in transferrins. Electrophoresis of egg-white from hens of the three transferrin phenotypes, carried out under the same conditions as for sera, is shown in Fig. 2. The conalbumins are the heavily staining bands corresponding with bands 16 and 17 of Lush[14]. (The other variation in Fig. 2 involves Lush's locus III.) The variation in conalbumins, parallels that in the transferrins, the *a* transferrin type having two faster-migrating conalbumin bands, the *b* type two slower bands, and the *ab* type three bands, with the slow band of conalbumin *a* and the fast band of *b* in the same position. In each type the faster band is less intense than the slower.

We have tested eggs from 3 type *a*, 12 *ab* and 23 *b* hens, and in every case the conalbumins showed the corresponding bands, as already described.

A. L. OGDEN
Animal Health Trust Livestock Research Centre,
Stock, Essex.

J. R. MORTON
D. G. GILMOUR
School of Agriculture,
University of Cambridge.

E. M. MCDERMID
Thornber Bros.,
Mytholmroyd,
Halifax, Yorkshire.

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VIROLOGY

A Sensitive and Precise Plaque Assay for Herpes Virus

Two techniques are at present available for the plaque assay of animal viruses. The most widely used involves the infection of monolayers of susceptible cells[1]: this method has been successfully applied to herpes virus by a number of authors using various cell systems[2–7]. The second technique, which has certain advantages over the monolayer method, uses agar-cell suspensions[8]: this method does not function satisfactorily with herpes virus in the cell systems tested (cf. ref. 3).

A method is described here which involves some features of both these techniques. The cells are infected in suspension and plated out to form monolayers in which plaques eventually appear. Variables affecting the production of plaques have been examined and the following procedure gives the optimal conditions for the assay.

Monodispersed cell suspensions are produced from monolayers by the action of a 0·02 per cent versene solution in phosphate-buffered saline or of a 0·05 per cent solution of trypsin in the 0·02 per cent versene solution. The cells are washed twice in Eagle's medium containing 10 per cent tryptose phosphate broth (Difco) and 10 per cent calf serum (ETC). Aliquots of the cells suspended in ETC containing 4 *n* million cells (where *n* is the number of 60-mm. assay plates required) are then centrifuged and the pellet of cells resuspended in *n* c.c. of an appropriate virus dilution in ETC. This cell-virus suspension is shaken at 37° C. for 20 min., when a further 4 *n* c.c. of ETC is added and 5 c.c. aliquots of the suspension plated into *n* 60-mm. Petri dishes. These are then incubated in a humidified atmosphere of 5 per cent carbon dioxide in air at 37° C. Secondary plaque formation is prevented by adding 5 c.c. per plate of an antibody containing medium, that is, Eagle's medium containing 10 per cent tryptose phosphate broth and 10 per cent pooled human serum, within 4–18 hr. after infection. Inhibition of secondary plaques can also be achieved by adding Eagle's medium containing carboxymethyl cellulose instead of ETC after the 20-min. adsorption period. This medium is prepared by adding 15 c.c. of a 5 per cent w/v solution of the sodium salt of carboxymethyl cellulose (L. Light and Co.) in water and 10 c.c. of calf serum to 75 c.c. of Eagle's medium (1·3 times normal strength). In this procedure, care must be taken not to move the assay plates during incubation.

Plaques 1–2 mm. in diameter are produced in 2–3 days, the time for maximum production of plaques depending on the type of cell infected. Plates are fixed with formol/saline, stained with Leishman's stain, dried and plaques counted using a hand lens (×4). The technique has been successfully applied with herpes virus (strain *HFEM*) to a line of HeLa cells adapted to calf serum; to a strain of baby hamster kidney cells, *BHK*21 clone *C*13 (ref. 9); to whole chick embryo cultures and to whole mouse embryo cultures.

The method has the advantages over present plaque assay methods of requiring only a short adsorption period and of producing the maximal number of plaques relatively quickly. A demonstration of the validity of this technique as an assay for the virus is seen in Fig. 1, where a linear dose response relationship exists over a wide range of plaque counts.

Table 1. COMPARISON OF PLAQUE AND POCK TITRES

Cells used	Plaque titre/ml.	Pock titre/ml.	Plaque/pock
HeLa	$7{\cdot}7 \times 10^7$	$1{\cdot}9 \times 10^7$	4·1
HeLa	$1{\cdot}9 \times 10^9$	$8{\cdot}0 \times 10^8$	2·5
HeLa	$5{\cdot}6 \times 10^8$	$1{\cdot}6 \times 10^8$	3·5
*BHK*21/*C*13	$4{\cdot}5 \times 10^{10}$	$1{\cdot}8 \times 10^{10}$	2·5
*BHK*21/*C*13	$4{\cdot}3 \times 10^9$	$1{\cdot}2 \times 10^9$	3·6
*BHK*21/*C*13	$1{\cdot}2 \times 10^9$	$5{\cdot}5 \times 10^8$	2·2
*BHK*21/*C*13	$4{\cdot}3 \times 10^9$	$1{\cdot}1 \times 10^9$	3·9
*BHK*21/*C*13	$9{\cdot}1 \times 10^9$	$2{\cdot}0 \times 10^9$	4·6
Whole chick cultures	$1{\cdot}7 \times 10^9$	$1{\cdot}1 \times 10^9$	1·5
Whole mouse cultures	$4{\cdot}0 \times 10^8$	$1{\cdot}1 \times 10^9$	0·4

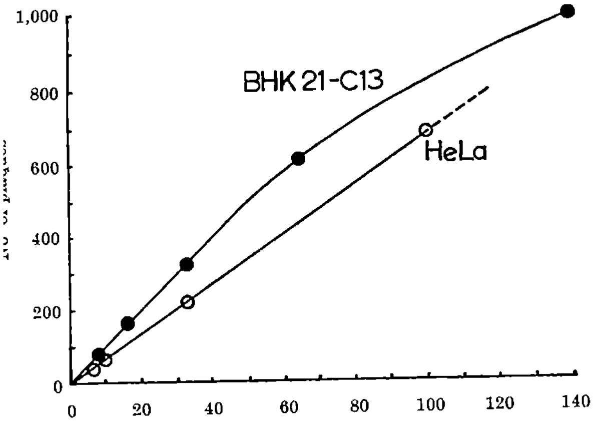

Fig 1 Relationship between plaque counts on HeLa and *BHK*21-*C*13 cells and relative virus concentration Different virus seeds were used in the two experiments

Further important advantages of the method are its reproducibility, sensitivity and precision The reproducibility is such that a given virus seed on assaying in *BHK*21-*C*13 cells in six different experiments making fresh dilutions to 10^{-7} each time shows a standard deviation of each measurement from the mean of 11 per cent Table 1 shows that with most cells tested the method is significantly more sensitive than the pock count on the chick chorioallantois The precision of the technique is illustrated in Table 2

Table 2 PLAQUE NUMBERS FROM REPLICATE DISHES

Cells used	Mean No of plaques per dish	Degrees of freedom	Variance	95 per cent fiducial limits of mean
*BHK*21/*C*13	12	15	12	1 8
*BHK*21/*C*13	429	11	311	11 4
*BHK*21/*C*13	119	5	78	9 4
*BHK*21/*C*13	55	12	71	5 0
HeLa	51	15	58	4 1
HeLa	263	15	304	9 3

The method has been shown to function successfully with other herpes strains and with vaccinia, pseudorabies and fowl plague viruses, secondary plaque formation being prevented by the addition of medium containing carboxymethyl cellulose

WILLIAM C RUSSELL *

Institute of Virology,
University of Glasgow

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* Lock Research Fellow of the Royal Society of Physicians and Surgeons of Glasgow

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Inactivation of Naturally Occurring Variants of Tobacco Mosaic Virus with Nitrous Acid

ALTHOUGH there is general agreement that mutant strains of tobacco mosaic virus (TMV) may arise during oxidative de-amination of the ribonucleic acid by nitrous acid, this view has been held without conclusive evidence eliminating selection as a possible cause for the appearance of new strains Soon after Gierer and Mundry[1] announced the results of their experiments in which the mutagenesis of nitrous acid was implied, Bawden[2] pointed out that their results did not, in fact, exclude the possibility of selection, and despite Mundry's counter-claims[3], the issue has remained unsettled

Bawden based his argument for selection on the known competitive behaviour of strains of TMV For example, when two strains of TMV compete for the same limited number of infective sites, the extent of competition is found to be a function of both the ratio of the two strains to each other, and the total concentration of infectious units in the inoculum[4] Thus, at any one ratio, the extent of competition encountered by one strain in the presence of the other depends on the concentration If the starting TMV preparations in the nitrous acid experiment contained a small amount of a variant strain competitively inhibited by the predominant strain, inactivation could reduce the extent of competition observed simply by reducing the total concentration of infectious units If, in addition, the predominant strain were inactivated at a faster rate than the variant, an absolute rise in the number of variant infections is possible without the predominant strain mutating As an example, a differential dilution following ultra-violet irradiation of a mixture of two strains of TMV was observed to simulate closely the results of a nitrous acid experiment[4] A comparison of the data from the following two experiments will show how different the results of simple dilution can be from a differential dilution

A mixture of two competing TMV strains *U*1 and *VM*, was prepared The ratio and concentration of the strains were adjusted so that competition reduced the number of *U*1 lesions appearing to approximately 10 per cent of the controls In one case, the mixture was diluted and in the other samples were irradiated with ultra-violet light for different intervals of time before the mixtures were used as inocula The dilution and irradiation results are shown in Fig 1 It can be seen that dilution reduced the number of *U*1 lesions appearing in the mixture, but not as much as if no interference were encountered Nevertheless, this effect was small, compared with the large absolute rise in the number of *U*1 lesions observed following irradiation More than five times the number of *U*1 lesions were engendered by the mixture that was irradiated compared with the unirradiated control It had already been established that *U*1 and *VM* differed in their *in vitro* ultra-violet sensitivity[4] by factor 5 With this information, plus the quantitative data on competition, it was possible to estimate the reduction in competition expected following the ultra-violet irradiation This estimate compares favourably with the observed results

Since all strains of TMV tested so far compete with each other, it is likely that any variant detected would also compete with the parent strain In the circumstances, the question of mutagenesis, or selection, arising from nitrous acid treatment of TMV can be resolved only after the sensitivity to nitrous acid of the variant or mutant strain has been determined For this reason, Mundry's argument[3] that "   differences in interference-induced selection may be considered to be excluded if treated material is compared with untreated controls of the same infectivity   " is an inadequate control An experiment was therefore designed in which the possibility of selection in the TMV system was investigated directly

One thousand single lesions produced by *U*1 on *Nicotiana glutinosa* were transferred to individual tobacco plants (*Nicotiana tabacum*) When systemic symptoms were apparent the plants were classified, and any plant displaying marked variation from the type strain was separated In all, 15 variants (1 5 per cent) were recovered Fourteen produced much milder symptoms than the parent common strain One was highly necrotic, causing severe stunting and distortion of the plant Transfers were made from each individual variant, and large amounts of each new strain were prepared All variants reproduced true to type Four of the milds and the one necrotic strain were selected for investigation

Infectivity dilution curves established that each variant had the same specific infectivity as the parent These five variants, the parent *U*1 strain, and two other related, but different TMV strains (*U*2 and *U*8) were then individually inactivated with nitrous acid utilizing the method described by Mundry and Gierer[1] Many kinetic investigations were made, the average results of which appear in Fig 2

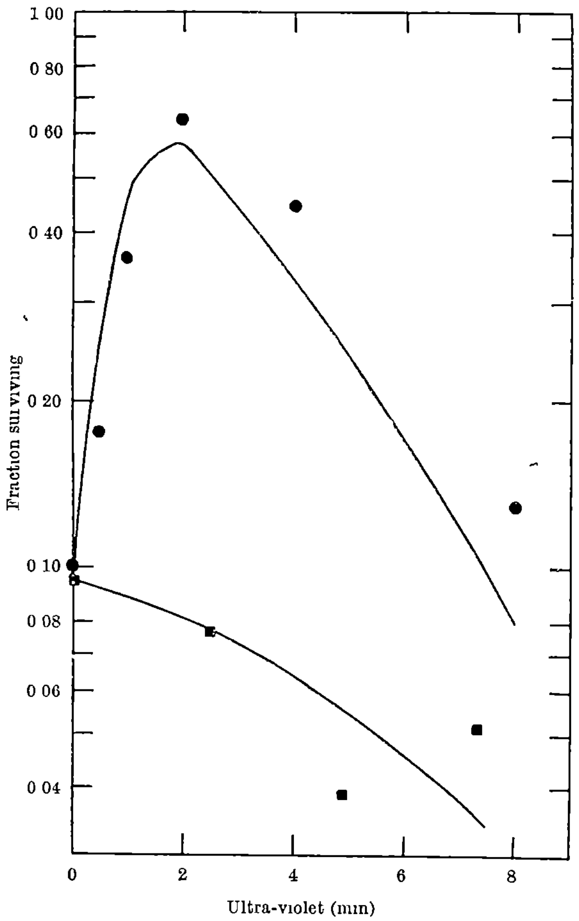

Fig 1 Competition between TMV strain *U*1 and *VM* (The irradiation data are taken from Rappaport and Wu, ref 5) *U*1 at 2 × 10^{-1} mg/ml was mixed with an equal volume of *VM* at 5 × 10^{-2} mg/ml One such mixture was irradiated *in vitro* with ultra violet light and then inoculated on leaves of *N glutinosa* The fraction of *U*1 lesions surviving appear in the upper curve Another mixture of *U*1 and *VM* as above was serially diluted in phosphate buffer before inoculation The fraction of *U*1 lesions observed in this case appears in the lower curve The dilution data are adjusted to fit the same abscissa as the irradiation data

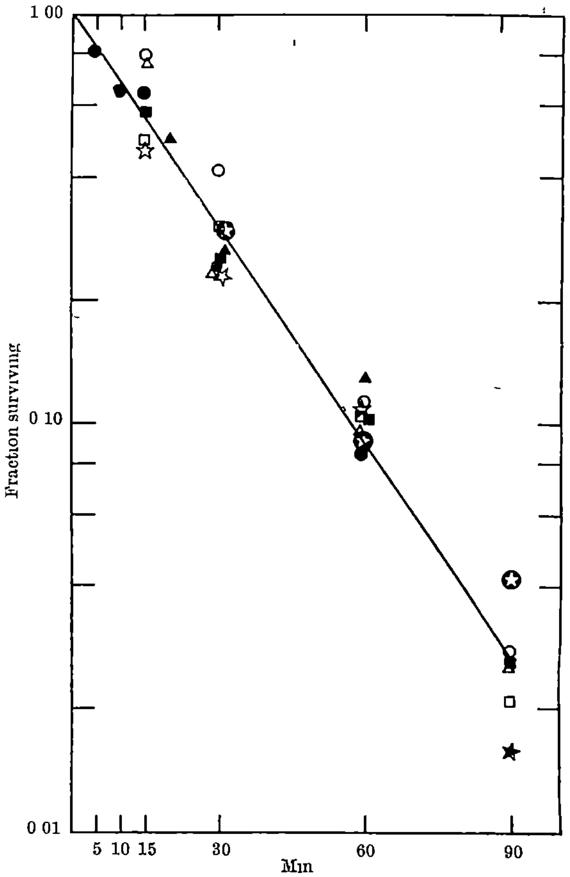

Fig 2 Nitrous acid inactivation of five variants, one parent and two other related TMV strains The data confirm those of Gierer and Mundry (ref 1)

Each TMV strain, regardless of its previous history, was inactivated by nitrous acid at essentially the same rate No significant differences were discernible This is in marked contrast to the different rates of inactivation of some of these strains by ultra-violet light Thus, inactivation of a mixture of two TMV strains with nitrous acid is equivalent to a simple dilution rather than a differential dilution Therefore, any absolute rise in the number of variants detected following a brief exposure to nitrous acid reflects induced qualitative changes in the starting material The results of Gierer and Mundry and of Siegel[6], who have investigated this phenomenon, all indicate that nitrous acid is mutagenic for TMV

This work was supported in part by a research grant *E*536(*C*8) from the National Institutes of Health, U S Public Health Service, and contract *At* (11-1)-34 Project 8 of the Division of Biology and Medicine, U S Atomic Energy Commission

Irving Rappaport*
S G Wildman

Department of Botany,
University of California,
Los Angeles 24

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* Present address La Rabida Institute, University of Chicago

[1] Gierer, A , and Mundry, K W , *Nature*, **182**, 1457 (1958) Mundry, K W , and Gierer, A , *Z Vererbungslehre*, **89**, 614 (1959)

[2] Bawden, F C , *Nature*, **184**, 27 (1959)

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[4] Mundry, K W , *Z Verebungslehre*, **88**, 115 (1957) Siegel, A , *Virology*, 8, 470 (1959) Wu, J H , and Rappaport, I , *ibid* , **14**, 259 (1961)

[5] Rappaport, I , and Wu, J H , *Virology* (in the press)

[6] Siegel, A , *Virology*, **11**, 156 (1960)

FORTHCOMING EVENTS

Wednesday, September 12

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S W 7), at 6 p m —Mr Russell Lowe (U S A) "Structural Damping Allied to the Isolation of Electronic Equipment"

Thursday, September 13—Friday, September 14

Biochemical Society (in the Department of Agricultural Biochemistry, Institute of Rural Science, Penglais, Aberystwyth)—Colloquium on "Biochemistry of Plant Pigments" followed by original communications

Thursday, September 13—Friday, September 14

British Nuclear Energy Society (at the Institution of Civil Engineers, 1–7 Great George Street, London, S W 1)—Symposium on "The Advanced Gas-Cooled Reactor"

Friday, September 14

Society of Non-Destructive Examination (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S W 1), at 3 30 p m —Dr M McCraig "Magnetic Factors in Testing Materials"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturer (graduate or equivalent qualifications, and with sound industrial and teaching experience) in Electrical Engineering, to be responsible, under the Head of the Department of Engineering, for the development and supervision of electrical engineering courses and laboratories—The Principal, Aylesbury College of Further Education, County Offices, Aylesbury, Bucks (September 14)

Electronics Designer, Experimental Officer or Senior Experimental Officer grade (with a degree in physics or electrical engineering, or comparable qualification) in the Department of Chemistry, to collaborate with members of the teaching staff in designing and constructing electronic equipment to be used in research work in the Department—The Registrar, The University, Manchester 13 (September 15)

Hospital Biologist (with a university degree in physiology, biochemistry or zoology, or similar qualifications) in the Department of Chemical Pathology, for routine bioassay and chemical procedures mainly in the field of clinical endocrinology, research facilities also available—The Deputy Director, Vincent Square Laboratories of Westminster Hospital, 124 Vauxhall Bridge Road, London, S W 1 (September 15)

Research Fellow (preferably with a knowledge of many-electron atom theory and of computing) in Theoretical Chemistry, to work with Dr H O Pritchard on exact calculations for lithium and beryllium using the Atlas computer—The Registrar, The University, Manchester 13 (September 15)

Hargreaves Research Assistant (graduate in agriculture, agricultural science or science) in Agriculture, to do research over a period of three years on fertilizer placement in beet and potatoes—The Registrar, The University, Leeds 2 (September 17)

Research Student (graduate in zoology) in Zoology, to undertake research in relation to the ecology of the food consumed by salmonid fish—The Secretary, The University, Exeter (September 17)

Senior Research Officer (honours graduate in agriculture or science, with tropical experience or other evidence of appropriate research and administrative ability) at the Forest Products Station, Kade, University of Ghana, to accept professional and administrative responsibility for the running of the Station in consultation with a Committee of Management composed of members of the Faculty of Agriculture—The Registrar, University of Ghana, P O Box 25, Legon, Accra or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (September 17)

Senior Lecturer in Economics in the Faculty of Social Science, Queen's College—The Secretary of the University of St Andrews, Queen's College, Dundee (September 22)

Assistant Experimental Officer (graduate, drawing ability an advantage) in the Nematology Department, to work on the taxonomy of plant-parasitic nematodes—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 24)

Bacteriologist, Scientific Officer or Senior Scientific Officer grade, at the Marine Laboratory, Aberdeen, to be responsible for statutory diagnosis of furunculosis and other diseases in salmonid fish, and to undertake research either in such diseases or in bacteriological aspects of marine productivity—The Establishment Officer, Department of Agriculture and Fisheries for Scotland, Room 73, St Andrew's House, Edinburgh 1 (September 24)

Lecturer or Assistant Lecturer in Bacteriology—The Registrar, The University, Leeds 2 (September 24)

Lecturer (graduate with a good honours degree) in Sociology at the University College of Cape Coast, Ghana—The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (September 24)

Assistant Librarian (graduate with university library experience, preferably male, age 30–45), for general, including administrative, duties—The Goldsmith's Librarian, University of London Library, Senate House London, W C 1 (September 28)

Senior Lecturer (with an honours or higher degree in chemical engineering and with research and teaching or industrial experience) in Chemical Engineering at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S W 1 (September 28)

Lecturer or Assistant Lecturer in Stability and Control of Aeroelasticity in the Department of Aeronautics and Astronautics—The Secretary and Registrar, The University, Southampton (September 29)

Physicist/Mineralogist (with a university honours degree in science, with adequate training in mathematics, physics and chemistry, preferably with postgraduate training to Ph D level or equivalent research qualifications in some field of X-ray studies) in the Mineralogy Section, Division of Soils, C S I R O , Adelaide, South Australia, to undertake research on soil problems by X-ray techniques —Mr P F Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2, quoting Appointment No 270/246 (September 29)

Lecturer (with qualifications in either pure or applied mathematics) in Mathematics at the University of Canterbury, Christchurch, New Zealand, to engage in research in his own field or to assist with the work in the field of rocket ballistics already in progress in the mathematics department—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (September 30)

Lecturer or Junior Lecturer in Social Anthropology at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (September 30)

Senior Lecturer and a Lecturer in the Department of Mathematics, University of the Witwatersrand, Johannesburg, South Africa —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, September 30)

Senior Research Fellow (with Ph D or equivalent postgraduate research experience) in the Department of Zoology, Royal College, Nairobi, for work on the nature of the host/parasite relationships of Ixodid ticks—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (September 30)

Lecturer in Philosophy at the University of Melbourne, Australia —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, October 15)

Lecturer in Physical Chemistry at the University or Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Lecturer (with postgraduate qualifications and research interests in sensation and perception) in Psychology at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Senior Lecturer in Social Anthropology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Physicist (with an honours degree in physics, and preferably with a special interest and aptitude in electronics) in the Physics Section of the Anti-Cancer Foundation, for work on problems arising from the application of ionizing radiation to the diagnosis and treatment of disease—The Secretary, The University of Adelaide Anti-Cancer Foundation, Adelaide, South Australia (November 1)

Bibliographic Assistant, to assist with literature search and abstracting in connexion with medical and scientific research—Prof A C Frazer, Department of Medical Biochemistry and Pharmacology, The University, Birmingham 15

Biochemist—(non-medical) for the Group Laboratory, Guildford —The Hospital Secretary, St Luke's Hospital, Guildford, Surrey

Biochemist (science graduate, preferably with an honours degree in chemistry) in the Chemical Pathology Department, for duties including routine investigations and participation in research—The Senior Consultant Pathologist, Queen Elizabeth Hospital for Children, Hackney Road, London, E 2

Chief Technician, to take charge of electronic medical workshop —The House Governor, The Hospital for Sick Children, Great Ormond Street, London, W C 1

Entomologist (Medical) (national of, and resident in, Great Britain or the Republic of Ireland, with an honours degree in Zoology, and preferably with postgraduate specialist experience in entomology) with the Government of Uganda, to work under the general direction of the Senior Entomologist (Medical) on the general problems of medical entomology and the control of vector borne diseases the duties will entail a great deal of field work involving considerable physical hardship—The Director of Recruitment, Department of Technical Co-operation, 3 Sanctuary Buildings, Great Smith Street, London, S W 1, quoting RC 205/153/018

Principal Biochemist (male, with a university science degree, associate or graduate member of the Royal Institute of Chemistry), for duty at H M Prison, Wormwood Scrubs, London—Establishment Officer (JB 652), Prison Commission, Horseferry House, Dean Ryle Street, London, S W 1

Research Scholar or Assistant (with a degree or diploma in technology, and with an interest in the applications of computers) in the Computer Centre, to engage in a programme of research in methods of numerical analysis suitable for a medium-sized computer, the preparation of an Algol compiler or an approved data handling topic—The Academic Registrar, Loughborough College of Technology, Loughborough, Leics, quoting reference 21/G

Scientific Officer or Senior Scientific Officer (honours graduate in applied mathematics), to work on problems in rheology theory of plasticity, theory of elasticity and thermal stresses—Dr N F Astbury, Director of Research, The British Ceramic Research Association, Queen's Road, Penkhull, Stoke-on-Trent

Technician (with suitable qualifications and preferably with a knowledge of photography or tissue culture) to assist in chromosome research—Dr Martin Bodian, Department of Morbid Anatomy, Hospital for Sick Children, Great Ormond Street, London, W C 1

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Proceedings at a Congregation of the University of Birmingham, 14 July, 1962 Degrees in Pure Science and in Commerce and Social Science Pp 20 Degrees in Arts and in Medicine and Dentistry Pp 16 Degrees in Applied Science and in Law Pp 15 (Birmingham The University, 1962) [187

Department of Scientific and Industrial Research Torry Research Station Annual Report 1961 on the Handling and Preservation of Fish and Fish Products Report of the Torry Research Station Steering Committee and Report of the Director of the Torry Research Station Pp iv+44+4 plates (London H M Stationery Office, 1962) 4*s* [187

Eighteenth Annual Report of the British Coke Research Association for the year 1961 Pp 35 (Chesterfield British Coke Research Association, 1962) [187

The Atomic Gardening Society *The Atomic Gardening Digest*, Vol 3, No 3 (June 1962) Pp 10 (Winchelsea Dr Thomas Gray, The Atomic Gardening Society,"Winnipeg", 1962) [187

Council for the Preservation of Rural Wales (Cymdeithas Doigelu Harddwch Cymru) Annual Report, 1959–1960 , 1960–1961 Pp 93+2 plates (Machynlleth, Montgomeryshire Council for the Preservation of Rural Wales, 1962) [237

Cement and Concrete Association Report for the year 1961 Pp ii+100 (London Cement and Concrete Association, 1962) [237

Science Books for a School Library, Supplement No 3, 1962 (A list of recent books selected from those sent by publishers to the Science Masters' Association) Pp 16 (London John Murray, 1962 Published for the Science Masters' Association) 1s [237

Magnanimity By Sir Charles Snow (Rectorial Address delivered before the University of St Andrews on 13 April, 1962) Pp 23 (St Andrews, Fife Students' Representative Council, The University, 1962) 2*s* [237

The Physics of Chemical Structure By R J Taylor (Advanced Series in Unilever Educational Booklets) Pp 40 (London Unilever Education Section, Unilever House, 1962) [237

The Nuffield Foundation Seventeenth Report for the year ended 31 March, 1962 Pp xiv+159 (London The Nuffield Foundation, 1962) [237

Bulletin of the Royal Society International Scientific Information Services, Vol 1, No 1 (July 1962) Pp 80 (London The Royal Society, 1962) [237

The Natural Rubber Producers' Research Association Twenty-fourth Annual Report Pp 39 (London The Natural Rubber Producers' Research Association, 1962) [237

Philosophical Transactions of the Royal Society of London Series B Biological Sciences No 720, Vol 245 (19 July, 1962) The Structure and Innervation of the Nuclear Bag Muscle Fibre System and the Nuclear Chain Muscle Fibre System in Mammalian Muscle Spindles By I A Boyd Pp 81–136+plates 42–54 (London The Royal Society, 1962) 45*s* [237

Department of Scientific and Industrial Research Research for Industry 1961 A Report on Work done by Industrial Research Associations in the Government Scheme Pp iv+140+12 plates. (London H.M Stationery Office, 1962) 10*s* net [237

Bulletin of the British Museum (Natural History) Entomology Vol 12, No 4 A Revision of African Laemophloeinae (Coleoptera Cucujidae) By L P Lefkovitch Pp 165—245 27*s* 6*d* Vol 12, No 5 Microlepidoptera from the New Hebrides Records and Descriptions of Microlepidoptera collected on the Island of Aneityum by Miss Evelyn Cheesman, O B E By J D Bradley Pp 247–271+plates 14–27 20*s* Vol 12, No 6 The Acridoides (Orthoptera) of Madagascar 1 Acrididae (Except Acridinae) By V M Dirsh Pp 273–350 26*s* (London British Museum (Natural History), 1962) [237

Other Countries

Universities Under Siege By Sir Eric Ashby (The Chancellor's Lecture) Pp 19 (Johannesburg University of the Witwatersrand, 1962) [237

Smithsonian Institution United States National Museum Bulletin 232 The Lithographs of Childe Hassam—a Catalog By Fuller Griffith Pp vii+66 55 cents Contributions from the Museum of History and Technology Paper 18 Excavations at Rosewell in Gloucester County, Virginia, 1957–1959 By Ivor N Hume Pp 153–229 70 cents (Washington, D C Government Printing Office, 1962) [237

The Natural History of Rennell Island, British Solomon Islands Vol 4 (Invertebrates, Pars, and addition to Vertebrates) By Torben Wolff (Scientific Results of the Danish Rennell Expedition, 1951 and The British Museum (Natural History), Expedition, 1953) Pp 120+18 plates (Copenhagen Danish Science Press, Ltd , 1962 Published for the University, Copenhagen, and the British Museum (Natural History), London) 35 Danish kr [237

Institut Royal des Sciences Naturelles de Belgique Bulletin Tome 38, No 1 René Verheyen (1907–1961) Notice Biographique Par René Van Tassel et Ludo Van Meel Pp 14+1 planche Tome 38, No 2 Les Denticulations Cardinales des Spiriferidae Par Antoine Vandercammen Pp 6+1 planche Tome 38, No 3 Heminia, Discinites et Tongshania Par Francois Stockmans et Yvonne Williere Pp viii+2 planches Vol 38, No 4 Contribution a l'Etude des Bostrychidae 31 Troisiéme Collection du Musèe Zoologique de l'Université Humboldt à Berlin Par Jean-Marie Vrydagh Pp 47 Tome 38, No 7 Sur la Presence du Prismotest dans les Brachiopodes Articules Par Antoine Vandercammen Pp 17+2 planches Tome 38, No 8 A Propos de l'Ordre des *Aphelophlebia* (Insects Palaeoptera) Par Georges Demoulin Pp 4 Tome 38, No 9 Mission A Collart en Espagne (1960) Diptera Bombyliidae Par Frans Joseph Francois Pp 8 Tome 38, No 14 Notes sur les Microlepidopteres de Belgique Par Edouard Janmoulle Pp 3 Tome 38, No 15 La Larve d'Orthotrichia Angustella MacLachlan (Trichoptere, Hydroptilidae) Par Serge Jacquemart Pp 8 Tome 38, No 17 Etudes sur les Mollusques de l'Afrique Centrale et des Regions Voisines 3 Quelques Espèces peu Connues de *Gonaxis* Par William Adam Pp 11±1 planche Tome 38, No 19, A Propos de l'Especie-Type du Genre *Yunnanella* Grabau, A W , 1923 Par Paul Sartenaer Pp 3 Tome 38, No 21 Etudes sur les Mollusques de l'Afrique Centrale et des Regions Voisines 4 Contribution à la Connaissance du Genre *Lameliger* Ancey, 1884 Par William Adam Pp 11+1 planche Tome 38, No 27 Notes sur les Trichopteres 3 Le Genre *Cheumatopsyche* en Afrique Par Georges Marleir Pp 9 (Bruxelles Institut Royal des Sciences Naturelles de Belgique, 1962) [237

Institutt for Atomenergi, Kjeller Quarterly Progress Report, January–March 1962 Pp 41. (KR 22) (Lillestrøm Institut for Atomenergi, Kjeller Research Establishment, 1962) [237

Publications de l'Institut National pour l'Étude Agronomique du Congo Flore du Congo et du Rwanda-Burundi Vol 8, 1 Spermatophytes Préparée par le Comité Exécutif de la Flore et par le Jardin Botanique de l'État Pp vii+214 (Bruxelles Institut National pour l'Étude Agronomique du Congo, 1962) [237

Republic of Iraq Ministry of Communications Directorate General of Civil Aviation Meteorological Department Meteorological Memoirs, Vol 1 Pp ii+165 (Baghdad Ministry of Communications, Meteorological Department, 1962) [237

National Aeronautics and Space Administration, Washington Technical Note D-1153 Star Catalogs on Punched Cards and Magnetic Tape By J. H Berbert Pp 6 Star Catalogs on Punched Cards and Magnetic Tapes—Details By J H Berbert and J D Oosterhout Pp 12 (Washington, D C ,and Greenbelt, Md National Aeronautics and Space Administration, 1961 and 1962) [237

United States Department of the Interior Geological Survey Water-Supply Paper 1329-B Waterpower Resources of the Wilson River Basin, Oregon By Donald W Neal With a section on Geology by David L Gaskill Pp iv+31–62+plate 3 Water-Supply Paper 1585 Effect of Irrigation on Ground Water in Southern Canyon County, Idaho By P R Stevens Pp iv+74+4 plates Water-Supply Paper 1702 Surface Water Supply of the United States, 1960 Part 1-B North Atlantic Slope Basins, New York to York River Prepared under the direction of E L Hendricks Pp xi+567 Water-Supply Paper 1708 Surface Water Supply of the United States, 1960 Part 5 Hudson Bay and Upper Mississippi River Basins Prepared under the direction of E L Hendricks Pp xii+588 (Washington, D C Government Printing Office, 1961 and 1962) [237

United States Department of Commerce Coast and Geodetic Survey Technical Bulletin No 19 Analytic Absolute Orientation in Photogrammetry By G C Tewinkel Pp 11+18 (Washington, D C Government Printing Office, 1962) 20 cents [237

United States Atomic Energy Commission Oak Ridge National Laboratory ORNL-3300, Volume 1 (Special) Publications, Reports, and Papers for 1961 from Oak Ridge National Laboratory Pp vi+82 (Oak Ridge, Tennessee Oak Ridge National Laboratory, 1962 Available from Office of Technical Services, U S Department of Commerce, Washington 25, D C) 2 dollars [237

United States Department of Commerce Coast and Geodetic Survey C and G C Publication 31-3 Surface Water Temperature and Salinity, Pacific Coast—North and South America and Pacific Ocean Islands Pp iii+71 (Washington, D C Government Printing Office, 1962) 45 cents [237

Tables for Complex Hyperosculatory Interpolation Over a Cartesian Grid By Herbert E Salzer, Genevieve M Kimbro and Marjory M Thorn Pp i+71 (San Diego, Calif General Dynamics/Astronautics, Division of General Dynamics Corporation, 1962) [237

Population Reference Bureau *Population Bulletin*, Vol 18, No 41 (July, 1962) The Common Market Pp 65–190 (Washington, D C Population Reference Bureau, 1962) 50 cents [237

Annals of the New York Academy of Sciences Vol 93, Article 10 Radio Telescope Structures By Jacob Feld Pp 351–456 (New York New York Academy of Sciences, 1962) [237

Transactions of the American Philosophical Society New Series, Vol 52 Part 2 Late Permian Terrestrial Vertebrates, U S A and U S S R By Prof Everett C Olson Pp 224 (21 plates) (Philadelphia The American Philosophical Society, 1962) 5 dollars [237

Smithsonian Miscellaneous Collections Vol 144, No 3 Revision of the Cassiduloid Echinoids By Porter M Mier Pp iii+262+44 plates (Publication 4500) Vol 145, No 1 Systematic Notes Concerned with the Avifauna of Panama By Alexander Wetmore Pp 14 (Publication 4501) Vol 145, No 2 Notes on Fossil and Subfossil Birds By Alexander Wetmore Pp 17 (Publication 4502) (Washington, D C Smithsonian Institution, 1962) [237

Western Australia Report of the Government Chemical Laboratories for the year 1960 (Extract from the Report of the Department of Mines) Pp 30 (Perth Government Printer, 1962) [237

Canada Meteorological Branch Department of Transport Division of Building Research National Research Council NRC 6521 A Bibliography of Canadian Climate, 1763–1957 Compiled by Morley K. Thomas Pp 114 (Ottawa Queen's Printer, 1961) 1 dollar [237

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EMPLOYMENT OF SCIENTISTS AND TECHNOLOGISTS

WHEN the report on the *Long-Term Demand for Scientific Manpower* (which the Statistics Committee of the Advisory Council on Scientific Policy prepared at the request of the Council's Committee on Scientific Manpower) was published last autumn, the Committee's conclusion that by 1965 the supply and demand of such manpower should not be much out of balance and that thereafter a surplus might exist caused some concern (*Nature*, 192, 293, 1961) The assumptions and conclusions of the Committee were indeed questioned, notably in a debate in the House of Lords on November 15, but apart from the fact that, as the Committee itself points out, the balance is a statistical one and that supply and demand in individual disciplines cannot be so easily equated, the Committee on Scientific Manpower, in presenting the report, suggested that in the educational profession and in those disciplines which involve a high degree of mathematical skill the present shortage of scientific manpower is likely to continue for a long time

While as yet the assumptions which the Committee made regarding demand do not appear to have been seriously challenged, its assumption regarding supply is certainly rendered dubious by the Government's policy towards the universities It now appears unlikely that the universities will be able to achieve by 1971 the expansion figure of 170,000 assumed by the Statistics Committee, and, even if there were no similar check to expansion of the colleges of technology and similar institutions, it is now problematical whether it will be possible to count on the 147,300 technologists and 107,700 scientists at the beginning of 1965–69, 149,300 scientists and 196,000 technologists at the beginning of 1970–74, and 206,000 scientists and 257,500 technologists at the beginning of 1975–79 postulated in the report unless there is a very early change in Government policy The universities must be given the tools as well as the time to do the job, and the question of quality is of increasing importance Prof M Stacey, of the University of Birmingham, who believes that the demand for chemists will remain high, has recently given some impressive figures of the existing strain on the capacity of the universities, and although the universities are prepared to take as many more entrants as possible without lowering standards, they are determined not to relax these standards

The report from the Appointments Board of the University of Birmingham for 1961 * suggests, however, that in the meantime the point when the output of graduate scientists exceeds demand may be nearer than that forecast by the Statistics Committee The report does not indeed refer to an actual shortage of vacancies for scientists, though it comments that some technologists may have to be prepared to take staff positions rather than the more usual apprenticeships Furthermore, it expresses the hope that more technologists will turn to teaching, either in schools or in higher technical education Some firms, however, found in October that they had fewer vacancies than were expected in the previous spring, and the Scientific Civil Service, the Atomic Energy Authority and the Patent Office all reported fewer vacancies for chemists than in previous years Demand for specialists, such as petroleum production engineers, has markedly fallen in consequence of many oil companies cutting down on exploration Here the Appointments Board suggests that, as they meet stiffer competition, oil and chemical companies are re-examining the staff structure, with a resultant decrease in vacancies for chemical engineers, in any event for the present

Nevertheless, the report notes a marked interest in flexible and adaptable scientists who are good at languages and of acceptable personality, since these can easily find posts on the commercial side of any industry interested in exports or the Common Market The Board's general impression, however, is that there are both fewer inquiries for graduates than there were in the previous year and also few vacancies in the organizations seeking recruits A report for 1961 from the Appointments Committee of the University of Glasgow †, however, contains no evidence in support of this, and in respect of the Women's Section states definitely that there has been no falling off in the number of vacancies notified The report seems somewhat preoccupied with whether or not the graduates find employment in Scotland, and refers to a continuing shortage of engineers of good quality and the desirability of shortening the training period where possible

If it be judged that these reports from university appointments committees or boards support the conclusions of the Committee on Scientific Manpower which have been held in some quarters to be unduly optimistic, it should be remembered, first, that the Committee made numerous reservations which may or may not be operative It was fully aware, for example, that the requirements of the Atomic Energy Authority were likely to contract Secondly, it should be remembered that in the Committee's view the possibility of a balance or even a surplus should be welcomed It should facilitate a rational use of the scientific disciplines, and it should mean that at last the Nation has a supply of qualified manpower with a scientific training for management, administration and the professions generally, in addition to those who hitherto have been drawn normally into vocational employment

That the Committee on Scientific Manpower is right in thinking that it will be to the benefit of the

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* University of Birmingham Annual Report of the Appointments Board for the year ending 31st December, 1961 Pp 6 (Birmingham University Appointments Board, 1962)

† University of Glasgow Appointments Committee Report for the year ended 31st December, 1961 Pp 20 (Glasgow University Appointments Committee, 1962)

Nation as an increasing proportion of those trained in specialized scientific disciplines find employment outside them is not to be doubted. Moreover, there is evidence in the Zuckerman Report on the management and control of research and development that that Committee also is thinking on the same lines (see *Nature*, **193**, 710, 1962). There are, however, two important points to be remembered if this situation arises. First, it will accentuate the need for some adjustments in the education of scientists and technologists, particularly in the direction of a broad and balanced education so as to encourage flexibility and avoid narrowness and rigidity.

The Committee on Scientific Manpower found some evidence that the universities are already thinking along such lines, and it is important that effective action should be taken before the expected surplus arises. On no account should the financial pressure which the Government has put on the universities be allowed to prevent such changes or developments. Secondly, however, and no less important, some changes are called for in the attitude of some of the professional associations.

It is clear from the Zuckerman Report and from what the Minister for Science has since said that changes are already contemplated in the Scientific Civil Service and the use made of scientists in the Government service which are both appropriate to the more effective use of trained manpower and in keeping with the situation visualized in the report on the *Long-term Demand for Scientific Manpower*. It follows that in such a situation it will not be so easy to equate supply and demand in terms of individual scientific disciplines. Demand must be interpreted with some elasticity as well as with judgment. Neither scientists nor technologists can expect to press for consideration for administrative appointments on equal terms with those qualified on other grounds and at the same time complain that their qualifications are wasted if they are expected to take up occupations in which the exercise of scientific skill as such is not a first requirement. Since the publication of the Zuckerman Report and the report on the *Long-term Demand for Scientific Manpower*, it is exactly this that some institutions and individual scientists seem inclined to do.

PROPERTIES AND STRUCTURE OF COAL

Coal. Typology—Chemistry—Physics—Constitution

By Prof. D. W. Van Krevelen. Pp. xviii+514. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1961.) 100*s*.

IN 1957 *Coal Science*, by D. W. van Krevelen and J. Schuyer, was published. While in a sense the present book is a new edition of the earlier one, it is much broader in approach, and more mature as a result of the great advances made in the past five years. van Krevelen has taken sole responsibility for authorship. Much of the content of *Coal Science* will be found in *Coal*, and a good deal more besides, but the weaknesses noted in the former (see *Nature*, 181, 4, 1958) have largely been eliminated. The strong theme that runs through the book and its planned integration make for interesting reading. The author propounds detailed views on coal constitution that explain qualitatively many of its properties, but several unsolved features of coal structure remain, some common to the many "amorphous brown substances" found in Nature. The achievement of a more-or-less satisfactory model structure for coal does not yet rule out the possibility of related, yet different, structures superseding it.

A good balance within the limits of space (the book is half as long again as the previous one) is reached between the following main topics: occurrence and systematics, geology and metamorphism, physical structure (151 pages), chemical reactions (153 pages), physical properties (123 pages), and coal constitution including the mechanism of carbonization (49 pages). The book incorporates a topical selection of new information and omits some that has been superseded, and overcomes difficulties always experienced in a work of this type, for example, the correct place in which to describe X-ray diffraction studies. By selecting for Parts 2–4 the titles "Coal Chemistry", "Coal Physics" and "Coal Constitution", respectively, a logical classification of subject-matter has been combined with an integrated discussion of the evidence, from whatever source, concerning a given molecular feature.

Part 1 consists of a straightforward and authoritative account of coals: their distribution and nature, their classification, and theories of their formation in both geological and chemical terms. The composition, properties and technological functions of macerals are fully considered, and there is a systematic and greatly improved account of porosity, internal surface and molecular sieve properties. In Part 2, on the chemical properties, functional groups are first considered; a somewhat optimistic impression is given of the quantitative agreement reached on hydroxyl contents, and there is an occasional ambiguity about references to carbonyl groups which relates to differences in spectra between the conjugated and unconjugated forms. A considerably improved and extended account of solvent extraction is given. The complex literature on hydrogenolysis and on oxidation by various reagents is admirably clarified and condensed, though necessarily with the omission of many references. This is followed by an account of methods for the investigation of properties important in coke-making, and of a mathematical model describing the observed changes, the skilful use made of graphs greatly helps the exposition.

When discussing the physics of coal in Part 3, the author is especially interested in the deduction of chemical parameters from physical properties by means of additivity principles (statistical constitution analysis). The section on X-ray diffraction has been considerably expanded and constitutes a connected and critical discussion of the available results. Optical properties, ultra-violet and infra-red absorption spectra are given adequate treatment. There follows a good account of conclusions from the high-resolution nuclear magnetic resonance spectra of soluble fractions of coal and the broad-line nuclear magnetic resonance spectra of solid coals that were available; important papers on these topics have appeared since the book was written. Mechanical properties are considered from the physical point of view Constitutional evidence from heat of combustion (Table 21, 2 *et seq*) has been superseded by conclusions reached in a very recent paper Part 3 concludes with sections on thermal properties

Part 4, on coal constitution, is also closely concerned with statistical constitution analysis, for macromolecular substances of non-uniform chemical structure this, with other physical methods, is a necessary field of study, since classical chemical methods are limited in application and interpretation, at least in respect of skeletal structure The summing-up in terms of values of the statistical parameters is unexpectedly disappointing For example, values of carbon aromaticity are basic, and quantitative estimates concerning other features of the molecule are sensitive to variations in them, the values selected range, in the rank group 80–90 per cent carbon content, from 0 8 to nearly 0 9, and I would not be alone in considering a radically different range, 0 65–0 75, as most compatible with up-to-date evidence Some values of other parameters that have been shown since writing to be incorrect have unavoidably been included in the summing-up In general, the values quoted are largely based on indirect methods

The last two chapters in Part 4 are concerned with the interesting and imaginative work of the author and the late Prof Waterman on condensation products of aromatic compounds with radioactively-labelled formaldehyde With the aid of these model compounds, the coking properties and decomposition yields with coal can be much better understood

The book concludes with an interesting and useful 13-page appendix "Historical Development of Fundamental Coal Research" In effect, this includes an up-to-date directory of coal research

The book gains in clarity from the adoption of a critically selective approach to the literature It is impossible to refer in detail to all errors noted, but very few are serious, and misprints are of minor importance The index of 17 pages seems to be adequate The good features of layout in the earlier work, *Coal Science*, have been retained, and the style is eminently logical and readable

Coal will provide both those concerned with the use of coal and new recruits to the study of coal science with a firm basis of fact and an excellent critical introduction to present-day thought on the subject Production is excellent and the price moderate The book is strongly to be recommended

I G C Dryden

PROBLEMS OF BLOOD TRANSFUSION IN JAPAN

Proceedings of the Eighth Congress of the International Society of Blood Transfusion

Tokyo, September 12–15, 1960 Edited by L Hollander (Bibliotheca Haematologica, Fasc 13) Pp xiv + 546 (Basle and New York S Karger, 1962) 85 Swiss francs

THE first international congress of blood transfusion to be held in the Far East was held in Tokyo in September 1960 All but two of the 125 papers delivered at this Eighth Congress of the International Society of Blood Transfusion are now published *in extenso* in a single volume of 546 pages

One of the pleasing things about this volume is that it is prefaced by two charming songs, in Japanese and with an English translation, entitled "On Blood Transfusion", by Her Imperial Majesty, Empress Nagako In how many other instances, one wonders, have the published proceedings of a scientific congress been graced in such a heart-warming manner? Noteworthy also is the fact that the contributors adopted a suggestion made by the President of the Society that the longer papers read should not exceed 2,000 words, and the shorter papers 1,000 words, when prepared for publication In consequence, this volume is not only less bulky to handle than that of many other proceedings, but also the salient facts are more readily assimilated by the reader

It is disturbing to note that blood for transfusion is supplied in Japan almost entirely by professional donors, many of whom are so poor that they donate blood to the commercially run blood banks when they lose their jobs or are faced with some urgent pressing need to obtain money The fact of rapid repeated blood donations has led to a dangerous increase in the number of persons no longer acceptable as donors because of anæmia In 1959, for example, 45 per cent of persons offering their services as professional donors had to be rejected as unacceptable on health grounds In reporting on this problem, Dr Y Azuma states that the Japanese Red Cross Society has earnestly appealed to the public to create and support a voluntary blood donor system, but that the response in 1960 still fell far short of the requirements of the hospitals

The proceedings of this congress contain the views held in 1960 by leading authorities in every continent of the world on many aspects of immunohæmatology and transfusion The nine sectional sub-headings include the transfusion problems of extracorporeal circulation, new aspects of blood group serology, studies of the serum groups such as the haptoglobins and transferrins, the preservation of blood cells, and problems of transfusion reactions This volume contains, therefore, much of interest and value to all concerned with blood transfusion, and the editor, Dr L Hollander, and the publishers are to be congratulated on the high standard of production

G H Tovey

VACUUM SCIENCE

Scientific Foundations of Vacuum Technique

By Saul Dushman Edited by J M Lafferty Second edition Pp xviii + 806 (New York and London John Wiley and Sons, Inc , 1962) 147*s*

SINCE 1949, when the first edition appeared, scientific workers all over the world have used "Dushman" as a valuable source-book However, the many advances of recent years have necessitated revision of this text Indeed, it highlights the rapid growth of vacuum science that, whereas the first edition was up to date, there is little or no mention in it of such recent innovations as the techniques of ultra-high vacuum, the getter-ion pumps Roots pumps, cryogenic pumping and alloy getters, while much progress has been made, for example, in studies of the interactions with solids of gases and gaseous ions of sorption and desorption, of diffusion processes, of measurements of partial pressure and of methods of detecting leaks All these topics and several more are included in this second edition

Dr. Dushman spent some thirty years compiling data on all aspects of the fundamentals of vacuum techniques. Now he has died it is unlikely that there is anywhere a successor with the breadth of knowledge and experience to undertake single-handed the writing of such a comprehensive treatise. Fortunately, the revision of this classic text has been entrusted to a team of eight specialists under the editorship of Dr. J. M. Lafferty, all of whom are members of the research staff of the General Electric Research Laboratory, Schenectady, N.Y., where Dushman was formerly assistant director. Sometimes books written by a team are badly co-ordinated and vexatious to the reader. Happily, this criticism certainly cannot be levelled at this second edition. The editor has most successfully woven the contributions of his team into the original text of Dushman to produce an up-to-date version which still retains the virtues of clear writing, comprehensiveness, full referencing and orderly development of the first edition.

As would be expected from its title, little appears in the book about applications. However, more detail of the scientific aspects of the design of very large vacuum plants would have been welcome. Again, stainless steel—increasingly used in modern ultra-high vacuum technology—receives little attention. Accounts of the kinetic theory are a little obscured by repetition of formulæ in various units, which would be preferably avoided by giving only basic formulæ and appropriate conversion tables.

But this is an excellent book. Dushman would have been proud of it. Like his own first edition, it will be indispensable for many years to come.

JOHN YARWOOD

MEASURING RADIOACTIVITY IN THE HUMAN BODY

Whole-Body Counting

Proceedings of the Symposium held by the International Atomic Energy Agency at the Neue Hofburg, Vienna, 12–16 June, 1961. Pp. viii+535. (Vienna: International Atomic Energy Agency; London: H.M. Stationery Office, 1962.) 60s.

THE whole-body counter is becoming increasingly important as a tool in health physics, to determine the amounts of radioactive materials which have entered the human body, generally as a result of an accident. It is, however, of greater interest than it appears to be at first sight; for example, its use can considerably extend many *in vivo* tracer experiments and the causes of obesity and similar disorders can be investigated by using it to determine the potassium-40 content of the body and hence the fat–lean ratio.

The earliest instruments of this type used ionization chambers, with low efficiency and poor geometry, but the development of the scintillation counter has brought tremendous advantages. Sodium iodide crystal counters have a higher γ-efficiency, and permit very good energy resolution for spectrometry but are restricted in size, thus limiting the solid angle. The geometry can be greatly improved by using plastic or liquid scintillators which are virtually unlimited in size, and a 4π counter using a liquid scintillator has already been constructed; these scintillators, however, have poor energy resolution.

Needless to say, the whole-body counter is very expensive, and considerable thought must be given to its siting, particularly in relation to the natural background and the type of shielding to be used. As a result, we are never likely to see large numbers of these instruments, but we should aim at making full use of those that are available, both for emergency measurements and for research.

Up to the present time, the method has been feasible only for measuring γ-emitters, but recent work in Sweden shows some promise of measuring body burdens of pure β-emitters by means of the associated bremsstrahlung. Another new development is the use of computer techniques for storage and processing of the data obtained in γ-spectrometry.

These are some of the problems discussed in detail in *Whole-Body Counting*. Those already in the field will probably be aware of progress elsewhere, but they will almost certainly find this book to be a useful survey. It will also be of considerable interest to health physicists, and to many users of radioisotopes who may well be surprised at the problems and possibilities of this type of instrument.

H. D. EVANS

ELECTRON MICROSCOPY

Traité de Microscopie Électronique

Sous la direction de Claude Magnan, avec la collaboration de Nina Carasso, Pierre Favard, Charles Fert, Angès Oberlin, Adrien Saulnier et Cyril Tchoubar. Tome 1. Pp. viii+1–650. Tome 2. Pp. viii+651–1301+33 planches. (Paris: Hermann, 1961.) 2 volumes 300 NF.

THINK of the most sumptuously produced scientific book you have ever seen—on any subject, animal, vegetable or mineral—double it, insert 33 full-page, glazed photographic prints, and you have the only complete treatise on electron microscopy in any language. The only comparable work has been *Electron Optics and the Electron Microscope* (1946) by a team colloquially known in the United States as 'We, the People'. But Zworykin, Morton, Ramberg, Hillier and Vance numbered only five and contained the subject within 760 pages, with very little on practical applications. The new French team of seven have need of almost twice the space, and at that, the electron optical part has been condensed into much less compass than the Americans allowed themselves; it is now the applications which have filled out to more than half the work.

Volume 1 describes the physical principles of electron microscopy and Volume 2 its applications. In addition to the basic optics, much attention is also directed to instrumentation—the theory of electron interaction with matter (including photography) as well as detailed descriptions of the many different types of electron microscope and their accessories. There are special chapters on the microscopy of solid surfaces, on electron interference effects and on electron diffraction, and on the aspects of crystallography with which these phenomena are concerned. Methods of specimen preparation are fully dealt with, and the first volume is completed by surveys of particle size and shape determinations and of physical and chemical transformations that can be followed by electron microscopy.

Volume 2 is devoted to the special preparation techniques for metallurgical and biological material, and surveys of the new knowledge which they have yielded about the very fine structure of biological tissues on one hand and of the texture of metals and alloys on the other The final chapter describes the role of electron microscopy in recent attempts at resolving the structural details of macromolecules, viruses and larger micro-organisms At the end of each chapter there is a list (usually very full) of references to original work, and the treatise has been given very extensive indexes by both author and subject

So far as random sampling could show, the treatment is comprehensive and thorough The French authors are all well known for their own contributions to the different fields on which they write, yet they take the wide view and do ample justice to work in other countries Inevitably, some aspects are stressed or neglected other than would have been done by a team under some other flag in Britain we should make more of metal physics, in the United States biological applications would loom even larger But the breadth and depth of electron microscopy are now so great that no country can be up on all the frontiers, and an editor has to pick the best team available Prof Magnan has done remarkably well in his task Anyone who has had to do with a composite work will know of the difficulty of collecting a team, ensuring reasonable uniformity of treatment and avoiding delay in publication This *Traité* appeared at the end of 1961, the references in most chapters are up to date to the end of 1960, and a few from 1961 are included

It is the only comprehensive, modern book on the subject and is certain to be the standard reference work for many years to come However, electron microscopy is still in rapid development, in techniques as well as applications, thin section-cutting in biology is barely ten years old, the preparation of electron-transparent films of metals only half this age Without in any way detracting from the magnificent achievement of our French colleagues, it may perhaps be queried whether so expensive a standard of production was really necessary Would it not have lowered the cost quite appreciably to use art paper only where needed for plates, and not for the whole text? We may be willing to pay £20 and more for an encyclopædia or art volume of permanent value, but no individual microscopist can face such a bill for a work that will need major revision within five years It is to be hoped, however, that libraries can be more open-handed, they may rest assured that the binding will stand up to the very heavy use any copy is certain to attract

V E COSSLETT

NEW METHODS FOR RAPID PUBLICATION

Proceedings of the Eighth Congress of the European Society of Hæmatology, Vienna, 1961

Part 1 Pp 1184 Part 2 Pp 1376 (Basel and New York S Karger, 1962) 172 Swiss francs the two parts

IT is usual to publish the Proceedings of international congresses, but during the past few years the advantages of this have been questioned The real benefit of congresses is to allow informal exchange of views between workers in allied fields who might otherwise never meet, for some participants can obtain a visa, or foreign exchange, only if their names appear on the programme as speakers It is difficult to exaggerate the value of these contacts, not only in the technical, but also in the social and political field, for, excepting the very carefully organized delegations which periodically appear, it is only during these congresses that ordinary workers from the two sides of the 'Iron Curtain' or the two sides of the Atlantic meet and talk freely

Although the reviews by famous speakers in plenary sessions are usually heard by a large audience and undergo simultaneous translation, original work by someone less well known is to be heard in a small lecture theatre by an audience of a dozen or so, half of whom have an imperfect understanding of the language used, and if the speaker is using a language not his own, the address may well be comprehensible to him alone This being so, publication of the Proceedings soon after the end of the congress is very desirable

However, in the past such publication has been slow, largely because very careful editing has been the rule, delays of a year or even eighteen months are usual, so that the authors of original work have already published it in the recognized journals—and indeed, it is quite impossible to give a detailed account of complicated experimental work in 1,000 words, which is as much as can be made comprehensible in the ten minutes the speakers are usually allowed

Messrs Karger have now cut the Gordian knot and produced the Proceedings only six months after the end of the eighth congress of the European Society of Hæmatology This feat has been accomplished by a radical re-organization of the editorial process—indeed, one might almost say by the abolition of the editor. Soon after the end of the Congress, all speakers received sheets of special paper on which to type the text of their contribution, after collation, these were photographically reduced on to offset plates and printed, special art paper being used wherever illustrations were required The results fully justify this departure from tradition, all the papers are reasonably legible, though some authors have submitted tables, graphs or line drawings so small that after reduction they are virtually illegible, should this process be used again this particular disadvantage may require special attention Those authors who use electrically operated typewriters have produced texts as legible as is type-set copy, but there are a few whose typists omitted the desirable cleansing of the type-face, with consequent reduction in legibility In fact, one of the most interesting things about these two volumes is the contrast in type-face, almost every type-face available on a typewriter is to be seen here, and one can see what a great increase in legibility has been obtained by using the new type-faces compared with the old and traditional styles, and how large a part is played by the spacing between letters

When 551 papers are contributed in 52 sections by about 800 authors, it is clear that they must represent a cross-section of present-day world opinion on the growing points of hæmatology, it is for this reason, rather than any single contribution, that one welcomes these volumes, even though at 285*s* they will be beyond the pocket of most individual contributors one would have hoped that at this price the publishers could have provided a proper binding instead of the thin card covering which they have used, but this is probably unavoidable with publication so quickly accomplished

G DISCOMBE

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

GOLDEN JUBILEE

By Prof. R. G. BASKETT, O.B.E.

Director

FIFTY years ago, with the aid of a grant from the Development Fund, a Dairy Research Institute was established at the then University College, Reading. The income of the Institute was of the order of £2,000 per annum and there were two members of staff, R. Stenhouse Williams, a research bacteriologist in dairying, and John Golding, a research chemist in dairying. The Institute was accommodated in the attics and cellar of a dwelling-house near the College. These were small beginnings, but the initial lack of facilities was balanced by the clear vision and energy of Stenhouse Williams. As a medical man he was aware of the high nutritional value of milk in the human diet, but he was equally aware of the bad conditions in which milk was produced on the farm and handled during distribution. The older generation of dairy farmers and dairy scientists will remember the almost missionary zeal with which Stenhouse Williams advocated the production of clean milk from healthy cows and will also remember the apathy and misunderstanding he had to combat.

The First World War inevitably slowed down the work of the Institute, but plans were laid for future development and after the War an appeal committee was formed under the chairmanship of the Earl of Iveagh. Funds were collected which, together with Development Fund grants and very generous help from Lord Iveagh, financed the purchase of the Shinfield Manor Estate. The old Manor House was suitable for conversion into laboratories, and the farm buildings and 350 acres of farmland provided facilities for field trials.

The government of the Institute was now delegated by Deed of Trust to a Board the members of which represented the College, the Ministry of Agriculture, the Ministry of Health, the Royal Agricultural Society of England, the National Farmers' Union, the British Dairy Farmers' Association and the staff of the Institute.

Thus, 1920 saw not only a new form of government for the Institute and new premises but also a change of name to the National Institute for Research in Dairying. By 1923 the Manor House had been converted into laboratories and the staff, numbering ten scientists, seven laboratory assistants, a librarian and a small secretarial staff, occupied their new home.

These were exciting years with so much worthwhile research waiting to be done against a background of financial stringency—a history common to most research organizations in the years between the Wars. Nevertheless, the Institute grew and embarked on a wider programme of research, but it was to suffer a sad blow in 1932 by the death of its first director, a man much beloved by his colleagues who, with them, had pioneered the application of science to the dairy industry.

Prof. H. D. Kay succeeded Dr. Stenhouse Williams and remained director of the Institute until he retired in 1958. It was under his guidance that the Institute grew to its present size and acquired new departments and laboratories which provided the scientific facilities essential for the very much expanded programme of research. Dr. A. T. R. Mattick, who for many years had been head of the Bacteriology Department and deputy director, succeeded Prof. Kay and was acting director until 1959 when I came to Shinfield as director[*]. The total staff of the Institute now numbered about 370 and included a scientific and experimental staff of 114. This staff was distributed over the departments of dairy husbandry, feeding and metabolism, physiology, radiobiochemistry, bacteriology, chemical microbiology, chemistry, physics, engineering and nutrition. There were also an experimental dairy, a statistics section and a unit concerned with liaison work with the dairy industry. The departments acted as hosts for about fifteen visiting workers from overseas. The Institute now farmed some 700 acres and even with this acreage had difficulty in providing sufficient dairy cattle for experimentation.

At the end of June, in celebration of the Jubilee, the Institute held two open days which were attended by some five thousand visitors. The staff of the ten departments staged more than eighty exhibits to illustrate the research on which they were engaged. Results of investigations relating to reproduction and lactation were shown and included those of workers in the Physiology Department who have made significant contributions to knowledge concerning the hormonal control of growth of the mammary gland and the formation and removal of milk. Exhibits from other departments covered work on the metabolism of synthetic œstrogens in ruminants and the counting and sizing of spermatozoa electronically.

Three departments staged exhibits on the formation of milk. That from the Chemistry Department dealt with the different routes by which the individual fatty acids are metabolized in the ruminant and how the end-products of their metabolism affect the rate of synthesis of milk constituents. The other two exhibits, one from the Radiobiochemistry Department and one from the Physiology Department, dealt respectively with studies in which radioisotopes were being used to determine the chemical form in which fat is transported to the udder and with research concerning some key enzymes involved in the control of mammary gland and liver metabolism.

The Departments of Dairy Husbandry, Nutrition, Chemistry, and Feeding and Metabolism staged ten exhibits which dealt with the results of experimental work on the feeding of dairy cattle. These ranged over calf-feeding trials, rumen development, development of the secretion of digestive enzymes in relation to utilization of nutrients by young calves and studies concerning the absorption of magnesium and calcium as affected by age. In recent years dairy farmers and others have been concerned by the variations occurring in the solids-not-fat fraction of cow's milk, and for several years the staff of the Dairy Husbandry Department have examined the effect of inherent and environmental factors and prepartum feeding on the solids-not-fat and fat contents of the milk from

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[*] See p. 1049 of this issue.

Fig. 1. The Manor House and Library at the National Institute for Research in Dairying, Shinfield, Reading

Friesian heifers. The results of these trials were demonstrated and had considerable interest for farmer visitors. Other exhibits dealt with requirements for energy and protein of growing heifers and factors affecting appetite.

For many years the staff of the Chemistry Department has been studying factors which affect the composition of cow's milk. More recently, the use of cows provided with rumen fistulæ has enabled quite rapid advances to be made in knowledge concerning the role of ruminal fatty acids in relation to milk composition, and these observations, together with the results of earlier experiments concerning diet and composition, were used to demonstrate how these findings may in time have a practical application in the formulation of feed mixtures. The same Department staged an exhibit based on work concerning genetic variations in milk proteins in the cow and related species and changes in protein distribution induced by stage of lactation.

Great interest was displayed in the prototype of an infra-red milk analyser designed for the automatic analysis of milk. This apparatus, developed by the Physics Department, is capable of determining fat, protein, lactose and solids-not-fat in a small milk sample in less than 1 min.

The Institute has made full use of its experimental dairy husbandry unit in developing and testing milking and management techniques, and visitors were able to see demonstrations relating to pipeline milking in parlour and cowshed, circulation cleaning, machine stripping, slatted floors and hygiene control of mastitis.

Staff in the Bacteriology Department staged exhibits to illustrate methods of evaluating disinfectants and bacteriological techniques used in mastitis investigations. Other exhibits dealt with the influence of different species of bacteria on cheese flavour and an attempt to relate flavour assessment by a tasting panel to the results obtained by an examination involving gas chromatography. Further demonstrations dealt with work relating to brown discoloration in Stilton cheese and the isolation of nisin and its characterization and practical application.

The staff of the Physics Department, in addition to demonstrating the infra-red milk analyser, showed apparatus which had been developed in the department for measuring the spreadability of butter and the results of work on the determination of the activity and purity of commercial rennets. A modified torsiometer for use in a cheese vat in measuring the 'firming up' of curd was also demonstrated.

The staff of the Nutrition Department, in addition to its interest in animal feeding and production, has been concerned with the assessment of the nutritive value of milk and other foods. Microbiological assay techniques have been developed for measuring vitamins and protein quality and these were demonstrated to visitors. In addition, the results were shown from comparisons which are being made of the nutritive value as between milk fat and various vegetable oils using rabbits and chicks as the experimental animals. A very extensive survey has been carried out in the Nutrition Department of the variations in vitamin A, carotene and tocopherol in milk fat throughout the year and scientific visitors were particularly interested in a newly adapted laboratory which has been air-conditioned for work involving the use of dangerous solvents.

Although the Institute is concerned mainly with the production and utilization of milk and milk products, a considerable amount of work has been done on pig nutrition, and the present work in the experimental piggeries was demonstrated and a display made of some practical applications of recent research findings in pig nutrition.

Recently, staff in the Nutrition and Bacteriology Departments have been using techniques involving the production of germ-free chicks. After preliminary work, a special unit has been built to house the 'germ-free' work, and it is hoped that these new facilities will enable staff to tackle further work on the role of micro-organisms in the nutrition of animals.

The staff of the Engineering Department design and manufacture many of the specialized instruments and pieces of equipment used in other departments, and specimens were on view. They also co-operate in many research projects, for example, in investigations of the spoilage of heat-treated milk and in problems associated with ultra-high-temperature sterilization of milk and the aseptic filling of this product into bottles.

Many departments in the Institute use the facilities of the Experimental Dairy. The staff of the Dairy, in collaboration with the Engineering Department, have developed an apparatus for enabling the continuous cheddaring of curd in the manufacture of Cheddar cheese. This apparatus was on view. Another process concerned with the continuous preparation of cheese curd has been developed by the staff of the Chemical Microbiology Department, and a film could be seen by visitors illustrating the work which has so far been done in connexion with the process.

The Institute possesses an excellent library which houses probably the largest collection of dairy literature in the world. It was erected as a memorial to Stenhouse Williams.

The demonstrations to visitors on these open days illustrate the distance which has been travelled since the early days when the staff of the Institute could be counted on the fingers of one hand. At this milestone in the history of the Institute it is only right to pay tribute to those who had the courage and vision to direct and carry out the research for which the Institute is held in such high regard the world over.

CÆSIUM-134 IN MAN

By KURT LIDÉN

Radiation Physics Department, University of Lund, Sweden

AND

INGVAR Ö. ANDERSSON

AB Atomenergi, Stockholm, Sweden

After the discovery of the high cæsium-137 body burden of Swedish Lapps in the beginning of 1961[1], measurements of larger groups of Lapps and other people on a similar diet have been performed both in Sweden and Finland. In August 1961, twelve Swedish Lapps were measured in the whole-body counter of AB Atomenergi, Stockholm[2]. With a small semi-portable whole-body counter, built in the Department of Radiation Physics, University of Lund, 160 persons were examined at Jokkmokk (66·6° N., 19·9° E.) in Sweden in September[3] and 190 persons at Inari (68·9° N., 27·0° E.) in Finland in October 1961[4]. In all these cases the whole-body γ-ray spectra contain the same peak or bump at 800 keV. as discussed in ref. 1. In Fig. 1 the γ-ray spectrum of subject L. B. measured at AB Atomenergi on August 16, 1961, with an NaI(Tl) crystal 4 in. in diameter and 4 in. thick clearly demonstrates a well-resolved peak at that energy. The net sum spectrum of 52 Finnish Lapps, each measured for 10 min. at Inari during October 20–24, 1961, is shown in Fig. 2. Also in this case the 800-keV. peak is present; the crystal size was 5 in. in diameter and 4 in. thick.

As the 800-keV. peak is not found in the γ-ray spectra of Swedes from the Stockholm and Lund areas at 59·3° N., 18·0° E. and 55·7° N., 13·2° E. respectively, it could be assumed that there was a relation between this peak and the increased cæsium 137 level in people on a reindeer meat diet in northern Sweden. Measurements at the Department of Radiation Physics during March–May 1961 of 3 Lapps by means of a 5 in. by 4 in. crystal showed that the mean ratio of the number of pulses in the full (extrapolated) 800-keV. photopeak and the full cæsium-137 photo peak was (1·65 ± 0·2) : 100. In reindeer meat, measured on March 11, 1961, the ratio was (1·42 ± 0·07) : 100. A study of 12 Lapps at AB Atomenergi in August 1961 gave a mean ratio of (1·2 ± 0·1) : 100 and no significant deviation between different human subjects. In Fig. 3 we have given the net γ-ray spectrum of reindeer meat extract obtained at AB Atomenergi on October 20, 1961; the potassium-40 contribution is subtracted. In this case the ratio is (1·10 ± 0·05) : 100. Thus in both series of measurements the 800-keV. peak to cæsium-137 ratio has the same value in meat and man within the statistical limits.

At AB Atomenergi an attempt was then made to determine the half-life of the 800 keV. peak[5]. 3 kgm. of reindeer meat was treated with concentrated nitric acid + 5 per cent sulphuric acid for about 15 hr., during the first 2 hr. at slightly increased temperature (50° C.). The fat floating on the surface was removed by filtering. The solution was then boiled until a black viscous liquid remained. It was treated with 30 per cent hydrogen peroxide at about 50° C. giving a colourless liquid. Water was added so that a sample of 1 litre of solution was obtained. A solid deposition remained at the bottom of the container. The sample was measured in an accurately defined geometry with a 4 in. by 4 in. NaI(Tl) scintillation spectrometer. The measurement was repeated after 130 days. The half-life was calculated both from the number of pulses in the 800-keV. peak and from the ratio of the number of pulses in the 800-keV. peak and the cæsium-137 peak. In the second case a correction has been made for the decay of cæsium-137. The average value of the half-life obtained from the experimental results is 2·4 ± 0·5 years.

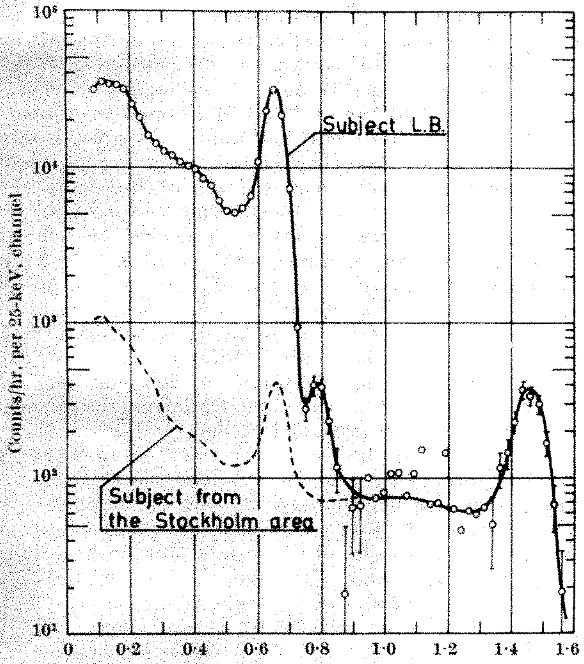

Fig. 1. Whole-body γ-ray spectrum of subject L. B. Cæsium-137 burden 0·60 μc.

When the 800-keV. component was found at the Department of Radiation Physics in March 1961, cæsium-134 was the first tentative interpretation of its origin but this hypothesis was rejected[1], since there was no known yield of cæsium-134 in nuclear weapons fission. However, results obtained from further investigations of the 800-keV. component, in the food-chain lichens–reindeer–man, strongly supported the first theory. A more direct approach in order to prove the theory was then made at the Department of Radiation Physics. The level scheme of cæsium-134, recently revised[6], shows that the most intense γ-ray lines at 605 and 797 keV. are emitted in cascade (Fig. 4). An attempt was then made to study the γ-ray spectrum of reindeer meat by a γ–γ coincidence technique. A 75-gm. sample of vacuum-dried meat, corresponding to 240 gm. fresh weight, in a cylindrical container 9 cm. in diameter by 1·5 cm. in height was placed between two crystals of NaI(Tl) 3 in. in diameter and 2 in. thick. One was connected to an integral discriminator (channel I) and the other to a 200-channel transistorized pulse amplitude analyser (channel II). Pulses in channel I corresponding to 530 keV. or more opened the gate of channel II. This coincidence system had a resolving time of 1 μsec. Two successful long-term runs were made, one at 45 hr. and one at 20 hr. The 45-hr. coincidence spectrum, corrected for background and potassium-40 contribution is plotted in Fig. 5. It shows two distinct peaks at 600 keV. and 800 keV. The increased counting-rate at lower energies is caused mainly by various Compton events. The random coincidence-rate is negligible even at the position of the cæsium-137 peak. A test with a cæsium-134 sample of the same size and density as the meat sample resulted in a coincidence spectrum of the same shape as for the meat.

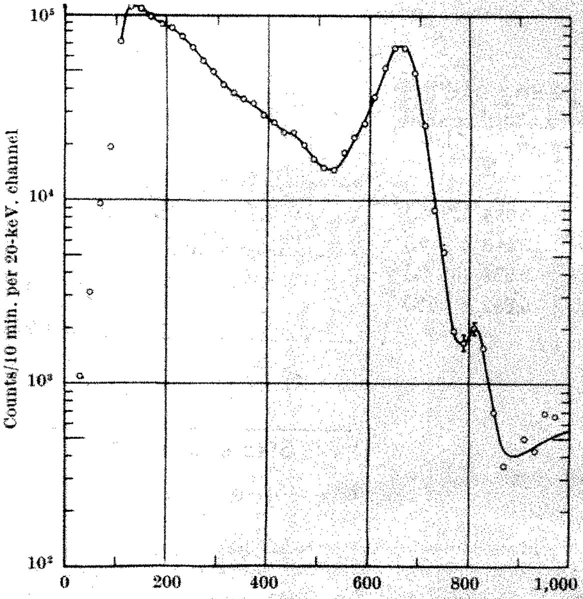

Fig. 2. Net sum spectrum of 52 Finnish Lapps. Average cæsium-137 burden 0·20 μc.

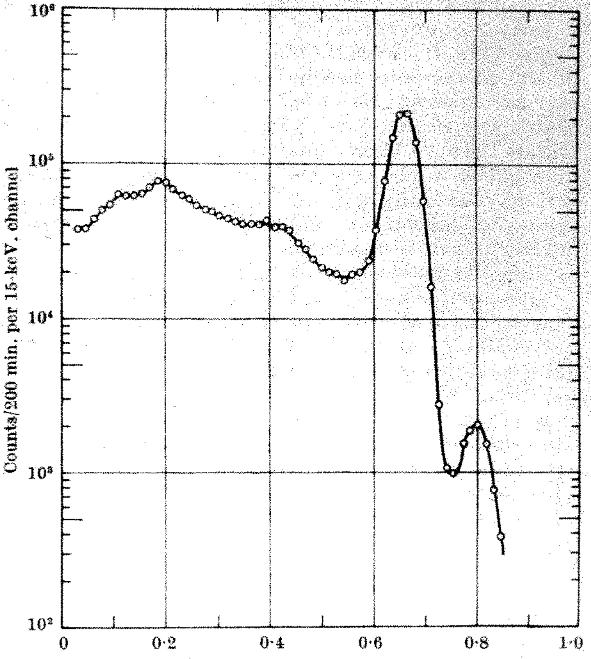

Fig. 3. γ-Ray spectrum of reindeer meat extract. Potassium-40 contribution is subtracted

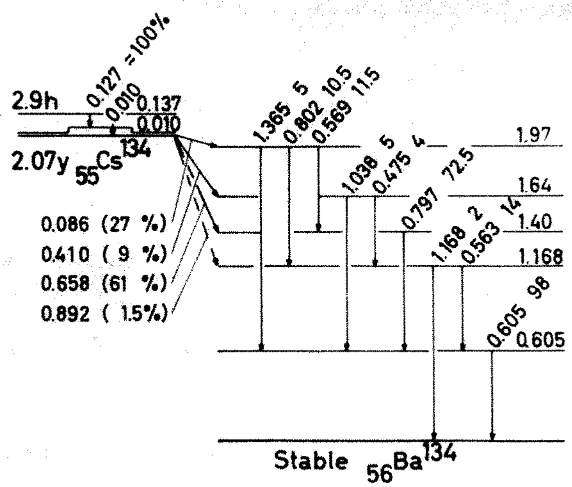

Fig. 4. Level scheme of cæsium-134

Thus these investigations have given us a knowledge of: (1) the energy of the 800-keV. peak; (2) its half-life of 2·4 ± 0·5 years, compared with 2·07 ± 0·02 years for cæsium-134 (ref. 7); (3) the presence of a 600-keV. γ-ray peak in coincidence with the 800-keV. peak. After correction for crystal efficiency the intensity ratio for these two peaks was found to be 10 : 6, in accordance with the level scheme for cæsium-134 from which the ratio 100 : 67 for the two groups 563 + 569 + 605 and 797 + 802 keV. is obtained (Fig. 4); (4) a metabolic behaviour of the 800-keV. component in reindeer meat and man very similar to that of cæsium-137.

That the origin of the 800-keV. peak in man's γ-ray spectrum is a cæsium-134 contamination of human tissue thus seems to be proved.

The origin of cæsium-134 in the biosphere is not known. There seem to be no figures in the literature concerning a direct cæsium-134 yield in fission. Indirect formation in the 134 mass chain by successive β-decay is prevented by the existence of stable xenon-134. Baerg *et al.*[8] found the independent yield of the metastable cæsium-134, half-life 2·9 hr., in thermal neutron fission of uranium-235 to be less than 1·4 × 10^{-5} per cent. From the measurements of March 1961 we have found the cæsium-134 activity in reindeer to be 1·6 per cent of the cæsium-137 activity assuming a total intensity of 0·83 photon per disintegration for the 800-keV. lines and correcting for crystal efficiency. This corresponds to a cæsium-134 yield of about 0·01 per cent.

An observation of cæsium-134 in soil samples taken close to the ground zero after an atomic balloon shot in Nevada is reported[9]. However, only a very slight indication of cæsium-137 was found, which is rather puzzling. Neutron-induction processes are suggested as a tentative interpretation in this case.

Cæsium-134 is produced in reactors by neutron activation of the stable fission product cæsium-133. There is no direct yield of cæsium-133, but it is created by the decay of other fission products. As this build-up of cæsium-133 is slow, it is not possible to interpret the biospheric cæsium-134 as a result of such an activation process in weapons tests. As cæsium-134 is produced in reactors, reactor release or reactor accidents might be the origin of this isotope in the biosphere. The isotopic composition of the fission product elements has been calculated for different irradiation times and three thermal neutron fluxes 10^{12}, 10^{13} and 10^{14} n./cm.²/sec. (ref. 10). If uranium-235 were irradiated at a neutron flux of 10^{13} for 6 months or at 10^{14} for 1 month the cæsium-134/cæsium-137 activity ratio would be about 1 : 3.

The Windscale accident in October 1957 was in fact detected in Sweden[11] and Norway[12]. A relatively heavy cæsium-137 fall-out was found at Bergen, Norway (60·4° N., 5·3° E.), during the period September–October 1957, which could be due to the Windscale accident. From that observation it can be estimated that about 10 per cent of the cumulative cæsium-137 fall-out up to August 1961 could be from that accident. If we assume that the cæsium-134/cæsium-137 activity ratio in the cæsium release from Windscale was 1 : 3, that ratio in the cumulative cæsium fall-out in August 1961 should be 0·8 : 100, a value which is the same, within a factor of two, as the ratio reported here. However, it should be emphasized that this is only a rough estimate. If the cæsium-134/cæsium-137 activity ratio in the Windscale reactor had been 0·05 as mentioned by Wheatley[13] our hypothesis will not hold. We would only wish to show that the release of activities from reactors has to be considered when discussing the possible origin of the biospheric cæsium-134. It remains to investigate other processes in nuclear weapons tests, for example, cæsium-134 fast neutron fission yield, multiple neutron capture, activation of non-fissionable material, etc.

Investigations of the ratio cæsium-134/cæsium-137 in biospheric samples from different parts of the world collected before September 1, 1961, are now in progress at the Department of Radiation Physics in order to check the proposed theory.

The radiation dose to man from cæsium-134 is small. From the newly proposed level scheme (Fig. 4) it can be calculated, using the proper geometric factor[14], that a uniform concentration of 1 μc. cæsium-134/gm. of tissue in a 70-kgm. standard man gives an average radiation dose of 1·43 rads/hr. (from β-rays 0·33 and from γ-rays 1·10 rads/hr.). For cæsium-137 the corresponding value is 0·91 rad/hr. (0·51 and 0·40). Thus, in this case the dose contribution from cæsium-134 is 1·6 times larger than from cæsium-137 per unit activity. In the case of Lapps with a total body burden of 0·5 μc. cæsium-137, which is not unusual, the radiation dose from cæsium-

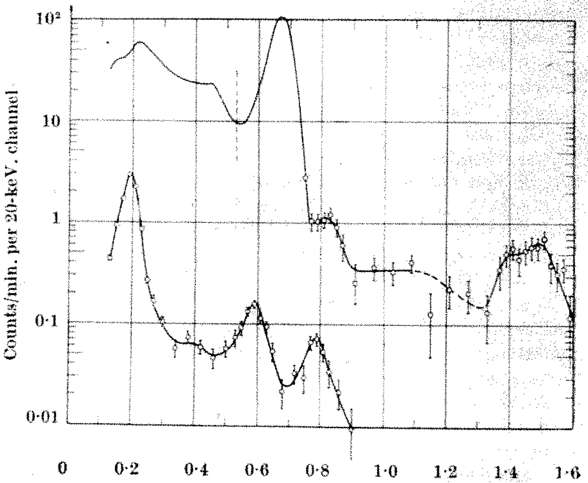

Fig. 5. γ–γ Coincidence net spectrum of a reindeer meat sample (lower curve, 45-hr. run) and a single crystal spectrum of the same sample in the same geometry (upper curve, 100-min. run)

137 is 57 mrad/year. An average cæsium-134 content of 1·5 per cent of the cæsium-137 level then contributes 1·3 mrad/year or approximately 2 per cent of the total radiocæsium irradiation. It should be added that the ICRP method[15] used in dose calculations for internal γ-ray radiation results in a 50 per cent higher dose than we have found for a standard man of 70 kgm., due to their approximation of the body as a sphere with 30-cm. radius.

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AN EVALUATION OF CONTROLLED-TEMPERATURE ENVIRONMENTS FOR PLANT-GROWTH INVESTIGATIONS

By Dr. A. JOFFE*

Division of Crops and Pastures, Department of Agricultural Technical Services,
Pretoria, South Africa

A DOZEN years have elapsed since the first large-scale controlled environment facility for plant growth was erected at the California Institute of Technology, Pasadena. Interest in the use of this type of facility (a so-called 'phytotron') has increased rapidly in many countries, and there are at present more than a dozen phytotrons in the world, while the number of additional facilities of one type or another must now number several hundred.

At approximately the same time as the Pasadena phytotron was erected, the late Prof. M. G. Mes was responsible for the construction of a smaller and somewhat different installation at the University of Pretoria, South Africa. After twelve years of use, certain fundamental principles of design and operation have become apparent, and in view of the ever-increasing demand for controlled-environment facilities, which are necessarily expensive, it appears timely to evaluate the validity of some of the assumptions which seem to have been tacitly accepted.

The present article will be mainly limited to the fundamental aspect of temperature control. The attainment of reliable temperature control in the dark is relatively simple, the main problem undoubtedly occurring under conditions of high light intensity, especially in glasshouses, but also in growth rooms where radiation effects have to be considered. It is presumed that the usual object of a glasshouse is to grow plants under conditions of high light intensity and as near natural light quality as possible. Thus, the use of shading or other devices that impede the entry of light is not considered.

Some of the engineering problems of temperature regulation in a glasshouse have been discussed by Went[1], who has also pointed out that although it would be desirable to control accurately the temperature of the whole plant, it is impossible to produce an even temperature throughout a land plant exposed to light. He states further that "in general, leaf temperatures are rather close to air temperature, because on the one hand radiation and transpiration hold each other more or less in check, and because on the other hand the leaves are so thin that through conduction and convection they exchange heat easily with the surrounding air".

The heat-transfer principles involved are fundamental to the whole concept of temperature-controlled facilities, and the view expressed here does not appear to be in harmony with the numerous investigations on leaf temperatures and transpiration which have been published since the appearance of Curtis's[2] analysis of the problem 25 years ago. Leaf temperatures between 5° and 10° C, or more, above ambient temperature have been consistently reported for thin-leaved mesophytes in sunlight in widely differing latitudes and under various conditions. Far higher values have been recorded in the case of succulent plants and thick plant organs. If temperature differences of the same order exist between plant and air in a phytotron, they can scarcely be regarded as insignificant.

So far as can be ascertained, no work has been published to date on the temperatures of plants or other objects in a so-called, constant-temperature glasshouse. Measurements obtained in the Pretoria glasshouses over a number of years, using 40-gauge thermocouples, have shown that the temperature of leaves of various thin-leaved mesophytes supplied with adequate water was consistently above the ambient temperature during high-radiation periods. Values of +3° to +10° C have been obtained for more than ten species when the leaves were horizontally exposed to insolation values of approximately 1·0 cal cm^{-2} min^{-1}, a wind speed of 80–120 f.p.m., wind direction obliquely from above, and relative humidities of 30–75 per cent at a dry-bulb temperature of 22° C. Though this radiation value is high, it may undoubtedly be encountered in glasshouses in many countries of the world. The wind speed of approximately 100 f.p.m. appears, on the other hand, to be somewhat higher than that prevailing in most research glasshouses.

Temperature measurements of sand and other objects are presented in Fig. 1 and Table 1, and from these it is evident that the actual temperature of objects in a glasshouse may bear little resemblance to the air temperature. Root medium temperatures

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* Present address: Margaretha Mes Institute for Plant Physiology and Biochemistry, University of Pretoria.

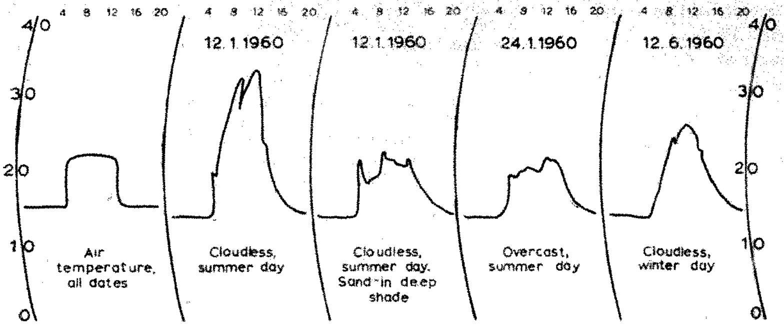

Fig. 1. Extracts from typical distance thermograph records of river sand in asbestos flats, 39 × 45 × 12·5 cm deep, in an air-temperature-controlled glasshouse. Thermograph elements 4 cm below sand surface. Water at 24° C applied at 8.00, 12.00 and 16.00 h in summer, and at 18° C applied at 12.00 and 16.00 h in winter. Wind speed, 100 f.p.m., direction obliquely from above

may be more than 11° C higher on a clear summer day than on an overcast day in a constant-air-temperature glasshouse (Fig. 1).

Certain controlled temperature experiments have been conducted in the glasshouses at this Institute in summer and have been repeated as exactly as possible in the winter: significantly different results were obtained. An analysis of the results suggested that one of the main variables was plant, and especially root, temperature rather than light intensity or quality.

Raschke[3] has recently stated "that it is surprising that the botanist neglects the significance of the infra-red radiation exchange; radiation laws have been known for 80 years and such contributions as Curtis's provide the proper emphasis". Curtis[2] had expressed the view as early as 1936 that, for interpretation of certain physiological experiments, air temperature readings alone may be highly misleading. Yet with the advent of highly efficient control equipment for air-conditioning purposes, the assumption is usually made that, once air temperature is controlled, everything else will look after itself. (The reliable control of air temperature is, in itself, not always simple under conditions of high insolation especially when low ventilation-rates are utilized. The air temperature in, for example, a return air-duct, where it is sometimes monitored, will not necessarily be the same as in the vicinity of the plants. Ideally, the reference air temperature should be that of the plant-growing space, this in fact being possible in practice.)

This approach is evidenced by the principles of design and construction sometimes used in phytotrons. In discussing these principles, it is necessary to stress that, for a given radiation-level and ambient temperature, the most important external and readily controllable factor affecting the temperature of plants and other objects is the rate of ventilation. The effect of wind speed on various objects exposed to high radiation in a glasshouse is presented in Fig. 2. The exponential nature of the curves has a great bearing on the thermodynamic and aerodynamic principles involved in the successful air-conditioning of a phytotron. It should be noted that, unlike the evaporimeter, the temperature of leaves and certain other objects approaches asymptotically the air temperature with increasing wind speed.

Table 1. SURFACE TEMPERATURES OF OBJECTS IN AN AIR-TEMPERATURE-CONTROLLED GLASSHOUSE MAINTAINED AT 22° C

Object	Surface temperature (°C)	Increase above ambient (°C)
River sand, wet	29	+ 7
River sand, dry	46	+24
Red loam soil, wet	31	+ 9
Red loam soil, dry	48	+26
Cotton wool, dull white	36	+14
Cotton wool, red	47	+25
Water-culture, in 1 l. white container	27	+ 5
Black seedcoat of *Phaseolus* sp. in moist, closed glass Petri dish	53	+31

Readings obtained with 40-gauge thermocouples under following approximate conditions: radiation, 1·0 cal cm^{-2} min^{-1}; wind speed, 100 f.p.m., direction obliquely from above; relative humidity, 50 per cent.

The usual method of air injection in most phytotrons appears to be from a perforated or slotted floor. It has been claimed that this system produces a uniform temperature régime in the glasshouse or growth room. With a carefully designed distribution system this is possible provided the chamber is not utilized for plant-growth purposes. As soon as plant containers standing on trolleys are introduced into the chamber the entire air-flow distribution pattern undergoes a radical change: high resistances to air flow are introduced and the ventilating air is channelled into passages between the trolleys. Thus, the wind speed is far higher in the non-growing spaces than in the space above the plants. This results in a zone of relatively stagnant air where the effect of radiation will be at a maximum. Of equal importance is the effect of this system on the microclimate immediately above an individual plant container; when air is passed in an upward direction between containers, a non-uniform flow pattern exists in the zone above the pots. This is due to the back-flow of air above the soil surface, resulting in the formation of vortices and 'dead-spot' areas.

The degree of inefficiency of the slotted floor ventilation system will depend on the size and spacing of the containers. However, even with liberal spacing, the position is aggravated by the presence of overhanging and intertwining foliage which is sometimes unavoidable. The cost of controlled environment space on a unit area basis is normally too high to permit the luxury of isolated plant containers, even if the 'dead zones' above the containers could be eliminated.

If the effect of incoming radiation on plant and soil temperatures is recognized and the nature of the effect of increasing wind speed on the heat balance is considered, one must come to the conclusion that the attainment of greatest uniformity of air, plant and soil temperatures is only possible if a relatively high wind speed and maximum uniformity of air movement in the correct direction are present.

The most satisfactory method of accomplishing this in a glasshouse or growth room appears to be the use of one or other lateral overhead distribution system. If the air distribution equipment is located at the maximum height on one of the long walls of a correctly oriented glasshouse, that is, the long axis east–west, then shadow effects are largely or entirely eliminated. Provided the width of the glasshouse does not exceed certain limits, air distribution is satisfactory with injection from one side only[4].

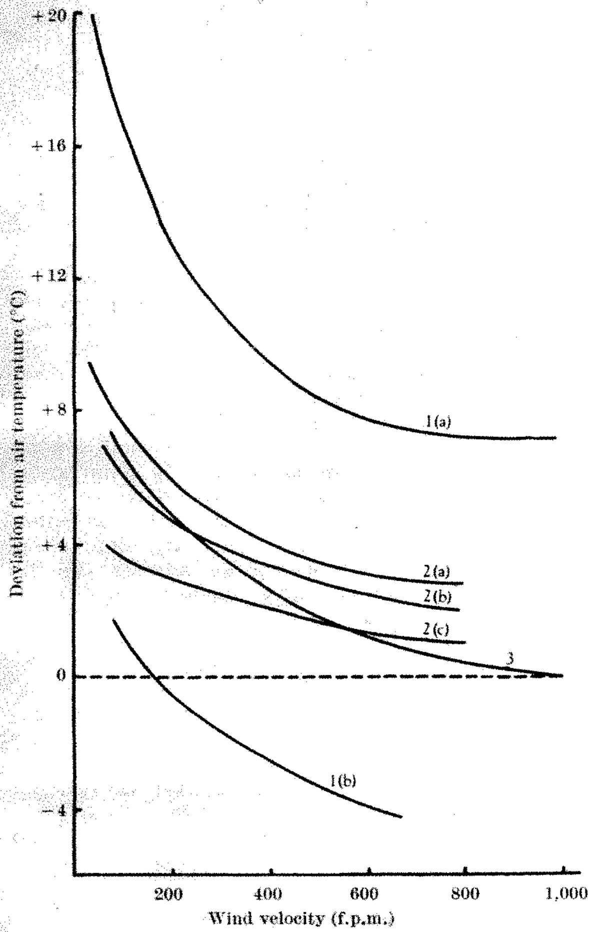

Fig. 2. Effect of wind speed on the temperature of objects in a glasshouse maintained at an air temperature of 22° C. Radiation, 1·1 cal cm^{-2} min^{-1}; humidity, approximately 50 per cent; wind direction, obliquely from above

1, green cloth, 3·5 cm square, (*a*) dry, (*b*) wet; 2, leaf temperatures, (*a*) *Fuchsia* sp., (*b*) *Fragaria* sp., (*c*) *Lotus corniculatus*, L.; 3, surface of wet river sand

Two methods of air distribution incorporating these principles have been used: (*a*) from one or more separate fans, usually in conjunction with evaporator coils and heating elements; or (*b*) by means of a continuous distribution duct with discharge slots, the duct being so designed as to produce a constant static pressure along its length. The advantage of the latter system lies in the fact that differences in wind speed and consequently temperature are at a minimum in the controlled area. These systems operate on the principle that relatively high-velocity jets of air are discharged in the unoccupied space above the plants in one direction, while air movement in the occupied space occurs in the opposite direction. Mixing of the incoming conditioning air occurs in the unoccupied space of the chamber and, in general, wind movement in the plant growing zone is predominantly lateral, but movement in the vertical direction is also present because of air entrainment. By using correctly chosen air velocities and slot areas to produce the desired entrainment ratios and terminal velocities, it has been shown that a high degree of uniformity with regard to wind speed and air temperature can be attained[4], thereby promoting uniform plant and soil temperatures. It must be stressed, however, that perfect conditions can really only be expected in a wind tunnel, and even then the presence of tall plants on the upstream side of shorter plants may create imperfect conditions.

It has been suggested that, because radiation effects are in any event present in Nature, it may as well be left that way in the phytotron. However, the main object of a phytotron does not appear to be to reproduce Nature but rather to obtain as complete control of the environment as possible, one of the objects being the reproduction of experimental conditions in time and in space both within and between chambers and even between institutes.

In the case of temperature control under high or variable radiation-levels, especially in a glasshouse, this is not entirely possible with any system. By making use of existing knowledge of heat-transfer principles it is, however, possible to reduce aberrations. From the nature of the curves presented in Fig. 2 it is evident that by simply using a uniform ventilation-rate of approximately 250–300 f.p.m. over plant and soil surfaces, the effect of radiation is considerably reduced (but not eliminated); wind speeds above this value become proportionately less effective for cooling purposes. Under conditions of adequate water supply, this wind speed is neither harmful to plant growth[5] nor difficult to attain in practice and ensures a satisfactory supply of carbon dioxide to plants for a given number of air changes per unit time.

In view of the nature of the heat transfer processes, one may well ask what purpose is served by installing expensive and highly accurate equipment under variable or high-radiation conditions. In the case of a glasshouse, the presence of a shadow from the structure or a passing cloud can considerably change the temperature of objects in a very short while. Even under constant insolation, temperature differences exist due to, for example, self-shading of leaves, partial shading of the soil and variations in long-wave radiation from other objects. Under conditions of net radiation loss, plant and soil temperatures will often be below ambient temperature (Fig. 1). These facts also serve to indicate the desirability of properly controlled root temperature facilities, independently from aerial temperature control and protected from radiation effects[6]. It is also evident that morphological and anatomical properties of a plant may influence not only the volume of the total heat exchange but also the predominance of one or other heat transfer process[3].

These facts show how pointless it really is to place a literal meaning on the usual statements that a phytotron experiment was conducted at a particular temperature plus or minus a negligible deviation.

Fortunately, radiation effects on objects in the phytotron are virtually independent of the air temperature, while the other components of the energy exchange are only relatively slightly affected by ambient temperature. Valuable comparative results of the influence of temperature can be, and have already been, obtained from phytotron experiments, and this should serve as an incentive to study in detail the many aspects of the control of the environment in modern installations.

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EXPERIMENTAL FOLDING IN ROCKS

By Dr. M. S. PATERSON and Dr. L. E. WEISS*

Department of Geophysics, Australian National University, Canberra

ONE of the most common features of bedded or foliated rocks seen in the field is the folding of surfaces which are presumed to have been initially plane. This folding may have arisen in a number of ways, depending on the circumstances, and it has been the subject of many theoretical and experimental investigations. Theoretical studies have been concerned mainly with the buckling of layers embedded in material of different properties (for example, Biot[1]). Most experimental investigations have aimed at simulating folding, using model materials such as wax, putty or 'Plasticine' (for example, Ramberg[2]). However, folding has not hitherto been produced experimentally in rocks except in a slight degree (ref. 3, Fig. 44).

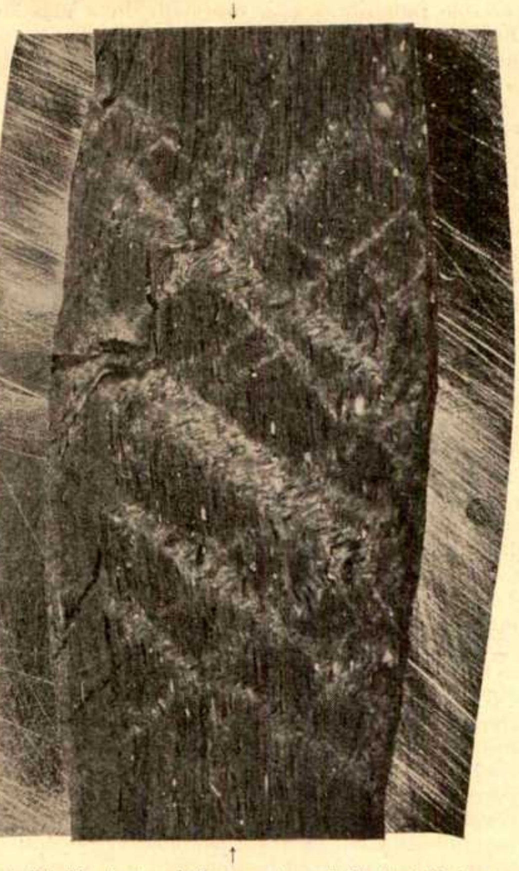

Fig. 1. Phyllite shortened 13 per cent parallel to the foliation in thick brass jacket, showing development of kinking. (Polished section, × c. 6.) (In all figures, arrows indicate direction of differential load)

Field study suggests that folding often involves flexural slip on the folded surfaces, a process analogous to bend gliding or kinking in crystals. Such behaviour is believed to be characteristic of the deformation of rocks such as slates, phyllites and mica schists which are already strongly foliated. We give here a preliminary account of experiments on a fine-grained phyllite and a mica schist in which prominent kinking and folding by flexural slip have been achieved, leading to microstructures that are strikingly similar geometrically to many structures observed in the field in such rocks.

The phyllite consists mainly of chlorite and sericite, with some fine quartzose layers and, in some specimens, an appreciable number of relatively large clastic quartz grains. Cylindrical specimens 7 mm. in diameter and 10 or 20 mm. long have been cut at angles of 0, 10°, 25° and 45° to the foliation. In the main series of experiments, the specimens have been sealed into thick brass or copper jackets of 10 mm. outside diameter.

The deformation experiments have been carried out at room temperature and at a confining pressure of 5,000 kgm. cm.$^{-2}$. The specimens were compressed between parallel platens, without a spherical seat, at a rate of shortening of the order of 1 mm. per min. The total strains corresponded to relative changes in length ranging from 5 to 80 per cent.

The following are the main observations on the phyllite specimens which were jacketed in thick brass or copper:

(1) Except in the most highly strained specimens, there is no substantial dimensional change in the cross-sectional direction parallel to the foliation. Therefore, since the behaviour remains elastic in this direction while large permanent changes in length occur in other directions, the rock is markedly anisotropic mechanically. This anisotropy is a reflexion of the dominant role of gliding or slip on the foliation as the mechanism of deformation.

(2) At small strains, narrow bands of kink folding appear at approximately 50° to the direction of shortening. In the specimens compressed parallel to the foliation, the kink bands are in symmetrically oriented conjugate sets (Fig. 1), and where the foliation crosses successively two conjugate kink bands

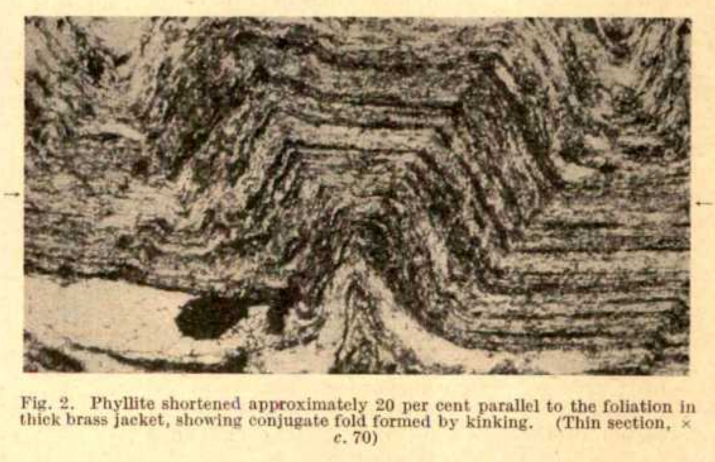

Fig. 2. Phyllite shortened approximately 20 per cent parallel to the foliation in thick brass jacket, showing conjugate fold formed by kinking. (Thin section, × c. 70)

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* On leave from the University of California, Berkeley.

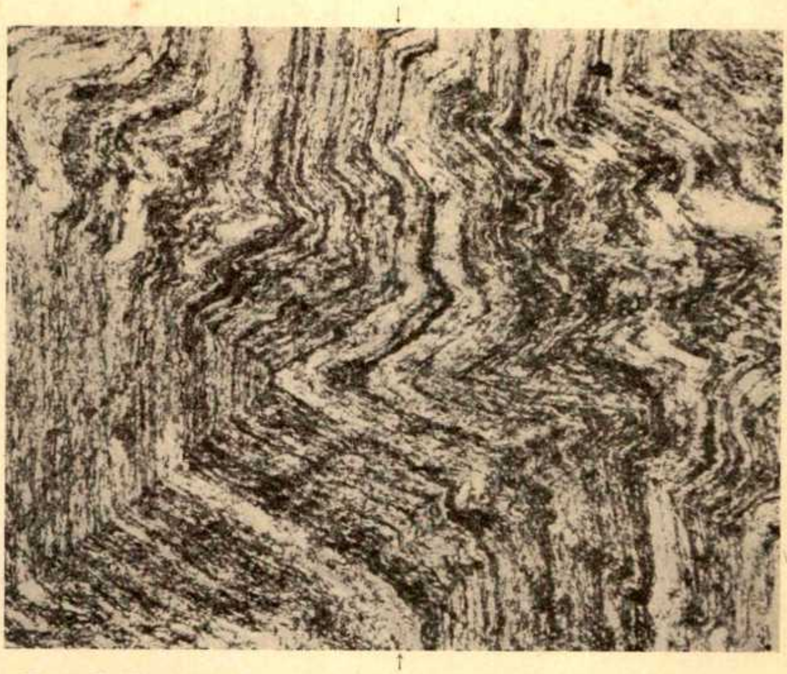

Fig. 3. Phyllite shortened approximately 20 per cent parallel to the foliation in thick brass jacket, showing folds formed at intersection of kinks. (Thin section, × c. 65)

Fig. 2) it shows the form of a conjugate fold[4]. In the other specimens, the kink bands are asymmetrically developed and in specimens compressed at 25° and 45° to the foliation only one set of kink bands generally appears. For all orientations, the kink bands grow in number and width as the strain increases, almost filling the whole specimen when 30–40 per cent shortening is reached.

(3) In the domains where kink bands intersect, folds appear in the foliation which are distinctly different in style and orientation from the folds forming the kink bands (Fig. 3). Whereas the kink folds are asymmetrical and open, with axial planes inclined to the specimen axis, these folds are commonly symmetrical in form and tightly appressed, with axial planes perpendicular to the specimen axis; they closely resemble the similar folds so widespread in strongly deformed rocks. The more highly strained specimens in which the coalescence of intersecting kink bands is nearly complete tend to contain only folds of this kind (Fig. 4). With further strain, these folds become more tightly appressed (Fig. 5). In contrast to the predominant similar folding in the phyllite, individual quartz layers which have deformed cataclastically show concentric folding (Fig. 5).

(4) In specimens compressed at 25° and 45° to the foliation in which only one set of kink bands develops, such a second generation of folds does not appear at moderate strains (Fig. 6). Instead, further deformation is by uniform gliding on the foliation accompanied by external rotation of the glide plane, similar to that observed in single crystals deformed by gliding (ref. 5, Plate 3).

In other experiments, specimens of mica schist were compressed parallel to the foliation in thick brass jackets. The mica schist consisted mainly of muscovite and biotite with some quartz and was of much coarser grain size than the phyllite. After deformation, it showed extensive kinking and small-scale folding, generally similar to that observed in the phyllite.

In some further experiments on specimens of phyllite jacketed only in rubber, the structural development was different:

(1) In specimens compressed parallel to the foliation, the deformation was mainly concentrated in two conjugate kink bands, one near each end of the specimen so that they intersected to only a small extent.

(2) In specimens compressed at 45° to the foliation, there was no kinking. The deformation took place inhomogeneously by gliding in the foliation within

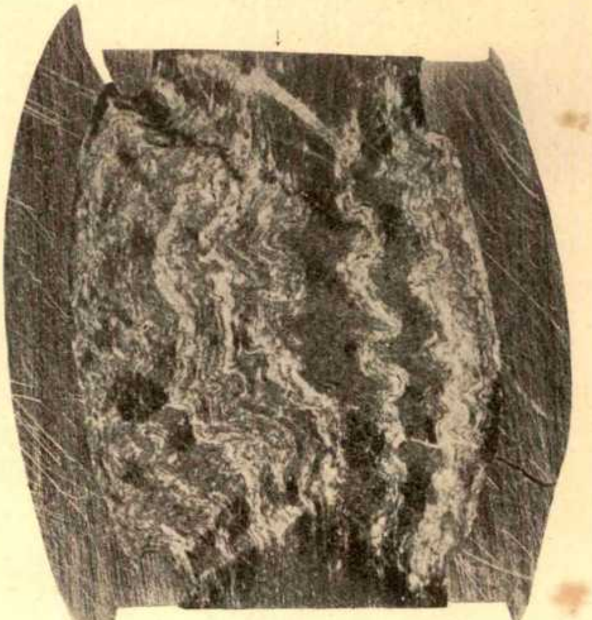

Fig. 4. Phyllite shortened 30 per cent parallel to the foliation in thick brass jacket, showing development of folding. (Polished section, × c. 5)

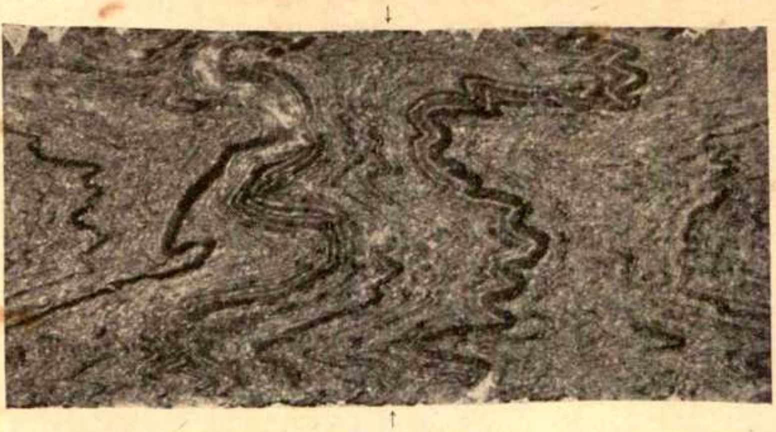

Fig. 5. Phyllite shortened 70 per cent parallel to the foliation in thick brass jacket, showing advanced stage of folding (central portion of specimen only). (Polished section, × c. 15)

central parts of the specimen, during which one end of the specimen slid laterally on the platen so that no change in the orientation of the foliation occurred (Fig. 7). This behaviour is the simplest expression of the basic mechanism of deformation in the rock in these experiments.

Clearly, therefore, the constraint offered by the thick brass or copper jackets strongly influences the structures produced by preventing the development of major inhomogeneity or arbitrary change of shape such as is observed in specimens jacketed only in rubber; that is, the role of the thick brass or copper jacket is to prescribe the gross strain in the specimen, which can only be realized by small-scale kinking and folding because the strong planar anisotropy restricts gliding to the foliation plane. In this prescription of the gross strain, the experiments go some way to simulate natural situations in which a given part of a body of rock undergoing deformation is not free to change its shape arbitrarily, but must conform with the deformation of neighbouring parts of the same body. It is presumably mainly for this reason that the structural features produced are similar to many occurring naturally in such rocks. Otherwise, it must be emphasized that the present combination of physical conditions (short duration, room temperature, confining pressure equivalent to that at about 15 km. depth) may not be representative of the natural conditions under which such types of folding are produced. However, it is believed that the observations in these experiments are of considerable value in aiding the geometrical understanding of the development of similar folding by flexural slip in foliated rocks, including such aspects as the orientation of kink bands and of axial planes of later folds relative to the mean strain ellipsoid and the close connexion between the generation of the similar folds and the intersection of kink bands.

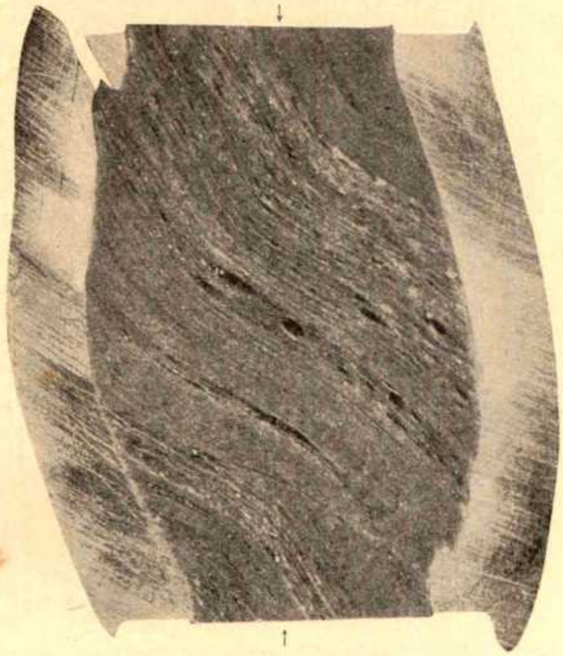

Fig. 6. Phyllite shortened 28 per cent in direction at 25° to the foliation in thick brass jacket, showing absence of small-scale folding in early stages of deformation. (Polished section, × c. 5)

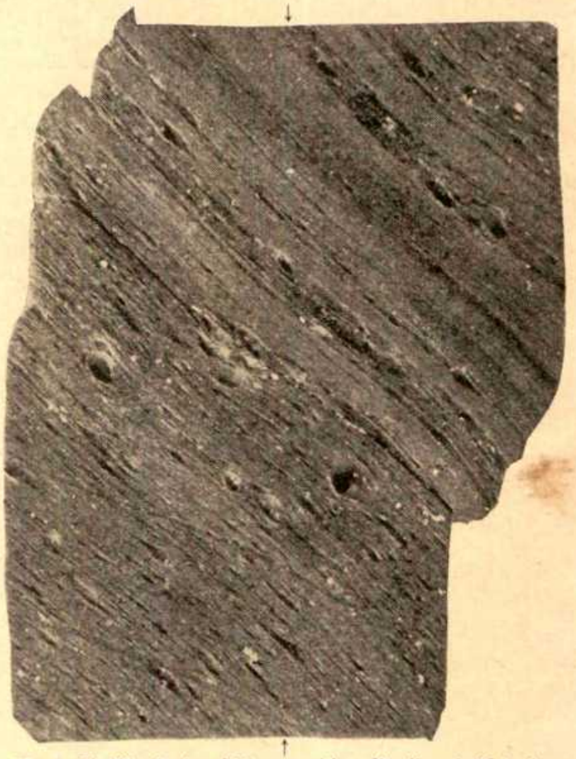

Fig. 7. Phyllite shortened 19 per cent in a direction at 45° to the foliation in rubber jacket only, showing simple gliding on the foliation. (Polished section, × c. 8)

A fuller description and more detailed analysis of the results will be published elsewhere. One of us (L. E. W.) thanks the Guggenheim Foundation for a fellowship which enabled him to visit the Australian National University and take part in this work.

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OBITUARIES

Dr R S Bassler

RAY SMITH BASSLER, who died on October 3, 1961, was born at Philadelphia on July 22, 1878 He was the last link with some of the earlier American geologists —he numbered James Hall among those he had known His childhood was spent in Cincinnati, where he came to know not only the abundant, local, well-preserved fossils but also, through them, such local (and later famous) geologists as J M Nickles, Charles Schuchert and E O Ulrich Their influence on his career was lasting

While Bassler was still at high school, Ulrich took him as a part-time assistant in his business of selling fossils (including thin sections of Palæozoic Polyzoa), and the boy learnt much After Schuchert had gone to be curator of invertebrate fossils at the United States National Museum in Washington, Bassler followed him there as assistant curator His scientific life was thus settled, and, after his retirement in 1948 as head curator, he was made associate in palæontology

Bassler's world-wide reputation rests mainly on his work on the Polyzoa He published several papers on the Palæozoic forms, notably on those of the Baltic Provinces (1911) and of Timor (1929) But it is by his voluminous joint papers with Ferdinand Canu on tertiary and recent Polyzoa that he is best known Their extensive research work, carried on for nearly twenty years from 1913, has profoundly influenced all later investigations into the systematics and structure of the post-Mesozoic Polyzoa

Bassler's duties at the U S National Museum necessarily entailed studies of other groups Thus he wrote (sometimes in collaboration with others, and especially with Ulrich) on echinoderms, ostracods, conodonts and sponges The first two claimed much of his attention, and he published a classification of the Edrioasteroidea in 1935 The last years of his life were largely devoted to a study of the Cystoidea, on which he had already published in 1943

Bassler had a flair for bibliographical compilation, and he published indexes of Polyzoa, ostracods, pelmatozoan echinoderms and Palæozoic corals Perhaps his best-known work of this kind is his *Bibliographic Index of American Ordovician and Silurian Fossils* (two volumes, 1915)

From his early days Bassler was keenly interested in stratigraphy, particularly that of the Lower Palæozoic, and in 1932 his *Stratigraphy of the Central Basin of Tennessee* appeared, based largely on his own field-mapping He also wrote the large *Cambrian and Ordovician* volume of the Maryland Geological Survey (1919)

Despite the great amount of time that Bassler spent on his official duties, he still found the energy to lecture at the George Washington University, where he was assistant professor during 1904–43 His friendly nature warmed to the difficulties and ambitions of youth, and he was regarded with affection by his students The University conferred on him an honorary D Sc in 1932

Besides filling various posts in American societies, Bassler was a correspondent of the Academy of Natural Sciences of Philadelphia Abroad, he was elected a foreign member of the Geological Society of London, an honorary member of the Natural History Society of Tartu, and a foreign correspondent of the Geologiska Foreningen, Stockholm

Energetic and vital as his work showed him to be, Bassler nevertheless could readily relax He welcomed visitors to the Museum and to his home, and delighted in showing them the sights of Washington, he was well read and cultured, with a tolerant outlook, and he never forgot his numerous friends.

H DIGHTON THOMAS

Dr A T R Mattick, C B E

DR A. T R MATTICK died at his home in Reading on July 2, 1962, nearly three years after his retirement from the National Institute for Research in Dairying.

Mattick was a student at University College, Reading, and obtained the Degree of B Sc as an external student of the University of London He served in the forces in the First World War and, after being demobilized, he returned to Reading to join the staff of the Research Institute in Dairying as an assistant for bacteriological research The Research Institute was, of course, the forerunner of the National Institute for Research in Dairying where Mattick spent his working life He became head of the Bacteriology Department and during 1946–58 was deputy director of the Institute On the retirement of Prof H D Kay from the directorship of the Institute, Mattick acted as director until Prof R G Baskett became director in 1959 [see also p 1038 of this issue] Mattick was a founder member and a president of the Society of Dairy Technology and served as president of the Society of Applied Bacteriology For twenty-seven years he was joint editor of the *Journal of Dairy Research* and acted as associate editor of the *Journal of General Microbiology*

Mattick entered the field of dairy bacteriology at a time when huge losses were being sustained by the dairy industry because of the lack of elementary hygiene In these early years the production of clean milk was a crusade, and Mattick as a worker, talker and fighter must have enjoyed every moment of it He and his colleagues fought and won the battle of dirty churns and they brought to a successful conclusion the campaign for bacteriological standards relating to designated milk His writings on the production and distribution of clean milk have become classics in their field

While Mattick had a clear understanding of the practical application of bacteriology to farm problems and the problems associated with milk distribution, his contributions to the advancement of the science of bacteriology were considerable and he played a leading part in the recognition and isolation of the antibiotic, nisin Shortly before his death the use of nisin was permitted as a food preservative

Mattick will be remembered as an outstanding character and a lively and interesting speaker whether on the platform or in private conversation On occasion he could appear brusque and he spoke and acted without fear or favour in the interest of what was just and right Those who knew him well recognized his essential humanity and he himself frequently asserted that people were more important than things His understanding of individuals was swift and accurate and his ready kindness could on occasion be tempered with more or less astringency according to the recipient

As he was a man of special and unique quality, so will be the affection with which his colleagues remember him

N J BERRIDGE

Prof W Rothmaler

PROF WERNER ROTHMALER, the well-known plant taxonomist, died at Greifswald, East Germany, in April 1962

Rothmaler was born in 1908 in Thuringia, he went to school at Weimar and then to the University of Jena At the age of twenty-five he emigrated to Spain, where he worked at the Botanical Institute in Barcelona, he later moved to Lisbon and worked there at the Estação Agronomica Nacional It was during this period that he made important contributions to the taxonomy and ecology of the flora of the Iberian Peninsula, and he retained an interest in this area all his life

In 1941 he was recalled to Germany and served as a soldier in Greece, where he was attached to a mapping unit In 1943, because of illness, he returned to Germany and after a short stay in Berlin found a home in Vienna with Prof Wettstein In 1944 he became director of the Botanical Department at Gatersleben and in 1945 went to Halle, where he became professor in 1950 In 1953 he made a final move to Greifswald, where he was appointed director of the new Institute for Agrobiology, and he remained there until his death, though he continued to travel widely

Rothmaler published many papers, and was known as an authority on the genera *Alchemilla* and *Antirrhinum* He recently produced an excellent short Flora of Germany, the final, critical volume of this was nearly complete at the time of his death and will probably appear soon He proposed as early as 1944 the preparation of a Flora of Europe, a project which had to be abandoned because of war-time circumstances, but he co-operated whole-heartedly in the new Flora Europæa and had already given much valuable assistance In this, as in other botanical research, both in his own country and internationally, his early death leaves a place difficult to fill

Rothmaler's interests both inside and outside science were unusually wide, he was an active exponent of the philosophy and history of science, and had expert knowledge of many branches of art and archæology British botanists, many of whom had got to know him during the past ten years, will miss him greatly To work with him in the field was an unforgettable experience, wise, witty and critical, he could always produce the right comment and the illuminating observation

He leaves a widow and four children

D H VALENTINE

NEWS and VIEWS

Scientific Attaché in Moscow

T Garrett

MR TERENCE GARRETT has been appointed scientific attaché to the British Embassy in Moscow and will take up his post this month He succeeds Dr D A Senior, who was the first to hold this post (see *Nature*, 183, 1018, 1959), and who is moving to the Department of Scientific and Industrial Research's Forest Products Research Laboratory on completion of his term of appointment As scientific attaché Mr Garrett will advise the British Ambassador (Sir Frank Roberts) on scientific matters, promote scientific contacts and report on Soviet scientific and technical development in the civil field Mr Garrett, who is 32, is at present senior lecturer in the Department of Mathematics and Physics at the Royal Military College of Science, Shrivenham Mr Garrett attended Alleyn's School, Dulwich, and then won an open scholarship to Gonville and Caius College, Cambridge, where he read mathematics He went on to take a diploma in mathematical statistics with chemical engineering as a special field of application Before going to Shrivenham he was, for a year, lecturer and head of the Mathematics Section at Ewell Technical College Mr Garrett, who speaks Russian and German fluently, has for some years been interested in the problems of translating technical literature and in the statistical analysis of languages He has written about education in the U S S R and the Russian Orthodox Church He has been to the U S S R several times, recently as interpreter at the British Trade Fair in Moscow

Biology in the University of East Anglia

Prof J Dainty

DR JACK DAINTY has been appointed professor of biology in the new University of East Anglia, Norwich Dr Dainty was a pupil at Mexborough Grammar School and obtained an Exhibition in mathematics at Queens' College, Cambridge, where he obtained firsts in the Mathematics Tripos Part I and Natural Sciences (Physics) Tripos Part II For six years he did research and teaching in nuclear physics at the Cavendish Laboratory, where he was in charge of the cyclotron After the Second World War he worked for a short time at Chalk River He came to the Natural Philosophy Department at Edinburgh in 1949 and, after some years spent building up the High Tension Laboratory, decided that biology was perhaps the most interesting branch of physics Appointed reader in biophysics at Edinburgh in 1955, he has since built up a research and postgraduate teaching department that is unique in Britain Dr Dainty's main interest is in the permeability and electrical properties of cell membranes, working particularly on the giant internodal cells of the Characeae He has developed and applied the as yet incomplete methods of non-equilibrium thermodynamics to permeability studies, and has stressed the serious errors that can arise from the use of simpler less rigorous methods Dr Dainty will no doubt attempt at Norwich to encourage a more rigorous and quantitative approach to biological teaching and research Dr Dainty has also wide interests in natural history He will be fully aware of the proper importance of field work and ecology, particularly in a county such as Norfolk

New Chemical Enzymology Unit for Shell Research

DR J W CORNFORTH, for some years a member of the National Institute of Medical Research, and Dr G J Popják, at present director of the Medical Research Council's Experimental Radiopathology Research Unit, will be jointly in charge of Shell Research's new Chemical Enzymology Unit They will be assisted by Dr Rita Cornforth, wife of Dr John Cornforth, and a group of experienced biochemists and technical assistants The Unit will have a new laboratory at Sittingbourne, in Kent (due for completion in April 1963) Its work will embrace both basic research and research on topics of shorter-range interest—mainly in the sphere of 'biological chemistry', on which Shell Research largely relies in the development of pesticides and herbicides

Babcock and Wilcox Nuclear Development Center

Dr W M Breazeale

Dr William M Breazeale, a nuclear scientist who holds the first reactor operator's licence issued by the U S Atomic Energy Commission, has been appointed director of the Babcock and Wilcox Co Nuclear Development Center Comprising a 3 5 million dollar test reactor and nuclear fuel laboratory, now under construction, and an existing critical experiment laboratory, the Center is being established near Lynchburg, Va Dr Breazeale will be responsible for providing laboratory and experimental support to the Atomic Energy Division, which has overall responsibility for the Company's nuclear power business Joining Babcock and Wilcox Co in 1956 as assistant manager in the Atomic Energy Division's Nuclear Engineering Department, Dr Breazeale became assistant manager of the Project Management Department in 1959, and in 1960 was made senior technical adviser for the Division Dr Breazeale received his first degree from Rutgers and later took his Ph D degree in physics at the University of Virginia at Charlottesville

International Academy of Astronautics

Corresponding Members

The following have been elected corresponding members of the International Academy of Astronautics of the International Astronautical Federation

Basic Sciences Section Prof Edoardo Amaldi, Istituto Física Università, Rome, Prof K O Kiepenheuer, Fraunhofer Institut, Freiburg i/B , Prof S K Mitra, national research professor, Institute of Radio Physics and Electronics, Calcutta, Dr Svein Rosseland, Institute of Theoretical Astrophysics, Oslo University, Blindern, Dr Harry Wexler (now deceased), U S Weather Bureau, Washington, D C , Dr John R Winckler, professor of physics, Institute of Technology, University of Minnesota, Minneapolis

Engineering Sciences Section Dr Sydney Goldstein, Harvard University, Cambridge, Mass , Dr William R Hawthorne, Engineering Laboratory, Cambridge, England, Dr Hideo Itokawa, Institute of Industrial Science, University of Tokyo, Dr W F Klemperer, Douglas Aircraft Co , Santa Monica, Cal , Dr Hans W Liepmann, California Institute of Technology, Pasadena Cal , Mr George P Sutton, manager, Long Range Planning, Rocketdyne, Canoga Park, Dr Herbert York, chancellor, University of California at San Diego, Cal , Mr M J Zucrow, Jet Propulsion Center, Purdue University, Lafayette, Indiana

Life Sciences Section Dr Pierre Biget, Centre d'Enseignement et de Recherches de Médecine Aéronautique, Paris, Dr E O Errebo-Knudsen, Institute of Aviation Medicine, Copenhagen, Dr O H Gauer, Freien Universitat, Berlin, Dr C J Lambertsen, University of Pennsylvania, Philadelphia, Mr Alfred M Mayo, manager, Advanced Systems Department, Astronautics Division, Chance Vought Corp , Dallas, Texas, Dr Hermann Rahn, Department of Physiology, University of Buffalo, N Y , Dr Cornelius A Tobias, Donner Laboratory, University of California, Berkeley, Dr F M Violette, Centre d'Enseignement et de Recherches de Médecine Aéronautique, Paris, Lt -Col Stanley C White, Life Science Division, National Aeronautics and Space Administration, Langley Air Force Base

The Academy now has a total membership of 140, from 22 countries The second meeting of the officers and trustees of the Academy will be held during the Thirteenth Congress of the International Astronautical Federation at Varna, Bulgaria, on September 25

Employment of Graduates

The sixteenth annual report of the University of Durham Appointments Board covering the academic year 1960–61 records that the demand for mathematicians continued to mount faster than supply, and that the similar position with physicists also worsened (Pp 12 Durham The University Appointments Board, 1962) On the other hand, the demand for chemists eased and the supply of chemical engineers began to catch up with demand, but while there were very few vacancies for geologists, especially in industry, the shortages of good mechanical and electrical engineers remained chronic Demand for arts graduates from all employers remained good, and more women found jobs in the chemical and allied industries, mainly in consumer research and information work The nationalized industries remained unpopular, but as a whole the public service gained more recruits, especially in central government departments, local government had little attraction Competition for the most outstanding engineers, scientists and arts graduates intensified, whereas the least-able and least-effective had more difficulty in locating suitable employment Grave concern is expressed over the numbers entering the teaching profession in arts and science alike there was a distinct drop in the numbers of both men and women going on to train as teachers, and the numbers of women science graduates and of men arts graduates taking up teaching posts untrained also decreased Comment is made on the ineffective character and handling of recruitment literature, the doubling of the number of men entering the private sector of employment, and the quadrupling of the number of men entering public service

The Australian Society of Limnology

At a meeting held at Monash University on May 26 a constitution was drawn up for an Australian Society of Limnology The objectives of the Society will be "To establish effective liaison between all persons interested in any aspect of fresh and brackish water research in Australia, and to promote the study of and encourage these interests in State and Commonwealth Research Institutes and Departments, and educational Institutions" The means of attaining these objectives will be "(*a*) The establishment and maintenance of a register of all persons working in appropriate fields in Australia and adjoining countries, giving details of their current interests and relevant publications, (*b*) The holding of an annual congress which will coincide with the annual general meeting, (*c*) The production of a *Newsletter* by an editor to be appointed by the executive" At the meeting the following officers were elected *President*, Dr A Nicholls, *Vice-President*, Mr J Lake, *Secretary-Treasurer*, Dr W Williams Further information and details of membership can be obtained from Dr. W Williams, Department of Zoology and Comparative Physiology, Monash University, Box 92 P O , Clayton, Victoria

Freedom in Science in the Nineteenth Century

Dr J R Baker's essay, "The Controversy on Freedom in Science in the Nineteenth Century", describing the debate between E Haeckel and R Virchow beginning at the Congress of German Naturalists and Physicians at Munich in September 1877, which originally appeared in the volume of essays, *The Logic of Personal Knowledge*, presented to Prof M Polanyi on the occasion of his seventieth birthday, has now been issued by the Society for Freedom in Science as *Occasional Pamphlet* No 22 (Pp 13 Oxford Assistant Secretary, Society for Freedom in Science, Department of Zoology, University Museum, 1962 1*s*) Dr Baker shows how radically this controversy differs from that which developed in the 1930's and 1940's, and notes how it led to the exclusion of biology from the curricula of all senior classes in schools—a result which neither Haeckel nor Virchow could have foreseen and which both must have deplored No one then had any intention of restricting the fredom of research workers to choose the subjects of their investigations, and, although it is not clear exactly what Virchow proposed, it is probable that he was concerned solely with the schools, where it is appropriate that instruction in science should be mainly factual

Idiopathic Clubbing of Fingers in the African

From time to time Africans are admitted to medical wards with a gross degree of finger and toe clubbing, it is difficult to determine its cause The clubbing is pronounced and cannot be missed, and, except for this, the patients enjoy good health Similar cases occurring in other members of the family are not mentioned On the other hand, clubbing is commonly met with in an African medical practice due to such diseases as chronic and sub-acute pulmonary disorders, notably bronchiectasis, lung abscess, bronchial carcinoma, empyema, silicotuberculosis and also, occasionally, in tuberculous disease of the lungs, when a bronchiectatic basis is a possible explanation Heart disease also accounts for a number of cases including congenital lesions, sub-acute infective endocarditis and aneurysm of the aorta Liver disease is often met with in the African, but Dr Michael Gelfand, physician to the Harare Central Hospital, Salisbury, is not satisfied that this is a factor in its causation Although steatorrhœa is given as a cause, this is rarely seen in the African The same causes of clubbing apply also to hypertrophic osteoarthropathy, in which an idiopathic variety has been described Dr Gelfand has not detected this and, in the *Central African Journal of Medicine* (8, No 6, June 1962) a case of clubbing is described for which no possible explanation can be found

Conservation of Marshes, Bogs and other Wetlands

Alarmed by the progressive loss of marshes, bogs and other wetlands through drainage and 'improvement' the Executive Board of the International Union for Conservation of Nature and Natural Resources proposed early in 1961 that the Union, in close co-operation with the International Council for Bird Preservation and the International Wildfowl Research Bureau, should develop a programme on conservation and management of temperate marshes, bogs and other wetlands to be called 'Project MAR' The final goals of the programme are to prepare a broad statement on the importance of marshes and wetlands to modern mankind and to give the widest publicity to this statement, to assemble all important data on means of conserving wetlands, to keep and improve them for wildlife through proper management, to restore them when debilitated and to make man-made aquatic habitats useful for wildlife, to make this information known and available to all those in a position to take action to advance the conservation of wetlands, to make an inventory and classification of all European and north-west African marshes, bogs and other wetlands of international importance and to offer technical assistance for the establishment of reserves in marshes, bogs and other wetlands classified as of international importance The initial 'Project MAR' is described in an issue of the *Bulletin* of the International Union for Conservation of Nature and Natural Resources (New Series, No 3, April–June 1962)

Myxomatosis Survey

D A Chapman, of Oundle School, has described investigations into myxomatosis (Oundle School Natural History Society, Annual Report 1961 Pp ii + 54 Oundle Oundle School Natural History Society, 1962) The survey was begun after several rabbits with the disease had been noticed on a small stretch of road between Warmington and Morborne Since then records have been made by many boys of the School and information collected from farmers and game-keepers within a five-mile radius of Oundle The aim of the survey was to find the effect of the disease on the numbers of rabbits within this area Chapman describes many observations on diseased rabbits "In the later stages I have seen rabbits crouching in the grass with their ears folded backwards over their backs and the fur ruffled by the wind They remain motionless for a long time like this, and I would not be surprised that if not interrupted they would stay like this until they die Before the eyes have completely sealed over the rabbits still eat, but after this they lose their appetite " "Before the eyes sealed the diseased rabbits seemed to prefer to keep together, but once they close they lose each other and would scatter I have never seen a diseased rabbit run into a burrow, and, where there is an infected warren, often the holes have not been kept clean, being semi-blocked by leaves and twigs Whether the diseased rabbits go into burrows or are not allowed by the still healthy ones I do not know In the advanced stages of the disease they sometimes take to wandering, and eventually die 11 to 18 days after infection " Nearly every farmer in the area affected north of Oundle pays a yearly membership to the Rabbit Clearance Society The rabbit clearance worker, whose territory covers the north-west of the area, is fully employed and estimates that there are no more than about 70 rabbits in his small district On February 8, ferrets were put into a set of warrens near Glapthorne Cow Pastures and only one rabbit was killed Before myxomatosis had been found, three ferrets were put into the warren and 47 rabbits were shot in a morning

Verbal Learning and Verbal Behaviour

ALTHOUGH new psychological journals proliferate almost as rapidly as the rats which feature so extensively in their contents, there is perhaps a need for a journal devoted to the special problems of human verbal behaviour *The Journal of Verbal Learning and Verbal Behaviour*, under the editorship of Dr Leo Postman and an all-American editorial board, should go far to meet this need and provide a focus for psychological work in this admittedly specialized field ((1, No 1 (July 1962) Pp 78' New York and London Academic Press, Inc, 1962 Subscription Volume 1 (6 issues), 1962, 15 dollars)) The first issue contains papers on one-trial learning (a matter of some significance for modern statistical learning theories), paired-associate learning, and some effects of grammatical category on rote learning and word association The appearance of this journal would no doubt have given warm pleasure to Hermann Ebbinghaus, from whose heroic labours in mastering strings of nonsense syllables the modern interest in verbal learning may be said to have sprung

The Harkness Fellowships of the Commonwealth Fund—New York

THIRTY Harkness Fellowships of the Commonwealth Fund—New York are tenable during 1963 for education and travel in the continental United States The fellowships are offered to candidates from the United Kingdom, Australia, New Zealand and Western Europe, who are British subjects, and either are graduates or have experience in Government service, the professions, the arts, branches of business or industry or other comparable careers or vocations, and have the equivalent of a degree in qualifications conferred by professional bodies or an attested level of professional competence Forms of application, which must be returned before November 15, can be obtained from the Warden, Harkness House, 38 Upper Brook Street, London, W 1, from whom further information can be obtained

Nuclear Reactor Chemistry and Technology

THE third conference on "Nuclear Reactor Chemistry" and the sixth conference on "Analytical Chemistry in Nuclear Reactor Technology", sponsored by Oak Ridge National Laboratory, will be held in Gatlinburg, Tennessee, during October 9–11 Subjects under discussion will include preparation of fuel, moderator, coolant and nuclear poison materials and their properties in the presence and absence of nuclear radiation, particle behaviour in nuclear reactor accidents, chemical problems of organic moderated and organic cooled reactors, the chemistry of corrosion processes in nuclear reactors, use of isotopes in analytical chemistry, instrumentation, analysis of organic moderators, spectroscopy, analytical developments in radiochemistry and nucleonics Further information can be obtained from Oak Ridge National Laboratory, P O Box X, Oak Ridge, Tennessee

Announcements

THE second interscience conference on "Antimicrobial Agents and Chemotherapy" will be held in Chicago during October 31–November 2 Further information can be obtained from the American Society for Microbiology, 19875 Mack Avenue Detroit 36 Michigan

THE second world congress of undersea activities, organized by the British Sub-Aqua Club and the World Underwater Federation, will be held in Westminster during October 18–24 Further information can be obtained from Kendall McDonald, 4 Pytchley Crescent, Upper Norwood, London, S E 19

A MEETING of the British Biophysical Society will be held in University College, London, during December 17–18 The programme will include two symposia physical aspects of electron microscopy, structure of the nucleus Further information can be obtained from Prof D D Eley, Chemistry Department, University of Nottingham, University Park, Nottingham

AN autumn meeting on metallurgy, organized by the Société Française de Métallurgie, will be held in Paris during October 15–19 The themes of this meeting will be high temperature-resisting metals and alloys, and the use of the vacuum and rarified atmosphere in metallurgy Further information can be obtained from La Société Française de Métallurgie, 25 rue de Clichy, Paris 9

THE sixth plenary meeting of the World Power Conference will be held in Melbourne during October 20–27 The technical programme will include energy resources, the production and amelioration of primary sources of energy, transformation of primary to secondary energy and transportation of energy, utilization of primary and secondary energy, economic evaluation of alternative energy sources Further information can be obtained from the Australian National Committee, World Power Conference, 1 Collins Place, Melbourne, C 1, Victoria

"SPACE SPECTROSCOPY" is the title of a meeting of the Spectroscopy Group of the Institute of Physics and the Physical Society to be held at the headquarters of the Institute and Society on October 12 Speakers will include Dr R Tousey (U S Naval Research Laboratory), Dr J Lindsay (U S National Aeronautics and Space Administration), Dr K A Pounds (University of Leicester) and Dr D W O Heddle (University College, London) Further information can be obtained from the secretary, Dr L Bovey, Building 329, Atomic Energy Research Establishment, Harwell, Didcot, Berks

THE second international symposium on "Phosphorylated Glucides" will be held in Milan during October 12–14 The programme will include biology of phosphorylated glucides, phosphorylated glucides and metabolism of the myocardium, phosphorylated glucides in cardiosurgery and anæsthesiology, phosphorylated glucides and metabolism of the myometrium, phosphorylated glucides in physiopathology and therapy of liver, phosphorylated glucides in physiopathology and therapy of the nervous system, metabolism diseases and phosphorylated glucides Further information can be obtained from the Organizing Committee of the Second International Symposium on Phosphorylated Glucides, Via Modica 6, Milan

ERRATUM In the article entitled "The Future Natural Sciences Programme of Unesco" by Dr Hilliard Roderick which appeared on p 215 of the July 21 issue of *Nature*, the percentages 50, 40 and 10 should be inserted in place of 56, 10 and 10 respectively in the fourth paragraph on p 221 (section iii)

THE UNITED KINGDOM ATOMIC ENERGY AUTHORITY

THE eighth annual report of the United Kingdom Atomic Energy Authority*, covering the year ended March 31, 1962, contains 73 pages, compared with 70 in the previous report However, the material is arranged in 16 instead of 13 chapters, the new chapters being on magnox reactors and on plasma physics (incorporating fusion research), as well as one on supporting technical developments, that on controlled thermonuclear research has now been dropped A chapter, "Isotopes and Irradiation Programme", replaces that on "Commercial Operations" Even more noteworthy, for the first time, an estimate is included of the Authority's expenditure on research and development which, over the next five years, is put at about £50 million per annum For 1961–62, the current expenditure on this programme was £33 million with £16 million on capital expenditure, of this, £21 million was expended on the reactor programme, £4 million on general reactor technology, £5 million on general research, £2 million on plasma physics and fusion research, and £1 million on isotopes research Out of 2,740 graduate or professional - engineers or scientists employed on the whole programme at March 31, the corresponding deployment at the end of the year was 1,460, 300, 520, 170 and 90 The level of general research is designed to provide the minimum necessary effort on fundamental problems in such fields as nuclear physics, chemistry and metallurgy, together with the essential scientific basis for the Authority's development activities, while that in the field of reactor technology includes work on materials for reactors and for their fuel

The Authority's non-industrial staff increased slightly during the year, from 20,234 to 20,543, while the number of industrial employees fell from 20,606 to 20,017 From the review of the Authority's forward civil research and development programme, and the known trends in the weapons and production fields, it seems probable that the total manpower will remain roughly steady for the time being Recruitment of non-industrial staff was generally satisfactory during the year, but the Authority did not secure quite as many qualified scientists and engineers from the universities as were needed It is suggested that the wide range of interesting work which will continue to be available for scientific and engineering graduates of many disciplines is not yet fully appreciated Recruitment of non-industrial staff has recently been confined to younger people, especially those leaving school or completing a first or research degree at university, and this will be the future general policy, so that reliance for the future succession to managerial posts is in the main placed on staff who have made their careers with the Authority

The net expenditure in 1962–63 is estimated at £67,875,000 compared with £78,071,000 in 1961–62, which included some £6 million for the National Institute of Research in Nuclear Science, and includes some £4 881,000 for research, development and design contracts with industry, the universities, etc (compared with £4,531,000 in 1961–62) Commercial sales during the year realized more than £20 million, with an additional £500,000 for consulting services and royalties Sales of isotopes yielded a return on capital of more than 7 per cent The first two contracts with the Central Electricity Generating Board for fuel elements were signed in May 1961, and a contract to supply fuel for the Latina nuclear power station in Italy in December 1961

The production reactors and processing plants continued to perform satisfactorily, process efficiencies were improved during the year, and progress on the advanced gas-cooled reactor supported the expectation that electricity generated from such stations will be as cheap as, and later cheaper than, electricity generated from conventional stations in the United Kingdom In the reactor development programme much experimental work was concentrated on the oxidation of the graphite moderator by the irradiated carbon dioxide coolant A vigorous programme of research aimed at an economically attractive marine propulsion unit is being carried out in conjunction with the United Kingdom industry, and a working group on marine reactor research has been set up to advise and make recommendations on the programme of research required to advance nuclear propulsion for merchant ships A team to consider the design of a marine reactor has been formed within the Authority, and a team of engineers has been seconded from the British Shipbuilding Research Association to study the special features of the design operation and safety of a nuclear ship

During the year, 332 unclassified reports were issued and 390 papers by members of the Authority's staff were published in periodicals From the topics selected for mention in the chapter on general research and development, only a few can be taken as illustrative Research at Culcheth on radiation damage in stainless steels containing niobium has given interesting results, and the investigations are continuing to obtain a clear understanding of the phenomena and to determine methods of fabrication and heat treatment which will eliminate embrittlement A wide programme of general research into ceramic fuels stimulated by their high promise for power reactors includes investigation of the release of fission product gases in irradiated oxide ceramic fuels, the stability of various phases in the uranium–plutonium–oxygen system over a range of temperatures including those used in fuel fabrication and in reactor operation, and the investigation of carbides of uranium and plutonium Small-scale exploratory studies are being made of the possibilities of direct conversion of heat to electricity, while in nuclear physics improved detectors for charged particles have been developed at Harwell from silicon which can discriminate between particle energies closely similar in value and respond rapidly to a particle, producing an output signal within a hundred-millionth part of a second Further development of these semi-conductor detectors is directed particularly at establishing production methods and finding ways of making larger detectors

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* United Kingdom Atomic Energy Authority Eighth Annual Report for the period 1st April, 1961–31st March, 1962 Pp ix+87+4 plates (London H M S O , 1962) 6s 6d net

Basic nuclear research is being carried out with the view of improving the imperfect understanding of the arrangement of the constituent particles within nuclei and of the forces that hold them together Work has continued at Harwell on the nature and properties of radioactive aerosols, with particular attention being directed to iodine A new laboratory designed specifically for remotely controlled experiments of plutonium critical assemblies came into use at Dounreay, and considerable progress was made at the Culham Laboratory site where the objective is the generation of power from fusion reactions In the extensive research in plasma physics, the key problem is the stability of the plasma and magnetic field system, and the emphasis of the experimental work is on those systems of magnetic confinement thought theoretically to be stable

THE GHANA SCIENCE ASSOCIATION

THE Ghana Science Association held its second annual congress during June 28–July 1, at the University of Ghana The highlight of the conference, the start of which coincided with the ending of the Accra assembly on "The World Without the Bomb", was the participation of a number of scientists from various other countries who were in Ghana for the Accra Assembly The visiting scientists had been informed of this meeting before their arrival in Ghana, and some of them read papers to the Association during the conference, including Prof J D Bernal (United Kingdom), Prof Ivan Supek (Yugoslavia), Prof Gerd Burkhardt (West Germany), Sir Robert Watson-Watt (U S A), Prof Alexjandro Lipschutz (Chile) and Dr H Marcovich (France)

The Ghana Science Association is a body in Ghana which is devoted solely to encouraging scientific and technological research and advancing both the understanding and teaching of science To this end, the Association provides a forum for scientists working in Ghana and guests from outside It also serves as a medium for informing the public of the part which science and technology are playing and can continue to play in the development of Ghana

The second annual congress was opened by the Minister Responsible for the National Research Council of Ghana, Mr Kofi Baako, who deputized for Osagyefo Dr Kwame Nkrumah, who is president of the Ghana Academy of Sciences—Prof J D Bernal having given a pre-conference open lecture on June 22 on "The Origin of Life" In his opening address, the Minister recognized that science was no magic wand with which to convert poor deserts to rich fertile lands, but that hard work was what was needed in all scientific endeavours The presidential address was given this year by Dr S R A Dodu In his speech, which set a high standard, Dr Dodu traced in outline the stages of development in the history of medicine He observed that contemporary primitive practices are often just echoing fragments of the past activities of pre-scientific man, and that by means of science we are able to dissipate superstitions and fears based on ignorance and to evaluate and assess the traditions and inherited customs by which we habitually order our lives and finally shape the very destiny of mankind

Later that evening, Dr H Marcovich, of the Pasteur Institute in Paris, gave an open lecture, the topic of which was, "Small Doses of Radiation, What are Their Biological Effects?" In his lecture, Dr Marcovich produced evidence to question the assumption in some quarters that small doses of radiation had no biological effects of any significance

There were two full sessional days, namely, June 29 and 30, and on each there were two morning sessions and one afternoon session, each session consisting of three papers The evenings were taken up by some open items

The first morning session on June 29 was under the chairmanship of Dr P M J Phillips, deputy chief medical officer It was devoted to subjects of medical importance such as "Drugs and the Organization of Mind", by Dr A C Mundy-Castle of the National Institute of Health and Medical Research, Accra, "Age and Tuberculosis Morbidity", by Dr E Laing of the Department of Botany, University of Ghana, and "Structure and Activity of the Steroid Hormones", by Prof J A K Quartey of the Department of Chemistry University of Ghana The first of these papers aroused great interest, and the speaker dealt with the varied characteristic disturbances evoked in humans by certain drugs and the investigations being carried out on the biochemical origin of madness He also showed cases where similar disturbances were induced without resort to chemicals In Dr Laing's paper an analysis of variance carried out on tuberculosis morbidity data in parts of Ghana showed a significant positive regression of morbidity with age Prof Quartey traced some of the chemical problems encountered in obtaining hormone substitutes in sufficient quantities for clinical hormone therapy, in particular that of determining the required structural features of the steroid molecule.

The second morning session concerned some aspects of agricultural research, under the chairmanship of K A Quagraine, chief scientific officer The first in this series was a paper by O Snow, adviser on agricultural matters to the Ministry of Agriculture on "The Economics of Agricultural Research" Recognizing that research is one item in the cost of production, Mr Snow considered, in an exploratory fashion, the problem in Ghana as to how much money can justifiably be spent on agricultural research, with the provisos that the research is well guided, gives results and is efficiently utilized The second speaker was P Ahn of the Faculty of Agriculture, University of Ghana, who read his paper on "Climate, Soils and Agriculture in the Forest Zone" (of Ghana) He showed in an elegant manner how the differences in rainfall in various parts of the forest zone are related to differences in the type of forest, both in floristic composition and in structure, and also to important differences in the soils The speaker emphasized that, as a result, both soils and natural vegetation can be correlated with agricultural belts The final paper in the series, on "Land Capability Classification of the Soils of Ghana", was read by Mr H B Obeng and Mr G K Smith, both of the Scientific Services Division of the Ministry of Agriculture The paper attempted to make a preliminary classification of the soils of Ghana under seven capability classes The classification, which is based on the American system, involves a critical evaluation of a number of important soil characteristics and the degree of limitation of each soil to mechanized farming and management It aimed at ensuring proper use of land and efficient crop and livestock production From the discussion that followed this excellent attempt, it became obvious that the scheme would have to be modified when more results became available concerning soils in Ghana

The afternoon session, under the chairmanship of Dr J Yanney-Wilson of the Department of Botany, University of Ghana, consisted of a mixed bag of topics The first speaker, H R B A Prempeh, acting regional agricultural officer for the Eastern Region, read a paper on "Improvement of Poultry in Ghana by Progeny Testing" He called for a programme by which the small number of imported birds which have been able to maintain their high breeding values could be 'progeny-tested' to form a foundation stock Dr J D Thomas, senior lecturer in zoology, University of Ghana, next gave his paper on "A Preliminary Account of the Life-cycle of *Mesocoelium monodi* (a trematode)" This parasite is widely distributed in West Africa and elsewhere in the tropical belt, and could be used as type specimen in tropical schools The last speaker was Dr O Davies, reader in archæology at the University of Ghana, and his topic was "The Stone Age in West Africa" The speaker, who is well known for his work in this field, gave a general account of the stone-age cultures in West Africa south of the Niger–Senegal, and showed a number of specimens that he had collected

There were two open items later that evening Prof Ivan Supek, professor of theoretical physics and philosophy at the University of Zagreb, spoke on "Science, Philosophy and Art", and after supper there was a symposium on "The Hopes and Dangers of Atomic Energy", under the chairmanship of Sir Robert Watson-Watt.

The second sessional day, June 30, commenced with the first morning session which was devoted to physics Under the chairmanship of Prof A Nunn May of the Department of Physics, University of Ghana, the session started with a paper by Prof Gerd Burkhardt of the Institute of Theoretical Physics, Hanover, West Germany He spoke on "Some Aspects of Plasma-Physics" Prof Burkhardt stated that the great interest now shown in research concerning the plasma-state of matter stems from the problem of controlling thermo-nuclear reaction The long-range forces between charged particles which make the kinetic behaviour of a plasma different from that of a neutral gas give rise to different modes of wave propagation, characteristic of a plasma, he then showed how these space charge waves could excite electromagnetic waves which are observed from the Sun and other cosmic bodies in the radio-frequency range The next paper was on "Atomic Reactor Research in Ghana", by Dr A H Ward of the Radio-Isotope Research Unit at Legon He gave a simple explanation of the construction and operation of reactors in general and, in particular, the *VVR–C* tank-type, and ended with an evaluation of the proposed 2,000-kW research reactor for Ghana in the light of its timing in the general development plans of Ghana, the capabilities of the reactor and its cost, including the problem of highly trained personnel The final speaker in the series was Father J R Koster of the Department of Physics, University of Ghana, who read a paper on "Some Recent Scientific Applications of the Doppler Shift" With illustrations of tape recordings, Father Koster showed how Doppler shift techniques are now being applied to some recent problems in ionospheric studies and satellite location

The second morning session, with Prof Hugh Nicol as chairman, had a zoological bias G C Rawson and J N N Adjetey, fisheries officers at Tema, read a paper on "The Development of Fishery Resources" in which they emphasized the need to tackle fisheries development from the aspects of biology, technology and economics E A Kwei, also a fisheries officer at Tema, read a paper on "The Spawning Migration of *Sardinella aurita* (Val)" He showed that the appearance of this very important fish near the shore coincided with its spawning, which thus has effect on the fishing season in Ghana Finally, W Pople of the Zoology Department, University of Ghana, gave a paper on "The Annual Cycle of a Lagoon in Ghana" He pointed out the features of the small blind estuaries of Ghana and described the annual features of the Kpeshi Lagoon of Accra

The afternoon session was chaired by O Snow, adviser on agricultural matters Dr W K Agble, deputy chief scientific officer, spoke on "Improvement of the Yields of Maize in Ghana" He reviewed the work done in this field since the adoption of a breeding programme on conventional lines as well as on emergent concepts of breeding tropical maize leading to the development of new synthetic hybrid maizes The next paper was that of Dr K Kadambi, formerly of the Indian Forestry Service, whose paper was read in his absence by Dr B J Harris It was on "Vegetative Propagation of Tropical Plants by Means of Air-layers" Finally, in this series was a paper by P Appiagyei-Danka of the Scientific Services Division on "Porosity Status of Some Major Soils of Ghana" The paper gave in much detail original investigations carried out by P Appiagyei-Danka on this vital aspect of the soil, and provided information that is much needed with the introduction of mechanization and intensive cropping throughout Ghana

The sessions for the day and for the whole conference were rounded off with a special short session of two papers read by two of the visiting scientists The first speaker was the 80-year-old Chilean experimental physiologist, Prof Alexjandro Lipschutz, who read a paper on "Some Progress with Hormones in Cancer Research—Experimental Aspects" The final speaker was Sir Robert Watson-Watt, who read a paper on "The Ethical Responsibilities of the Scientist" In the evening there was a film show

The final day of the conference, July 1, was devoted to excursions to Tema, Akosombo and Nungua Station The conference was rounded off with the annual dinner of the Association in the evening

There is no doubt that the second annual congress constitutes an improvement over the first one in many ways, particularly in respect of the participation of a number of leading scientists from other parts of the world Most of the papers read at the congress are to be published in the forthcoming issues of the *Ghana Journal of Science*

J Yanney-Wilson

THE TROPICAL PRODUCTS INSTITUTE

REPORT FOR 1961

DURING the next five years, the Department of Scientific and Industrial Research's Tropical Products Institute should expand its facilities to meet the increasing need for advice and investigatory work which is expected to result from technological and industrial development in tropical countries*.

Every year the Institute receives an increasing number of requests for specialist staff to visit tropical countries to give on-the-spot advice on problems connected with the use of natural products. The present commitments at the Institute often prevent the release of existing staff for this work. The Institute's Managing Committee considers that additional staff should be recruited so that experienced scientists will be available at relatively short notice for overseas investigations and, possibly, for the training of overseas laboratory staff in their own countries. Many tropical countries are beginning to set up their own research organizations with facilities for undertaking some investigations which previously would have been carried out at the Tropical Products Institute. However, the lack of suitable trained staff is still a problem, and it is recommended that opportunities for training them should be provided at the Institute in London.

The Director reports more than 960 inquiries for advice and assistance from 65 countries during 1961. About 20 per cent of these inquiries related to marketing, trade and economic aspects of various tropical commodities. A further 30 per cent were concerned with processing methods and machinery, packing, storage and transport and the capital, plant and labour required for the establishment of small industries.

Among the investigations was one which may lead to a new coffee industry in British Guiana. For the first time an 'instant coffee' has been made from Liberica coffee beans grown in British Guiana. This was done during an investigation to find out if a local 'instant coffee' industry could provide an additional market for the increasing production of Liberica coffee beans, almost the only remunerative crop that will grow in the peaty soils of the British Guiana plantations. Coffee infusions were prepared at the Tropical Products Institute from two blends of Liberica and Robusta coffees. The powders proved to be of good flavour, the objectionable tang normally associated with Liberica coffee being largely removed during the processing operations. It would seem, therefore, that an acceptable 'instant coffee' could be manufactured from this coffee bean, with or without the addition of Robusta coffee. As a result the British Guiana Government has asked the Institute to obtain an estimate of the cost of a machine to prepare 'instant coffee', and plans for a suitable factory building, including machinery layout.

Other research workers examined an African weed to see if it could be used as a new source of oil. The seeds of *Trichodesma zeylanicum* (a member of the borage family), which grows as a pernicious weed in many parts of Africa, and especially on maize farms in Northern Tanganyika, may now prove to be a commercial source of oil. Investigations have shown that the seed contains about 30 per cent of a drying oil, similar to linseed oil, which, if it could be produced economically, might be used in the paint, linoleum and leather industries. This oil content is low compared with major oil-producing crops such as groundnuts (about 50 per cent), copra (some 60 per cent), and linseed (about 40 per cent), but it might be improved by plant breeding, as has been done with linseed. As the seed contains supinine, an alkaloid which causes liver damage, the extracted meal is not suitable as an animal feeding material.

Cloves are Zanzibar's biggest export, providing about three-quarters of the world's supply. The islanders are largely dependent on this spice for their livelihood, and efforts have been made to find new uses for cloves so that the local economy can be improved. Clovebud oil, which is responsible for the aroma and flavour of cloves, is now being re-examined at the Tropical Products Institute with the use of new chemical techniques which may reveal the presence of components overlooked or wrongly identified in earlier analyses.

Although regular spraying of tea bushes with DDT is not a normal practice, it is important to know to what extent DDT persists in tea, both before and after processing, and in any infusion prepared from it, since such sprays are occasionally used. Research workers at the Tropical Products Institute have found that, while a large proportion of DDT present in the green leaf persisted throughout all stages of processing, only very low levels could be detected in infusions. This was so even when levels of DDT were much higher than those likely to be encountered in commercial practice. A similar study of sacks of whole root ginger, sprayed with DDT in Jamaica, showed that the levels of DDT persisting on the ginger after storage were sufficiently high to suggest that this insecticide should not be used for the protection of large stacks of ginger without further investigation. Tests with malathion, however, showed that the persistence of this insecticide was much lower than that of DDT and that contamination of stored ginger would be only slight if the insecticide were applied at normal rates.

Experiments at the Tropical Products Institute may lead also to a new industry in West and Central Africa, South America, India and other sub-tropical countries. Large quantities of groundnut shells, or husks, have no industrial use at present and disposal is difficult. They do not burn easily, nor do they rot quickly enough when returned to the land. Preliminary trials have produced an experimental particle board which is technically adequate and cheap to produce.

There are three important reasons why this industry could be developed in these countries with economic advantage. There is an abundant supply of the raw material in areas where there is often a shortage of low-priced building and furniture materials. No machinery would be required to reduce the shells to suitable sized particles, and the shells would be sufficiently dry when produced to be made into board without further drying. Preliminary trials are also in progress using rice hulls, coir waste, bagasse and other materials.

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* Department of Scientific and Industrial Research. Report of the Tropical Products Institute 1961: the Report of the Tropical Products Institute Committee with the Report of the Director of the Tropical Products Institute. Pp. vi+37. (London: H.M.S.O., 1962.) 3s. net.

TRAINING OF BIOLOGISTS

A CONFERENCE arranged by the Institute of Biology to discuss the training of biologists in all spheres of their activity was held during April 17–18 at The Polytechnic, Regent Street, London

The problems discussed during the first session were largely those concerned with the early training of biologists in the schools The first speaker was Mr Hugh P Ramage, chairman of the panel which framed the proposals of the Science Masters' Association and the Association of Women Science Teachers[1] Their view was that all pupils should pursue a balanced course of scientific study up to the sixth form In the sixth form, regardless of their special aims, all should continue with a broad 'general study' of science, if they were scientists the detailed factual content of their Advanced Level courses should be reduced Dr L H Finlayson (University of Birmingham), discussing the Biology Panel report in the Gulbenkian inquiry[2], endorsed the view commonly shared that the separate disciplines of botany and zoology should be abandoned in favour of the single subject biology, this would allow better opportunity for young biologists to acquire essential collateral training in chemistry, physics and perhaps mathematics Both he and Mr D A Coult (University of Liverpool) discussed the as yet unconfirmed proposals of the Northern Universities Joint Matriculation Board Biology Panel for a syllabus in which a synthesis of these two reports had been attempted It had been accepted that the basic language of biology could well be taught by reference to mammals and flowering plants, but that thereafter the Northern Universities Joint Matriculation Board Panel had adopted (with modification) the Gulbenkian proposals, whereby essential biological principles were taught with complete freedom of choice of illustrative material on the teacher's part Only by this means could individual enthusiasms be encouraged, it was planned to provide fairly extensive notes to help teachers who needed more guidance Many speakers expressed anxiety about the text-book situation and the need not only for good 'narrative' accounts of biological fields in which exciting advances have been made, but also for help in teaching biology as an experimental science Considerable criticism was voiced about the value at this stage of the demand for detailed dissection of the rat It was pointed out that even if the demand for a detailed dissection could be justified, the rat was almost an economic necessity for all but the small examining bodies

Prof C T Ingold (Birkbeck College, London) and Prof B M Jones (Aberystwyth) discussed university requirements for entry into botanical and zoological departments respectively Both agreed that most freshmen would be all the more acceptable for having received some training in mathematics and the physical sciences in addition to biology, but that it would always be a pity if this precluded entry for the naturalist with no aptitude for the physical group of sciences Both agreed also that a wide spread of at least three, if not four, Advanced Level subjects was desirable, and, should this prove too great a load, the proper remedy was surely to reduce the factual content of syllabuses Prof Jones, supported by Dr S. J Pirt (Queen Elizabeth College, London), advocated the reintroduction of subjects at a lower level (effectively the old subsidiary level of the Higher School Certificate) in order to make the spread more realizable It was greatly to be hoped that by this means sixth-form pupils with a flair for chemistry and physics could also be introduced to biology

The second session of the conference was concerned with the teaching of biology in the universities, and was opened by Prof J W S Pringle (Oxford), who analysed the problems of teaching in twenty-nine departments of zoology Variations were to be seen in respect of exemption from first-year work on the basis of good Advanced Level qualifications, and on the amount of specialization allowed in the last (third or fourth) year of the course Generally speaking, he said, a measure of specialization was found inevitable, but should never be allowed to prejudice the essentially broad training for a first degree Nevertheless, comparative anatomy should always leave time for teaching those non-taxonomic aspects of zoology which gave true integration to the course The problem, as he saw it, was to reconcile the slower-moving evolutionary studies, attractive to the naturalists, with the newer experimental fields in which the pace is at present being set He then outlined the projected first-year biology course at Oxford, which includes physics, chemistry and geology for biologists, who in their own right also take a course including genetics, general physiology, taxonomy, evolution and ecology This can then lead on in subsequent years to more specialized studies of plants and animals, of which systematic study is only one aspect It was planned also to provide for instruction in biology to postgraduate physicists and chemists

Prof W O James (Imperial College of Science and Technology, London) said that his Botany Department was concerned with the training not only of teachers and botanical technologists and the research workers of to-morrow, but also of those who were unlikely to become professional botanists He spoke strongly in favour of catering more meaningfully for the needs of our teachers, as for potential professional botanists, he pointed out that training must make it possible for such students to understand the language of the cognate sciences, so that communication and co-operation between the sciences might become more fruitful He was not in favour of a special project in the final year, which as low-grade research often got out of hand, but preferred the student to make a choice of one out of three options, namely, ecology with taxonomy, physiology with plant biochemistry, and mycology with plant pathology All such options also involved advanced formal teaching and long-term practical work

Prof H E Street (University College of Swansea) turned to consider the postgraduate training of biologists He strongly criticized the system of three years' training for a doctorate, first, in that it did not involve enough compulsory formal tuition and, second, because there was such a high pass-rate He did not believe in the attitude of 'sink or swim' for postgraduates any more than he believed in boosting a poor performance, at the last, with all the skills of the supervisor, a middle course was infinitely preferable He would prefer to see the Ph D candidate selected after an M Sc year consisting of broadly based formal teaching and an initiation into research, and he deplored the fact that too many candidates with 'lower seconds' were being given postgraduate degrees or diplomas in topics such as microbiology and biochemistry, etc , on the basis of formal tuition alone Prof D J Carr (Queen's, Belfast) thought that Prof Street's views might not easily apply to small departments Dr K Mellanby (Nature Conservancy) thought that suitable research stations might play quite an important part in postgraduate training Prof A H Bunting (University of Reading) asked for a better liaison between schools, universities and research institutions in order to promote better vocational orientation toward the satisfying careers available especially in applied biology Mr T A Oxley (Civil Service Commission) directed attention to the fact that students could gain valuable experience by taking vacation jobs in Government research establishments, while at a higher level a research fellowship scheme was operated Details of these could be found in a pamphlet recently published by the Civil Service Commissioners and entitled *The Scientific Civil Service*

The third session, held on the morning of April 18, fell into two parts Mr H Tristram (University College, London) spoke first on the teaching of microbiology in the universities He thought that there was some justification for a first degree in microbiology, provided that micro-organisms were used to illustrate the fundamental properties of living organisms, and that a full coverage of all types of micro-organisms was available, cutting across all the traditional boundaries He emphasized the value of micro-organisms in biochemical teaching, each organism, too, occupied its own ecological niche and demanded a 'naturalist' approach Practical work should concern itself with fundamental principles, and with experimental techniques which were a means to an end, and not an end in themselves Entrance requirements for such courses as exist all show a demand for initial training in chemistry, and for preference also in physics and mathematics Dr S J Pirt (Queen Elizabeth College, London) in a joint paper with Dr J R Postgate (Microbiological Research Establishment, Porton) discussed the desirable training for research in economic microbiology Having outlined the scope of economic microbiology, he underlined Mr Tristram's case for non-medical microbiology as a separate and specialized discipline, rather than as a branch of botany Posts available up to now had largely been filled by chemists, who picked up their microbiology second-hand A proper training course was to be preferred and should include advanced instruction in chemistry and the physical principles appropriate to aseptic engineering techniques, and enough mathematics to handle quantitative data and to design experiments that could be statistically controlled He deprecated ancillary training in the macrobiological aspects of botany and zoology, while welcoming the comparative biochemistry of higher plants and animals To the microbiological disciplines advocated by the first speaker he would add the techniques of tissue culture, and he thought that optional courses in microbial genetics, cytology and ecology would be indispensable The evolution of microbiology was fast and exciting, and it demanded a considerable flexibility of approach for the solution of its problems Prof D G Catcheside (University of Birmingham) thought that, as time was so limited, each course in whatever department should be built around a theme In Birmingham, for example, microbiology was developed about the theme of the genetic material and its expression As to the demand for more physics, chemistry and mathematics in biology, he thought that biologists without such training would be squeezed out of a larger increasing part of biology

Mr J B Copp (general secretary, Institute of Biology) discussed the means whereby biological laboratory assistants in industry and Government service might proceed by an alternative route to full professional status as biologists At present there were two levels, Ordinary and Higher, of the National Certificate Since 1957 the Ministry of Education has joined with the Institute in endorsing these certificates for biology In Scotland candidates have been able to sit for National Certificate examinations in biology since 1961 In England it is proposed to replace the Ordinary National Certificates at present available in scientific subjects with a generalized Ordinary National Certificate in Science The Ministry has been asked to introduce a Higher National Certificate in biology for subsequent training of those specializing in biology

Dr T G Onions (Brunel College of Technology) reviewed the institution and progress of the Diploma in Technology in applied biology This Diploma was open to entry both from schools ('college-based students') and from biological industry ('industry-based students') The first group predominated and received some of their training with the active co-operation of industry though as yet industry was not sending many of its own employees for sandwich courses The academic course was completed by a project and thesis, often the result of ideas stimulated by work with the host organization

Dr Onions commented on the need for liberal studies during this period and especially for continued training in the writing of good English He pointed out that the Diploma in Technology was intended to have the equivalent of graduate status, whether for purposes of proceeding to higher degrees, for full membership of the Institute of Biology, or generally for grading in the Scientific Civil Service

In discussion at the end of this period, a variety of opinions was expressed about the proposed Ordinary National Certificate in Science so far as biology, now much reduced in content, was concerned Mr D O Weitzel (Coventry Technical College) felt that a complete revision of syllabuses was required at both Ordinary and Higher Levels Mr E Norris (Ewell County Technical College) suggested a synthesis in which a good background of chemistry, physics, mathematics and English was deliberately slanted towards biology, even though specialization in biology itself was deferred to a later level It might, however, be necessary, he said, to combine this early general training with a general qualification in biological technology

The final session was opened by Mr T A Oxley (deputy commissioner, Civil Service Commission), who dealt with the requirements of the Civil Service for biologists He explained the classification of scientific officer, experimental officer and scientific assistant, pointing out that these categories were also in use in the Nature Conservancy and the Agricultural Research Council, although these bodies were strictly separate from the Scientific Civil Service He gave an analysis of the distribution of biologists of all grades throughout the various Governmental services He commented on the greater degree of specialization among biologists, which made it more difficult for them to change their field of employment than for, say, physicists He urged the importance of training in clear thinking and writing of reports There was a difficulty, he said, in persuading physicists or mathematicians to enter biological research teams, and one remedy would be for biologists with the appropriate flair to read a second degree in either subject

Prof F L Milthorpe (University of Nottingham) followed with a paper on the type of training suitable for agricultural research He sketched out the ramifications of the whole agricultural system and stressed the need in a successful course for presenting the basic principles of each of the interrelated disciplines, while keeping the integrated complex in mind The need for the use of teachers of so many disciplines helped to avoid the inbred narrowness of botany and zoology As for postgraduate training, he was certain that such training should be by research, that recruitment was to be preferred from a lower level such as plant physiology into a higher level such as agronomy, and lastly, that projects framing a very restricted part of an extensive programme are rarely satisfactory for training in research

Dr F Kingsley Sanders (director Medical Research Council Virus Research Unit) gave his views on the necessary qualifications for research in virology and molecular biology Biologists appeared to occupy a rather lowly position in his scheme for selection, unless they had undergone considerable training in physics beforehand

The final contribution from Dr W A Bain (Smith, Kline and French) dealt with the use of biologists in research and development in the pharmaceutical industry He thought that pharmacologists were in particularly short supply Working conditions in industry, he said, compared very favourably with those in universities, both in respect of emoluments, equipment and promotion prospects He paid tribute to the work of the biological technicians, and voiced the view that industrial firms were less liable to make the mistake of the universities, who had largely failed to appreciate the importance of technical staff in their scientific departments He congratulated the Institute of Biology, which, in conjunction with the Ministry of Education, had promoted the Higher Endorsed Certificate in biology, which should prove invaluable in this field

In the discussion at the end of the fourth session, disquiet was expressed that in the specialist demand for chemistry, physics and mathematics, biology would be even more than at the present squeezed out of its rightful place in the school Dr J C Smyth (Paisley Technical College) thought that the Institute's most important task was to improve the public image of biology, especially in the schools, by presenting it as a more rigorous scientific discipline Mr D O Weitzel (Coventry Technical College) thought that an Advanced Level course of 'background science for biologists' would meet the needs of the schools better than training in Advanced Level mathematics, physics and chemistry

Mr R Weatherall (Avery Hill Training College) spoke of the need for research into the whole educational process Were it more possible with the aid of the educational psychologists to detect and train real scientific ability, the way would be open for the already urgent reform of the examination system, which in its turn would bring more freedom at all levels to scientific teaching

The conference was well attended throughout and it was abundantly clear that there is a widespread and lively interest in improving the ways and means of training all categories of biologists during this present explosive period of expansion in biology

D A Coult

[1] *Biology for Grammar Schools* (A Section of Part I of the Science and Education Report of the S M A and A W S T) (John Murray, 196)

[2] *Report of an Enquiry into the Suitability of the G C E Advanced Level Syllabuses in Science as a Preparation for Direct Entry into First Degree Courses in the Faculty of Science* (Section *A* Report of the Biology Panel) (University of Birmingham, 1959)

ASPERGILLUS FLAVUS AND TURKEY X DISEASE

Isolation in Crystalline Form of a Toxin responsible for Turkey X Disease

THE toxin responsible for the so-called Turkey *X* disease has been described by Sargeant *et al* [1] as a metabolite of the mould *Aspergillus flavus* Link ex Fries Elsewhere[2] we have described the examination by thin-layer chromatography of toxin-containing extracts of groundnut meals and cultures of *Aspergillus flavus*, and presented evidence which suggests that such extracts may contain more than one substance toxic to ducklings This communication is concerned with the isolation in crystalline form of one of these substances from cultures of *Aspergillus flavus*, and gives an account of some of its physico-chemical properties

Mould cultures grown on a medium of wetted, crushed sterile groundnuts were extracted with chloroform/methanol (2 1), and the residue remaining after evaporation of the solvent defatted by partition between 90 per cent aqueous methanol and light petroleum The methanol-soluble material was chromatographed on a column of 'Silicagel' ('Mallinckrodt') in chloroform/1 per cent ethanol, and the zone fluorescent in ultra-violet light further purified by thin-layer chromatography on 'Kieselgel *G*' (Merck), column chromatography over 'Kieselgel *G*'/'Celite', and finally, filtration through a short column of alumina After crystallization from chloroform/ethanol, the recovered material was obtained as gleaming, almost colourless, anisotropic, monoclinic platelets (Fig 1) which had no sharp melting-point but decomposed at about 220° C Seventy-two chromatoplates yielded 40 mg

The same substance, which pending complete structural characterization we refer to as FB_1, was more simply isolated from liquid cultures of the mould in glucose/ammonium nitrate medium Chromatography of a chloroform extract of the culture medium

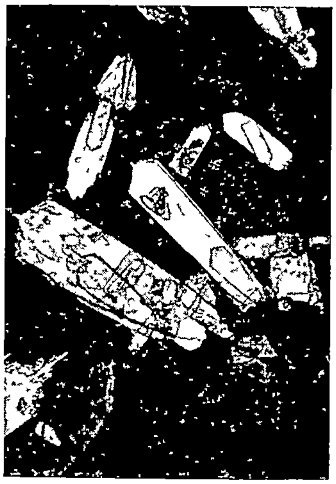

Fig 1 Crystalline toxin (× c 84)

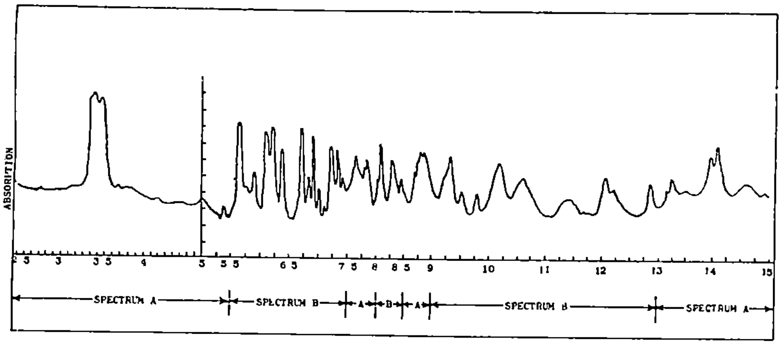

Fig 2 Infra-red absorption spectrum of fraction FB_1

State of sample

(*A*) Mulled in nujol	(*B*) in bromoform
2 5 μ–5 5 μ	5 5 μ–7 5 μ
7 5 μ–8 0 μ	8 0 μ–8 5 μ
8 5 μ–9 0 μ	9 0 μ–13 μ
13 0 μ–15 μ	

on a column of a 'Kieselgel *G*'/'Celite', developed with chloroform containing 0 25 per cent ethanol, provided a fraction which exhibited a blue-violet fluorescence in ultra-violet light, and which gave a single fluorescent spot on a chromatoplate coated with 'Kieselgel *G*' Evaporation of the solvent yielded a residue which readily crystallized from chloroform/ethanol, this crystalline substance was identical with FB_1 obtained from cultures on groundnut medium

The LD_{50} value of FB_1 to ducklings (one-day old Peking ducklings), 55 g mean body-weight, single dose (observed for 48 h), was 30–50 μg

Infra-red, nuclear magnetic resonance, and ultra-violet absorption spectra of FB_1 are shown in Figs 2–4, *E* (1 per cent, 1 cm) at λ_{max} 360 mμ was calculated to be 660

Under certain conditions, FB_1 is apparently photosensitive. In hydroxylic solvents such as water, methanol, ethanol, acetic acid, and particularly in the presence of oxygen, irradiation with ultra-violet light caused fading of the fluorescence, and a change in the ultra-violet absorption spectrum The change brought about by brief exposure is shown in Fig 4, curve II, prolonged exposure resulted in the disappearance of the peak at 360 mμ Moreover, solutions of FB_1 in these solvents which before irradiation gave a single spot on a chromatoplate afterwards were resolved into several additional fluorescent spots of lower R_F values

Treatment of FB_1 with alkali caused a shift of λ_{max} 360 mμ to λ_{max} 370 mμ, the shift being reversible on immediate acidification For the irradiated material, the shift was from λ_{max} 353 mμ to λ_{max} 401 mμ, this change was reversible on immediate acidification, but after prolonged contact with alkali, the peak at 401 mμ disappeared, and the 353 mμ maximum was not regenerated on acidification

The interpretation of ultra-violet, infra-red and nuclear magnetic resonance and mass spectroscopic results (and certain chemical data not reported here) in terms of chemical structure is in hand

We thank the Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, Wey-

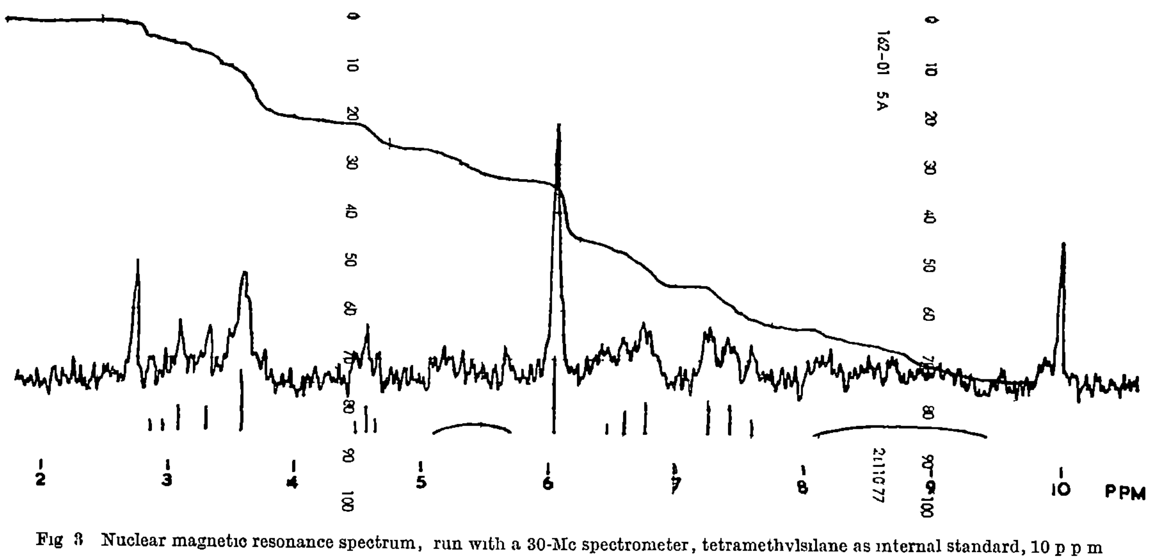

Fig 3 Nuclear magnetic resonance spectrum, run with a 30-Mc spectrometer, tetramethylsilane as internal standard, 10 p p m

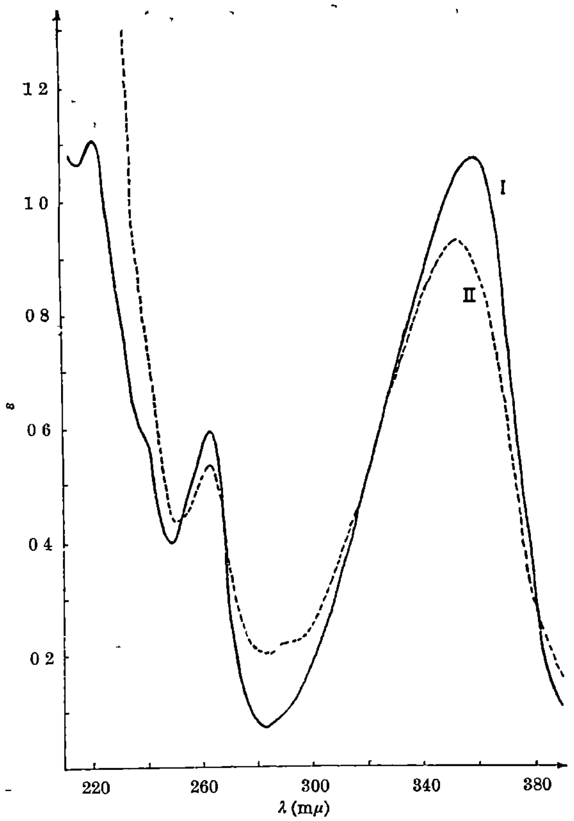

Fig. 4. Ultra-violet absorption spectrum of fraction FB_1 (in ethanol)

bridge, for the supply of cultures of *Aspergillus flavus*, and for extracts of cultures grown in liquid medium.

Note added in proof. Since submitting this communication we have obtained evidence, as a result of isolating larger quantities of material, that the crystalline toxin still contains some impurities. This evidence is from ultra-violet absorption spectra and from mass spectrometry, and will be described in a later communication.

A. S. M. van der Zijden
W. A. A. Blanche Koelensmid
J. Boldingh
Unilever Research Laboratory,
Vlaardingen, The Netherlands.
C. B. Barrett
W. O. Ord
J. Philp
Unilever Research Laboratory,
Port Sunlight, England.

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[1] Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R. B. A., *Nature*, 192, 1096 (1961).

[2] Barrett, C. B., de Iongh, H., *et al.*, *Biochim. Biophys. Acta* (in the press).

Toxic Metabolites of *Aspergillus flavus*

Further work has been carried out on the purification and characterization of the toxin produced by certain strains of *Aspergillus flavus* and described by Sargeant *et al.*[1]

The fluorescent material which was described as running on paper in *n*-butanol–acetic acid at R_F 0·7 has been further resolved on alumina chromatoplates in 98·5 per cent chloroform/1·5 per cent methanol[2]. Two spots which fluoresce in ultra-violet light are obtained, one running at R_F approximately 0·6 and having a violet-blue fluorescence and the other running slightly more slowly and having a green fluorescence. For convenience, these are referred to as Aflatoxin '*B*' and Aflatoxin '*G*' respectively.

Mixtures of these two compounds in varying proportions have been obtained both from sterilized groundnuts inoculated with the toxic strain of *A. flavus* and from Czapek's Dox medium with added zinc sulphate on which the fungus had been grown for 5–7 days at 27° C. The toxic mixture was isolated from the nuts by extraction with hot methanol, the methanolic extract was then concentrated, diluted with water and defatted with 40°–60° petroleum ether. The methanol was distilled off and the toxin extracted into chloroform, the chloroform extract was concentrated and passed down a column of silica gel. The fluorescent fraction from the column was evaporated to dryness under reduced pressure and the residue recrystallized from benzene or methanol. Crude crystalline toxin was obtained from the synthetic culture by direct extraction with cold chloroform, followed by chromatography of the concentrated chloroform extract on silica gel.

Aflatoxins '*B*' and '*G*' have been separated from one another by counter-current distribution in chloroform/carbon tetrachloride/water/methanol, 2 : 2·5 : 1 : 3 (200 transfers). After recrystallization from methanol, Aflatoxin '*B*' was obtained as irregularly shaped plates which melted with decomposition at about 270° C with prior softening at 250°–260° C. Elementary analysis gave C = 65·6, H = 4·4, O = 30·9 per cent and mass spectrometric examination gave 312 or 314 for the molecular weight. $C_{17}H_{12}O_6$ requires C = 65·4, H = 3·9, O = 30·7 per cent, mol. wt. 312. The ultra-violet spectrum showed peaks with maxima at 223 mμ, 265 mμ and 363 mμ, the molar extinction coefficients being 23,400, 12,700 and 20,500 respectively (assuming a molecular weight of 312). (In earlier work, blue fluorescent material was obtained with higher molar extinction coefficients for the ultra-violet peaks, in particular $\varepsilon_{363\ m\mu} = 44,500$. The other characteristics of this material were closely similar to those of Aflatoxin '*B*'.) The fluorescence spectrum showed a peak at 429 mμ, the absorption peak at 363 mμ in the ultra-violet being the excitation peak for this fluorescence. The infra-red spectrum in chloroform indicated that OH groups were absent, unless chelated; there were two peaks in the carbonyl region at 1,755 cm⁻¹ and 1,688 cm⁻¹. Measurement of optical rotation in chloroform gave $[\alpha]^{25}_{D} = -562 \pm 15$ ($c = 0·115$).

Preliminary biological tests on day-old ducklings indicate an LD_{50} of less than 20 μg.

Aflatoxin '*G*' has been purified by rapid filtration through a column of acid alumina and repeated recrystallization from methanol, giving fine, colourless needles with melting-point 247°–250° C. Elementary analysis gave C = 61·8, H = 4·0, O = 34·4 per cent, and mass spectrometric examination indicated a mol. wt. of either 326 or 328. $C_{17}H_{12}O_7$ requires C = 62·2, H = 3·7, O = 34·1 per cent and mol. wt. 328. The infra-red spectrum was closely similar to that of Aflatoxin '*B*', the major difference being a shift of a peak at approximately 1,125–1,140 cm⁻¹. The ultra-violet absorption spectrum has peaks with maxima at 265 mμ and 363 mμ (molar extinction coefficients 10,500 and 18,700 respectively, assuming a mol. wt. of 328). The fluorescence peak is at a slightly longer wave-length (450 mμ) than that of Aflatoxin '*B*'. The value for the optical rotation, $[\alpha]^{25}_{D}$, was -533 ± 5 ($c = 0·133$).

Biological tests on day-old ducklings indicate an LD_{50} of about 60 μg for this material

Work is in progress on the further characterization of the compounds described and on the elucidation of their structures

We thank Mr R B A Carnaghan of the Central Veterinary Laboratory for the biological tests Miss H Brown for technical assistance, Dr R I Reed of the University of Glasgow and Dr H Powell of the British Petroleum Research Centre for mass spectrometric determinations and Dr V Thaller of the University of Oxford for the measurement of optical rotations

Note added in proof. Since submitting this communication we have obtained evidence that Aflatoxin '*B*' is a mixture of two closely similar compounds

Brenda F Nesbitt
J O'Kelly
K Sargeant
Ann Sheridan

Tropical Products Institute,
London

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[1] Sargeant, K, Sheridan, Ann, O'Kelly, J, and Carnaghan, R B A, *Nature*, **192**, 1096 (1961)

[2] Shone, G (private communication)

LOSS OF INFECTIVITY ON DRYING VARIOUS VIRUSES

By F. E BUCKLAND and D A J TYRRELL

Medical Research Council, Common Cold Research Unit, Salisbury

ALTHOUGH certain viruses survive drying in air, others do not, and it has been shown recently by Hemmes, Winkler and Kool[1] that the survival of the latter is affected by the relative humidity of the air Poliomyelitis virus type 1 and one strain of influenza virus type *A* were sprayed into a chamber and the rate of decline of infectivity was measured The rate of poliovirus decline was low in air of a relative humidity greater than 50 per cent but higher in drier air the rate for influenza virus was low when the relative humidity was below 40 per cent and high when it was above 50 per cent These observations have in general been confirmed by Harper[2]

We have examined the effect of drying a number of viruses by means of a much simplified method Viruses were grown in eggs or in tissue culture and for test were suspended in or diluted in 2 per cent calf serum and 0 25 per cent lactalbumin hydrolysate in Hanks's saline containing 0 09 per cent sodium bicarbonate and antibiotics Twenty 0 0001 ml drops of the suspension were distributed with a microsyringe on each of four clean glass slides which were put at once into (*a*) a desiccator containing a saturated solution of potassium acetate (relative humidity of air 20 per cent at 20°), (*b*) a desiccator containing a saturated solution of potassium bromide (relative humidity of air 84 per cent at 20°), (*c*) a Petri dish containing wet filter paper maintained at room temperature (as were *a* and *b*), (*d*) a similar Petri dish kept at 4°

After 2½ h, the drops in *a* and *b* had dried while those in *c* and *d* were wet The slides were then washed down thoroughly with 1 ml of suspending medium and this was immediately titrated for infectivity the influenza viruses were titrated in embryonated eggs and the other viruses in tissue cultures

Preliminary experiments showed that no detectable virus was lost on to the slide by this procedure, whereas there were considerable losses if the drops were spread on cotton, or nylon

The results of a representative experiment are summarized in Table 1 The titres in *c* and *d* were usually very close, but we detected marked thermal inactivation of RS (respiratory syncytial) virus at room temperature under these conditions and so this virus was not studied further Experiments were usually repeated two or three times and the results are given in Table 2 This shows that although conditions of drying were different from those used by Hemmes *et al* we were in general able to confirm their results, in that the infectivity of all three serotypes of poliovirus was more unstable at 20 per cent relative humidity than at 84 per cent relative humidity, the other six enteroviruses tested gave a similar result Strains of influenza *A* were, on the other hand, more stable at low relative humidity

Rhinoviruses and adenoviruses behaved like enteroviruses and survived better at high relative humidity Parinfluenza and *REO* viruses survived as well at high as at low relative humidity The arbor virus we tested seemed to survive best at low relative humidity but this result must be viewed with caution since Harper's data (his Table 1) suggest that the arbor virus he studied (*VEE*) survived best at high relative humidity up to 4 h and at low relative humidity afterwards We note that the viruses that

Table 1 TYPICAL DRYING EXPERIMENT

Amount of ECHO 11 virus recovered from drops left on glass slides for 2½ h under conditions stated

Temperature	Room	Room	Room	4°
Relative humidity	20 per cent	84 per cent	100 per cent	100 per cent
	(*a*)	(*b*)	(*c*)	(*d*)
ID_{50}	$10^{1\ 5}$	10^3	10^3	10^3
Log reduction in titre log *c*/*a* or *c*/*b*	1 5	0	—	—

Table 2 REDUCTION OF TITRE OF VIRUSES ON DRYING

Virus used	Mean log reduction in titre of virus recovered from drops dried at room temperature for 2½ h at relative humidity of 20 per cent	84 per cent
Myxoviruses		
Influenza *A* (W S)	1 7	3 5
,, *A* (swine)	1 4	2 6
Para-influenza 2	0 8	0 6
Enteroviruses		
Poliovirus 1	1 6	0 6
,, 2*	1 6	0 4
,, 3	2 1	0 8
Coxsackie *A*21 (Coe)	0 8	0 4
,, *B*4	2 2	1 7
ECHO 7	1 0	0 5
,, 11 prototype	1 5	0
,, 11 "U"	3 7	0 5
,, 12	1 9	0
,, 28	1 2	0 2
Rhinoviruses		
*B*633	0 9	0 2
Adenoviruses		
Type 1	1 6	1 0
,, 3	1 7	0
S V 17	1 0	0
Reoviruses		
Type 2*	0 5	0 7
Arborviruses		
Semliki Forest	1 3	1 8

* Owing to the low titre of the inoculum a smear of 0 02 ml was used

lack lipids, namely, enteroviruses and adenoviruses, all survived best at high relative humidity while those which have lipid components, namely, influenza viruses and the arbor virus, survive best at low relative humidity The method seems, therefore, to subdivide the viruses we tested into the same groups as the ether sensitivity test[3], although the results with parainfluenza and *REO* viruses are indeterminate However, vaccinia is ether stable and Harper[2] found that it survived better at low than at high relative humidity His data also show (Table 1) that at relative humidity 50 per cent inactivation of poliovirus was more complete and rapid than at relative humidity 35 per cent or 65 per cent It is of interest that some viruses which survive less well at low relative humidity, for example, poliovirus, cannot be successfully freeze dried, while others, for example, adenovirus, can It is clear, therefore, that it is premature to suggest physiochemical explanations for these phenomena

Hemmes *et al* suggested that the survival of viruses at high and low humidities might account for their seasonal prevalence In winter, when the relative humidity indoors became low because the air was artificially heated, poliovirus would survive badly This difference might account for the prevalence of influenza in winter and of poliovirus in summer We consider that the effect of relative humidity on virus survival may indeed influence seasonal prevalence but it cannot determine it completely, for Table 2 shows that some respiratory viruses such as *B*633 [4] and adenovirus type 3, which are particularly common in the cold months of the year, have a stability pattern like that of poliovirus In addition, Hemmes *et al* calculated, from the recorded outdoor humidities and the temperatures indoors and outdoors, that indoor humidities of 40 per cent occurred in Britain in the winter This, however, does not take account of the water vapour produced by human beings and by domestic activities In the winter of 1960–61 the lowest relative humidity recorded in this centrally heated laboratory was 47 per cent In a centrally heated house nearby the relative humidity was between 50 and 60 per cent Similar findings have been reported by Hope-Simpson[5] In the exceptionally cold dry spell in March 1962 measurements in a centrally heated house and in the kitchen of a cottage heated by a range showed a relative humidity that never fell below 40 per cent Readings taken in the local theatre at that time showed that the relative humidity rose from 46 to 53 per cent during a performance and fell back to 45 per cent in 15 min when the theatre emptied Hutcheon[6] has shown that even in the Canadian winter the relative humidity inside a house may be much higher than that calculated from conditions outdoors It is therefore probable that in Britain, except in unusually dry cold winters, the relative humidity in living rooms and public places, where virus transmission probably takes place, rarely falls below 50 per cent, the level at which the factors demonstrated by Hemmes *et al* may be expected to operate

It is possible that changes in relative humidity affect the reaction of the host to virus infections, but we feel that in future any attempt to make a complete analysis of the effect of the environment on the epidemiology of a virus will have to include the demonstrated effect of relative humidity on virus survival outside the body We thank Dr D Taylor-Robinson for help in certain experiments

[1] Hemmes, J H , Winkler, K C , and Kool, S M , *Nature*, 188, 430 (1960)

[2] Harper, G J , *J Hyg* (Camb), 59, 479 (1961)

[3] Andrewes, C H , and Horstmann, D M , *J Gen Microbiol* , 3, 290 (1949)

[4] Taylor-Robinson, D , and Tyrrell, D A J , *Lancet*, i, 452 (1962)

[5] Hope-Simpson, R E , *Proc Roy Soc Med* , 51, 267 (1958)

[6] Hutcheon, N B , *Humidity in Canadian Buildings*, Canadian Building Digest No 1 (National Research Council, Ottawa, Canada, 1960)

EFFECT OF THE MATERNAL ENVIRONMENT ON STRAIN-SPECIFIC DIFFERENCES IN THE OVARIES OF NEW-BORN MICE

By DR ESTHER C JONES and PROF P L KROHN*

Department of Anatomy, The Medical School,
Birmingham, 15

WE[1] have reported that inbred strains of mice differ widely in the stage of development reached by their ovaries at the time of birth Thus, most of the oocytes in *CBA* strain ovaries have already entered the dictyate stage whereas in *A* strain ovaries the majority of oocytes are still in meiotic prophase The question arises whether these strain differences are due to inherent differences in the ovaries themselves or whether the maternal environment in which the ovaries develop can influence the course of oogenesis One way of analysing the problem would be to examine the stage of ovarian development reached at birth by mice derived from fertilized eggs of one strain transferred to the Fallopian tube or uterus of the other An alternative is to use orthotopic transplantation of the ovaries from one strain to another to produce pure-strain litters from genetically alien mothers[2] The discovery of methods of inducing immunological tolerance[3] has made it possible to overcome the obstacles which the homograft reaction ordinarily places in the way of this approach, and we have preferred it, since it allows the oocytes before ovulation, as well as the ova after ovulation, to develop in the foreign environment

CBA female mice have, therefore, been made immunologically tolerant of *A* strain tissues and vice versa, by the usual technique of injecting an appropriate spleen cell suspension either intraperitoneally or intravenously into the new-born The foreign ovaries were transplanted orthotopically[4] as soon as a test skin graft on the host revealed a state of complete tolerance (survival in perfect condition for 75 days) After recovery from the operation, *A* strain hosts were first test-mated with an *A* strain male, to provide a colour marker check that ovariectomy of the host had been complete,

* Nuffield Senior Gerontological Research Fellow

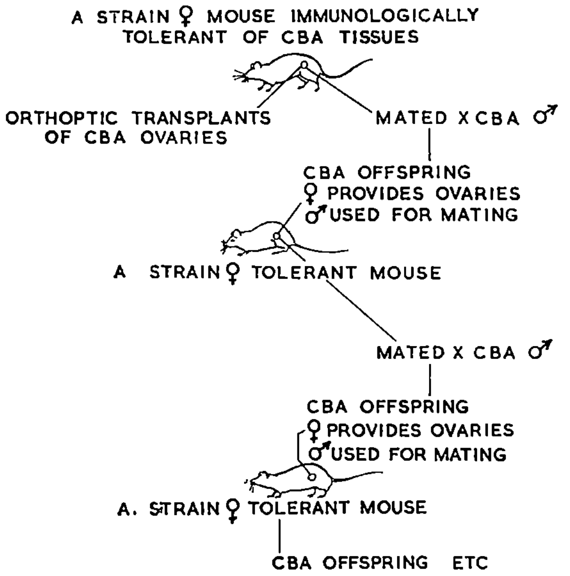
A STRAIN ♀ MOUSE IMMUNOLOGICALLY TOLERANT OF CBA TISSUES
ORTHOPTIC TRANSPLANTS OF CBA OVARIES
MATED X CBA ♂
CBA OFFSPRING
♀ PROVIDES OVARIES
♂ USED FOR MATING
A STRAIN ♀ TOLERANT MOUSE
MATED X CBA ♂
CBA OFFSPRING
♀ PROVIDES OVARIES
♂ USED FOR MATING
A. STRAIN ♀ TOLERANT MOUSE
CBA OFFSPRING ETC

Fig 1 Scheme of experiment

and were afterwards mated with a *CBA* male to produce pure *CBA* offspring This preliminary step was unnecessary for the *CBA* female hosts bearing *A* ovaries, since any remaining *CBA* ovarian tissue would immediately reveal itself in the form of agouti hybrid offspring from the mating with an *A* male Once the experiment was under way, the females of the first litters born provided donor ovaries for further transplants to other tolerant mice and the males were used for mating with these later generations The general scheme is laid out in Fig 1

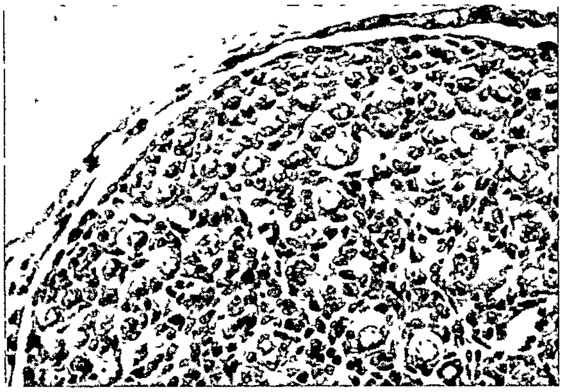

Fig 2 Ovary of a normal new-born *CBA* mouse (× *c* 100)

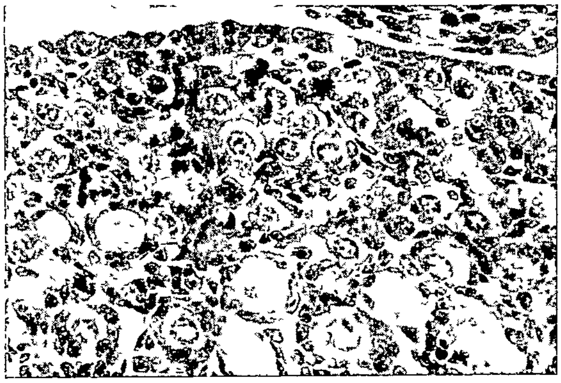

Fig 3 Ovary of a new-born *CBA* mouse derived from a *CBA* strain ovary transplanted into a tolerant *A* strain mouse (× *c* 100)

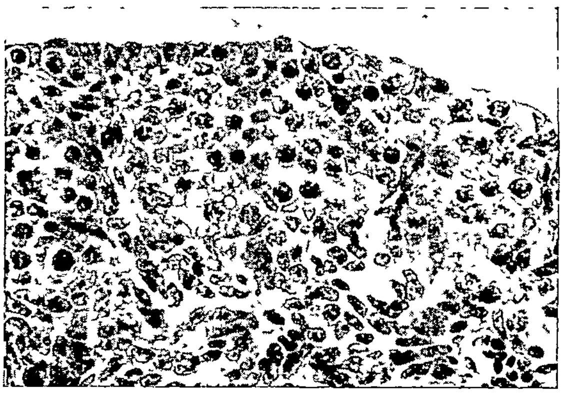

Fig 4 Ovary of a normal new-born *A* mouse. (× *c* 100)

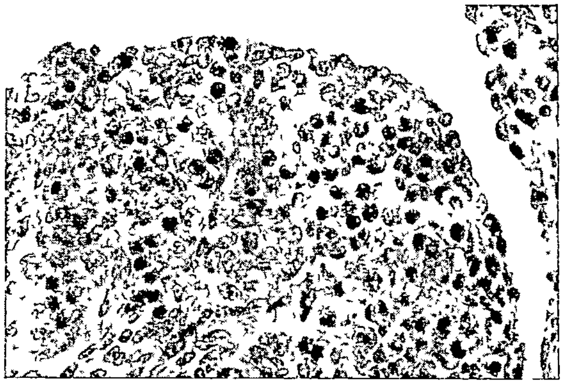

Fig 5 Ovary of a new-born *A* mouse derived from an *A* strain ovary transplanted into a tolerant *CBA* strain mouse (× *c* 100)

Seven *CBA* strain and 7 *A* strain female offspring from these immunologically tolerant mothers were killed immediately after birth and the ovaries removed and fixed Serial sections were prepared and counts made on the coded slides of the total number of oocytes, the proportion of normal to atretic cells and the proportion which were still in meiotic prophase The general histological appearance was always unequivocally that of normal *A* strain or of *CBA* strain ovaries, using the presence or absence of a large proportion of meiotic figures to divide the slides into two categories These observations, together with the results of the counts (Table 1), show that all the ovaries from new-born genetically *A* strain mice were entirely *A*-like and had been unaffected by the *CBA* environment The same was true of *CBA* ovaries, which all remained *CBA*-like (Figs 2–5)

The offspring, the ovaries of which have been examined, derived from ova which developed from the primary oocyte stage after the first transplantation, or the germ cell precursors in the second and subsequent passages, in a foreign environment Apart from the possibility of endocrine differences between strains, which might have altered the rate

Table 1 NUMBERS AND CONDITION OF OOCYTES IN THE OVARIES OF NEW-BORN *CBA* AND *A* STRAIN MICE

Strain	Environment	No of mice	Total oocytes	Normal oocytes (per cent)	Pre-dictyate oocytes (per cent)
CBA	*CBA*	3	10,900	87	55
CBA	*A*	7	10,700	85	55
A	*CBA*	7	21,100	90	93
A	*A*	8	17,200	87	91

of development of the ovaries, there are considerable opportunities during maturation and growth of the follicle for the transfer of large molecules (possibly strain specific) from the host's blood vessels to the oocytes in the grafted ovary via the closely apposed and interdigitating surfaces of granulosa cells and egg[5], and transfer could possibly have continued after fertilization both in the tube and in the uterus There are no indications, however, that even passage of *CBA* ovaries through six generations of tolerant *A* hosts has modified the specific pattern of development of the offspring Failure to show any effect also rules out the *a priori* possibility that the usual strain differences depend on differences in gestation time and hence of post-conceptional age at the time of birth

It may be concluded that even prolonged and repeated sojourn in the environment provided by a foreign strain cannot modify the pattern of oogenesis This retention of specific characteristics has also been observed for the histocompatibility antigens which determine the fate of grafted tissues Six members of earlier generations of *CBA* offspring and 13 members of the most recent generations have been tested with *A* strain skin to see whether the normal homograft reaction had been at all modified There has been no detectable deviation from the normal response Transplants of skin from the experimental *CBA* mice to 16 normal *A* strain mice have also been destroyed in the usual way

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MODIFICATION OF THE STRUCTURE OF HÆMOGLOBIN BY CHLORAMBUCIL

By J. H. LINFORD

Department of Biochemistry, University of Manitoba, Winnipeg

A RECENT report[1], concerning changes that have occurred in the *p*K of the carboxyl groups in protein as a result of acetylation of a large proportion of the amino-groups, directed attention to the lack of data concerning the effect of chemical modification of a protein on the electrical character of the molecule In this connexion it may be of interest to recount an investigation into the reaction with hæmoglobin of *p*-N, N-di (β-chorethyl) aminophenylbutyric acid (chlorambucil), as it has been shown[2-4] that the carboxyl groups in protein are esterified by this compound Hæmoglobin, molecular weight 64,450, contains 8 38 × 10^{-7} equivalent of acid carboxyl groups per mg[5] Chlorambucil, molecular weight 304, contains 65 8 × 10^{-7} equivalent of reactive chloro groups per mg, 25 mg of the latter is therefore equivalent to 196 mg of hæmoglobin

Solutions of human adult hæmoglobin which contained 1,000 mg of hæmoglobin. and which contained 300 mg of hæmoglobin, were prepared Chlorambucil solutions were prepared at 5° C by dissolving 25 mg of the compound in 3 5 ml of 0 025 N sodium hydroxide The solutions of chlorambucil and of hæmoglobin were mixed together and adjusted to the required *p*H with the 0 025 N alkali The volume was made up to 20 ml with water and the mixture was shaken at 37° C Control mixtures of the same concentrations of hæmoglobin only were prepared, adjusted to the required *p*H, and shaken at 37° C At measured intervals of time, the *p*H was adjusted to the initial value by addition of measured volumes of the standard alkali, as shown in Fig 1

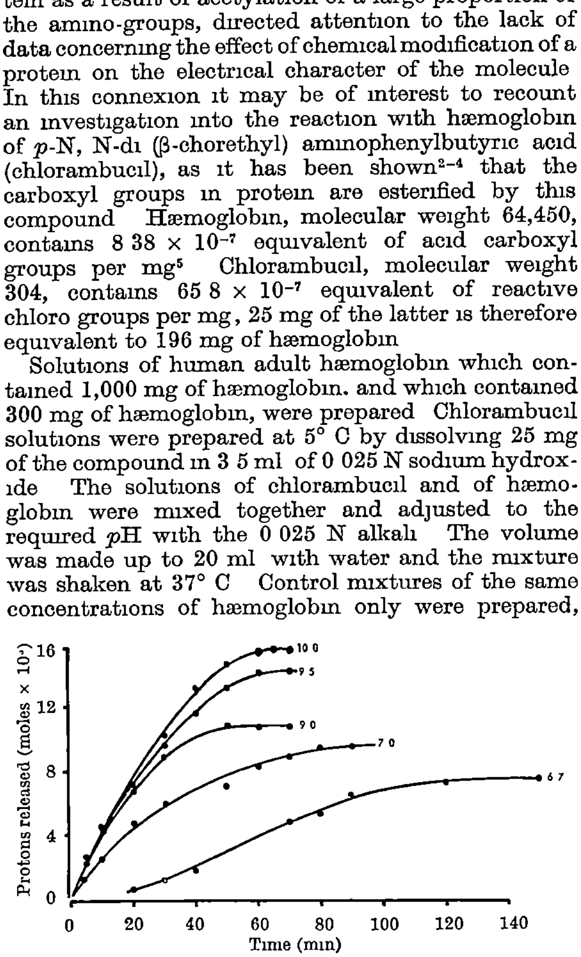

Fig 1 Rate of release of protons from 1,000 mg of human adult hæmoglobin during reaction with 16 4 × 10^{-5} moles (25 mg) of chlorambucil Numbers refer to *p*H values

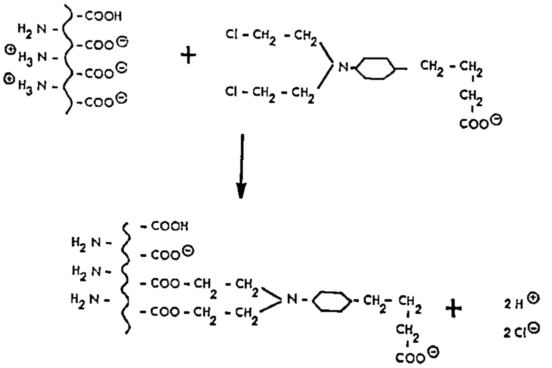

Fig 2 Diagrammatic representation of the reaction of protein with chlorambucil

Above *p*H 8 0 an appreciable change occurred in the *p*H value of the control solutions At *p*H 10 the corrected total amount of added alkali was 16 × 10^{-5} equivalent The 25 mg quantity of chlorambucil present was 16 4 × 10^{-5} equivalent It may be inferred that the esterification reaction at *p*H 10 0 was accompanied by the release of two hydrogen ions from protonated basic groups for each molecule of chlorambucil that was utilized (Fig 2) At *p*H 9 5 and below, dissociation of the basic groups was incomplete relative to the number of modified carboxyl groups

The control solutions and those containing the esterified protein were evaporated by freeze-drying, and each residue was dissolved in 5 ml of 0 15 M potassium chloride The protein which was precipitated by the addition of 8 volumes of ethanol was separated by centrifuge and was dissolved in 5 0 ml of N/5 hydrochloric acid Each acid solution was then back-titrated with 0 080 N sodium hydroxide The protonation of the protein that was esterified at *p*H 10 0 required one more equivalent of acid per mole of combined chlorambucil than was the case with the unmodified control protein (Fig. 3). At pH 9.5 and below, the amount of acid used for protonation was decreased in proportion to the reduced ionization of the charged basic groups. The amounts of chlorambucil remaining in the supernatant alcohol solutions were measured[6]. In the presence of 1,000 mg of hæmoglobin, 2 per cent of the chlorambucil remained in the uncombined reactive form; in the presence of 300 mg of hæmoglobin 2 per cent of chlorambucil was recovered in the reactive form and a further 8 per cent was recovered in the hydrolysed form. The basic groups from which the hydrogen ions were released were almost certainly the guanidyl groups in arginine, or the amino-groups of lysine, which have pK values of 12.5 and 10.5. The percentages of charged basic groups that dissociate per equivalent of modified carboxyl groups are plotted against the pH of the solutions in Fig. 4, which shows that the degree of dissociation of the affected groups approached 50 per cent at pH values near 7. The shape of the curve indicates that the degree of dissociation was directly proportional to the concentration of hydroxyl ion in the solution.

Reduction of the amount of hæmoglobin to 300 mg led to an apparently greater degree of dissociation of the charged basic groups at a given pH (Fig. 4). However, this apparent increase is accounted for by the amount of acid released during the hydrolysis of the chlorambucil. Therefore, over the range of equivalent ratios of hæmoglobin to chlorambucil from 5 : 1 to 1.5 : 1, the same change in pK occurs in certain charged basic groups which are equivalent in number to the amount of combined chlorambucil.

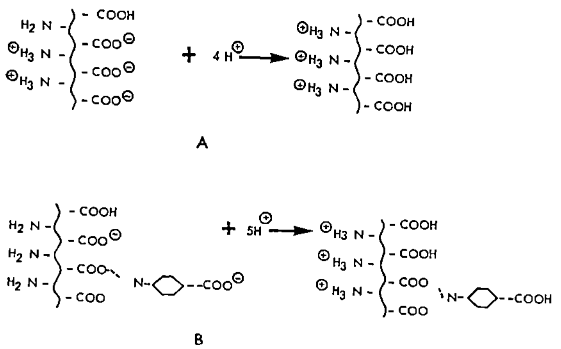

Fig. 3. Diagrammatic representation of the reaction of hydrogen ion with (*A*) protein, and (*B*) protein combined with chlorambucil

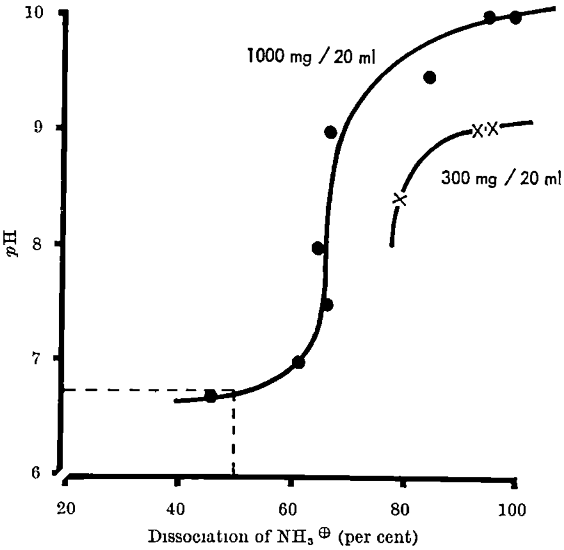

Fig. 4. Curve showing the relationship between the pH of the reacting mixture and the per cent dissociation of charged basic groups of hæmoglobin relative to the number of equivalents of combined chlorambucil

This work was supported by a grant from the National Cancer Institute of Canada.

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AMINO-ACID COMPOSITION OF GRAMICIDIN

By KIYOSHI OKUDA*, CHIN-SHIN LIN and PROF. THEODORE WINNICK

Department of Biochemistry and Biophysics, University of Hawaii, Honolulu

It is more than twenty years since Hotchkiss and Dubos[1] isolated the antibiotic substance tyrothrycin from an organism identified as *Bacillus brevis* and fractionated this material with organic solvents into gramicidin and tyrocidine. Later, Craig *et al.*[2] demonstrated by the countercurrent method that both of these peptide fractions were in turn heterogeneous. Tyrocidine *A* has the structure[3]:

$$\begin{tikzcd}
\text{L-valine} \arrow[r] & \text{L-ornithine} \arrow[r] & \text{L-leucine} \arrow[r] & \text{D-phenylalanine} \arrow[r] & \text{L-proline} \arrow[d] \\
\text{L-tyrosine} \arrow[u] & \text{L-glutamine} \arrow[l, "\gamma"'] & \text{L-asparagine} \arrow[l, "\beta"'] & \text{D-phenylalanine} \arrow[l] & \text{L-phenylalanine} \arrow[l]
\end{tikzcd}$$

In tyrocidine *B*, the L-phenylalanyl is replaced by an L-tryptophanyl residue.

The exact composition and amino-acid-sequence has not been determined for the gramicidins. The major *A* component contains glycine, L-alanine, L- and D-valine, D-leucine, L-tryptophan and ethanolamine. Gramicidins *B* and *C* differ from *A* in containing L-phenylalanine and L-tyrosine, respectively, in place of one or more tryptophans[2].

It is of interest that gramicidin *S*, produced by a special strain of *B. brevis*[4], has the identical pentapeptide sequence from L-valine to L-proline as does tyrocidine *A* (but repeated twice).

The need to investigate further the chemistry of the gramicidins arose in connexion with present investigations[5] on the biosynthesis of polypeptides in the Dubos strain of *B. brevis*. Two gramicidin preparations have been subjected to quantitative amino-acid analysis with the Beckman/Spinco model 120 automatic analyser: a commercial sample kindly supplied by the Wallerstein Laboratories, and gramicidin from a culture of *B. brevis* (A.T.C.C. 8185, Dubos strain *BG*). This latter gramicidin was isolated from tyrothrycin by chromatography on a charcoal–cellulose powder column[5], and then purified further by passage through a column of silica gel[6]. The two polypeptide preparations had identical ultra-violet absorption spectra[5]. Samples were hydrolysed with glacial acetic acid/concentrated hydrochloric acid (1:1 by vol.) at 110°–112° in evacuated tubes for specified times. After removal of acid, samples corresponding to 1.2 mg of original material were analysed with both the short and long 'Dowex' resin columns[7].

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* On leave from the Department of Biochemistry, Osaka City University Medical School, Osaka, Japan.

The results are summarized in Table 1. Tryptophan was progressively destroyed during the acid hydrolysis. However, a reliable value for this amino-acid, based on an analysis by Fraenkel-Conrat *et al.*[8] of an alkaline hydrolysate of Wallerstein gramicidin, is included for purposes of calculation and discussion.

Table 1. Amino-acid Analysis of Gramicidin

Amino-acid	Commercial gramicidin: Amino-acid/mg peptide (μmoles) 24-h hydrolysis	Commercial gramicidin: Amino-acid/mg peptide (μmoles) 48-h hydrolysis	Commercial gramicidin: No. of residues relative to glycine*	Gramicidin 8185 48-h hydrolysis: Amino-acid/mg peptide (μmoles)	Gramicidin 8185 48-h hydrolysis: Residues relative to glycine
Glycine	0.515	0.530	1.00	0.479	1.00
Alanine	1.030	1.042	1.985	0.927	1.935
Valine	2.010	2.102	3.93	1.837	3.835
Leucine	1.961	2.071	3.86	1.884	3.935
Isoleucine	0.0695	0.076	0.139	0.062	0.130
Phenylalanine	0.036	0.036	0.070	0.166	0.347
Tyrosine	0.118	0.127	0.235	0.075	0.156
Tryptophan	1.865		3.62		
Ethanolamine	0.27	†	0.52	0.30	0.62

* Average of 24- and 48-h values.

† Could not be calculated, due to excessive merging with the ammonia peak.

It may be seen that there was good agreement between the 24- and 48-h values for the commercial gramicidin, proving that the hydrolysis was complete. Very similar results were obtained with the gramicidin derived from the A.T.C.C. 8185 culture, but these values may be less accurate in an absolute sense, since the determination of the initial quantity of peptide was based on an indirect spectrophotometric measurement, rather than on weighing. If the μmoles of each amino-acid are multiplied by the residue weight (minus water), the total μg (including ethanolamine) accounted for, per mg polypeptide, is 932 for the commercial gramicidin. This recovery is fair in view of unavoidable losses due to handling and to partial destruction of ethanolamine.

In analyses utilizing the short 15-cm column, ethanolamine preceded ammonia as a discrete peak with the 24-h hydrolysate. In the 48-h samples, the size of the ammonia peak was increased, and the latter was not well resolved from the amino-alcohol. Hence accurate calculations were not possible. It should be mentioned that, in addition to traces of ornithine, variable quantities of other ninhydrin-positive substances appeared in the chromatograms with the 15 cm column. These could not be assigned to any known amino-acids, and they probably represent artefacts resulting from the destruction of tryptophan (and possibly from ethanolamine). With the 150 cm column, aspartic acid, glutamic acid and proline (components of tyrocidines) were detected in only very small, or in negligible, concentrations.

James and Synge[9] have devoted considerable effort to the determination of the ethanolamine content of gramicidin. We have observed that the amino-alcohol labelled with carbon-14 is quite stable to prolonged heating in acetic-hydrochloric acid, in the presence of gramicidin. However, the ethanolamine which occurs bound to gramicidin (possibly through the indole nucleus of tryptophan) is destroyed to a considerable extent during acid hydrolysis[9]. The 24- and 48-h values for ethanolamine in Table 1 are not quantitative chiefly for this reason. The average of the two reported determinations corresponds to an ethanolamine/glycine ratio of 0.57. The corrected data of James and Synge, taken in conjunction with various direct measurements on the gramicidin molecule, gave a value of approximately 0.60 μmole ethanolamine per mg polypeptide[9]. This represents close to one amino-alcohol per glycine and is very probably correct.

On the basis of the best analytical values available at the time, Synge[10] proposed that (mixed) gramicidin contained 2 glycyl, 3 alanyl, 5 valyl, 6 leucyl, 6 tryptophanyl and 2 ethanolamine residues. From the quantitative analyses in Table 1, it may be seen that glycine, alanine and valine actually occur in a 1:2:4 ratio in gramicidin. The leucyl plus isoleucyl residues total close to 4. Likewise, the sum of tryptophanyl, phenylalanyl, and tyrosyl groups is near 4. Since the chief component, gramicidin *A*, appears to contain no tyrosine or phenylalanine[2], its minimum molecular composition is 1 glycine, 2 alanines, 4 valines, 4 leucines, 4 tryptophans, and 1 ethanolamine. However, there are reasons for proposing that the gramicidin molecule has twice this number of residues.

(1) Since L-valyl glycine and D-leucyl glycine have both been isolated from a partial hydrolysate of gramicidin[11], the polypeptide must contain at least two glycine residues.

(2) The calculated molecular weight for gramicidin *A*, assuming 30 amino-acid residues plus 2 ethanolamines, is 3,725. This value is in good agreement with the figure of 3,800, derived from crystallographic data[12].

(3) Apart from æsthetic considerations, an even number of amino-acid residues seems likely, in view of similar situations in the cases of gramicidin *S* and tyrocidines *A* and *B*.

The presence of a small proportion of isoleucine in mixed gramicidin preparations is noteworthy and merits further study. It is possible to estimate the proportions of gramicidins *A*, *B* and *C* in the commercial preparation, from the data for phenylalanine, tyrosine, and tryptophan, by making certain assumptions.

If it is postulated that gramicidin *B* contains 3 phenylalanines plus 5 tryptophans, and gramicidin *C* 3 tyrosines plus 5 tryptophans, the μmole of *B* is 0.036/3 or 0.012 and the μmole of *C*, 0.122/3 or 0.0407, per mg of mixed gramicidin. Subtracting the tryptophan in *B* (0.036 × 5/3) and in *C* (0.122 × 5/3) from the total μmole, 1.865, the remaining tryptophan assignable to gramicidin *A* becomes 1.60. This last figure is equivalent to 0.20 μmole of *A*. On this basis, the percentages of *A*, *B* and *C* are approximately 79, 5 and 16. If these values are compared with those calculated from Craig's counter-current separation data ($A:B:C = 78:16:6$)[2], in both cases the *A* component represents almost 80 per cent of the total gramicidin. However, the proportions of *B* and *C* are reversed in the two situations. The explanation for this discrepancy is not clear at present. It will be noted that gramicidin 8185 has a higher phenylalanine and a lower tyrosine content than the Wallerstein sample, so that its content of gramicidins *B* and *C* would agree more closely with the counter-current ratios. Studies are in progress in our laboratory to determine the amino-acid sequences and exact structures of the gramicidins.

In summary, the Dubos strain of *B. brevis* contains two remarkable and contrasting groups of polypeptides, the basic tyrocidines with 10 amino-acid residues and molecular weights of about 1,300, and the neutral gramicidins with three-fold larger molecules. It will be of much interest to elucidate the mechanisms of biogenesis of these substances, and to compare the pathways with those of other classes of peptides and of proteins.

We thank Prof. K. T. Yasunobu for lending us his analytical equipment.

This investigation was supported by research grants from the U.S. National Science Foundation and the U.S. Public Health Service.

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ORIGIN OF EXINE

By Prof. J. HESLOP-HARRISON

Department of Botany, University of Birmingham

It has been known for practically a century that during the early development of pollen, refractive particles accumulate on the inner and to some extent also on the side faces of the tapetal cells[1]. These particles, which may take the form of flattened plaques, cups or hollow spheroids, show all the staining properties of exine and respond in the same manner to several microchemical tests[2,3], from which it seems probable that they are chemically the same. Their formation has been interpreted either as revealing a homology between tapetal cells and the microspores[4] or as giving evidence of the role of the tapetum in contributing to the development of the pollen exine[5].

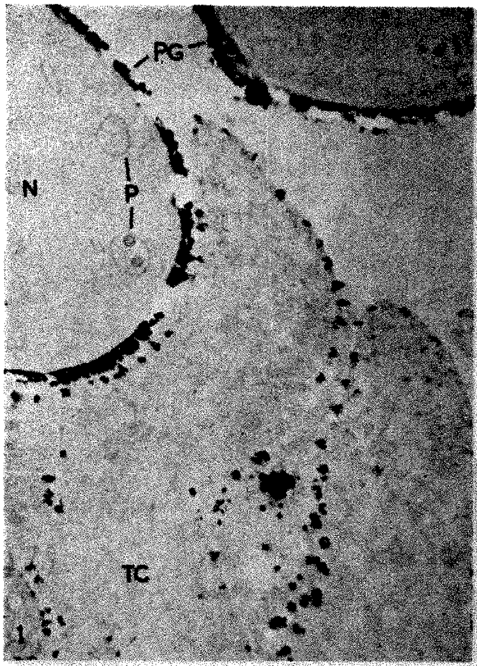
Recently, with the beginning of electron microscopic studies of pollen and tapetum, these particles have been redescribed in various flowering plants and gymnosperms by Rowley, Mühlethaler and Frey-Wyssling[6] and Chambers and Godwin[7]. Their similarity in density in the electron beam following osmic fixation and their resistance to acetolysis provide additional evidence of chemical identity with the exine material, and the affinity is further emphasized by the correspondence in surface features and fine structure.

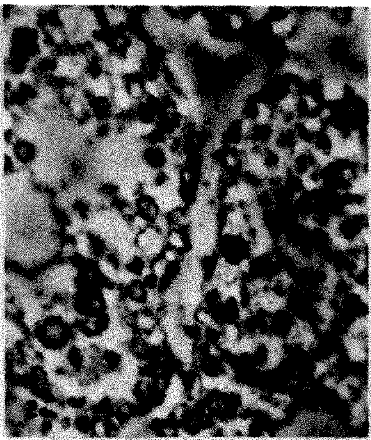
In the course of a study of tapetal function in normal and experimentally induced male sterile plants of *Silene pendula* and *Cannabis sativa* (hemp), the origin of the tapetal particles has been traced. Material for electron microscopic examination was fixed in 1 per cent unbuffered osmic acid for 1½ h at 2°–3° C, or in 2 per cent potassium permanganate for 1–2 h at room temperature, followed by alcoholic dehydration and embedding in 'Araldite'. Sections were cut on a Cambridge Instruments Huxley pattern ultramicrotome, and the observations were made with Metropolitan Vickers *EM*6 and Akashi microscopes.

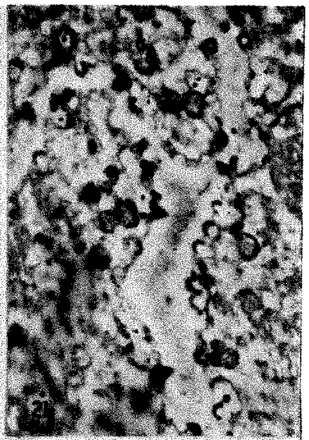
The particles and their association with the exine of the young microspores are strikingly illustrated with short period permanganate fixation in hemp (Fig. 1). With this fixation the tapetal organelles give little contrast, but the particles show high electron density. They most commonly reveal an O- or U-shape, suggesting that they are in fact shallow cups presenting one or other of these appearances according to the angle of sectioning. At the outer surface of the tapetal cells they show a regular orientation with the open end of the cup facing inwards. In some cases direct continuity between the outer surface of the cup and the exine of adjacent microspores may be seen.

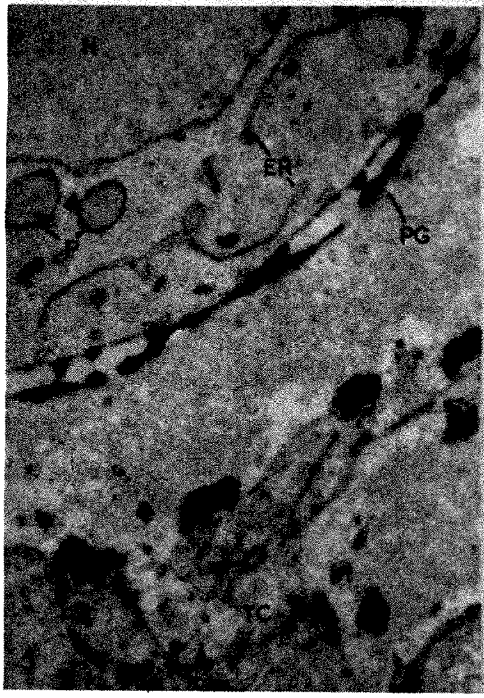
The significance of the cup shape becomes evident when the organelles near the plasmalemma of the tapetal cells are examined after osmic fixation (Fig. 2*a*, *b*). Just within the protoplast spherical or ellipsoidal bodies are present which show a cap-like accumulation of electron-dense material around one pole. These bodies have the dimensions of mitochondria, and there is occasionally sufficient evidence of cristæ to suggest that this is what they are. At the surface of the protoplast the caps of electron dense material appear in regular rows, while within the cell the membranes of the other pole are seen in various stages of dissolution.

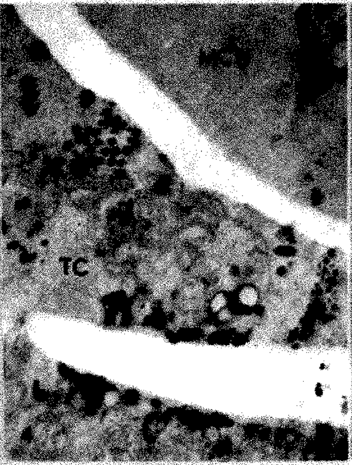
In *Silene pendula* the granules on the outer faces of the tapetal cells are larger, and can in fact be resolved with the optical microscope. With permanganate fixation they possess great electron density, and reveal a distinctive internal structure consisting of spherical cavities (Fig. 3). These large granules seemingly arise through the aggregation of smaller ones, since they are preceded in the early development of the tapetum by groups of electron-dense particles, sometimes with an annular appearance, ranging up to 600 Å in diameter (Fig. 4). Moreover, in the older tapetum a sequence of sizes can be traced in the interstices between the anticlinal faces of the tapetal cells from the large granules lying adjacent to the theca to particles nearer the anther wall of the size of those present on the young tapetum (Fig. 5). Again, material of the same electron density can be detected within the protoplast, contained in membrane-bounded organelles. While in hemp the material forms caps at the poles, in *Silene* it is in the form of discrete particles (Fig. 6). The presence of the particles obscures structure to some extent, but once more it seems probable that the organelles are mitochondria. An origin in plastids can be ruled out, since these are readily distinguishable in the tapetum of *Silene* at this stage, frequently characterized by their amœboid form.

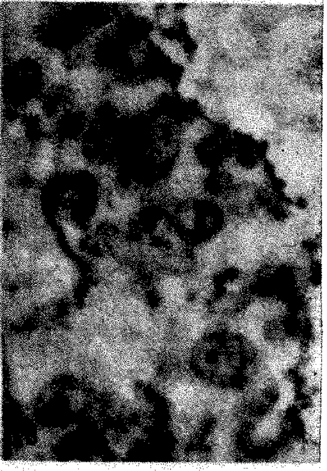


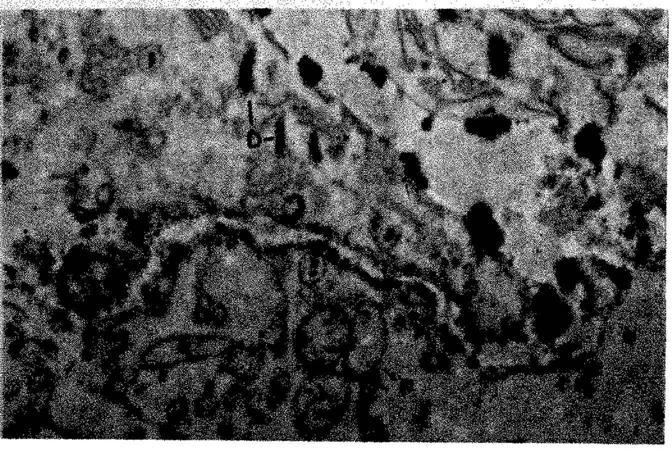












Figs. 1 and 2. *Cannabis sativa*

Fig. 1. Tapetal cells (*TC*) with adjacent pollen grains (*PG*). Conspicuous plastids (*P*), some with starch grains, are present in the pollen. *N*, nucleus. The pollen wall has been torn in a few places during sectioning. (× *c.* 2,000; permanganate fixation)

Figs. 2*a* and *b*. Two parts of a cleft between adjacent tapetal cells, showing the cap-forming organelles within the protoplast, caps aligned at the protoplast surface, and residual membranes. (*a*, *c.* 7,300; *b*, *c.* 6,800; osmic fixation)

Figs. 3–6. *Silene pendula*; all permanganate fixation

Fig. 3. Tapetal cell (*TC*) with adjacent pollen grain (*PG*). *P*, plastids; *ER*, endoplasmic reticulum; *N*, nucleus. (× *c.* 6,000)

Fig. 4. Tapetal cell (*TC*), partly in surface view, with adjacent microspore tetrad still invested in the mother cell wall (*MCW*). Particles in different stages of aggregation are visible. The white band at the base of the picture is a wall between neighbouring tapetal cells, in the course of dissolution. (× *c.* 9,000)

Fig. 5. Cleft between adjacent tapetal cells beginning near the theca (top centre) and extending inwards towards the anther wall. The organelles, presumed to be mitochondria, containing the dense particles may be seen throughout the picture, and large numbers of the particles are present adjoining the cleft, where residual membranes may be seen. *P*, plastids; *D*, dictyosomes. (× *c.* 4,500)

Fig. 6. The particle-containing organelles near the protoplast surface at higher magnification. (× *c.* 13,000)

Whereas in hemp the electron-dense material is presented at the plasmalemma as a cup, base first, in *Silene* the particles seem to be discharged at the cell surface in a random manner by the sacrifice of the organelles within which they are contained, aggregation following later in some as yet obscure way.

The question arises whether this mechanism in the tapetum is that through which all the exine material of the sporogenous mass of the anther is synthesized. On the whole it seems probable that it is. In *Silene*, the formation of exine material in the tapetum begins during the early stages of meiosis, and appreciable accumulations are present at the tetrad stage, when the microspores are invested in the mother cell wall and are without exine. There are no interstitial cells in the anthers of the two genera examined, and a careful search has revealed no evidence of the formation of exine material in the organelles of the microspores—indeed, as will be reported more fully elsewhere, there is every reason to believe that the exine material is applied from without. By what mechanism it is transported from the tapetal cells to the young spores remains a mystery, since continuities such as those seen in Fig. 1 can obviously be present only with peripheral grains. It is noteworthy, however, that exine can sometimes be seen to develop first on the side of pollen grains lying towards the tapetum, a fact which has been taken as evidence of the role of the latter tissue in the synthesis of exine material[5].

Finally, it is significant that exine formation is not necessarily severely reduced in sterile pollen of *Silene pendula*. Notwithstanding an early degeneration of the protoplast after the break-up of the tetrads in anthers rendered sterile by abnormal photoperiod régimes[8,9], exine is formed and applied to the spore walls. The tapetum, which does not generate the elaborate mass of endoplasmic reticulum found in fertile anthers, nevertheless produces the superficial granules. In a comparison of normal and male-sterile maize, Edwardson[10] has reported the presence in tapetal cells of particles of the type described here, and has suggested that there may be consistent differences in the sizes of the particles and the persistence of their enveloping membranes in fertile and sterile anthers. If the role of the particles is as suggested here, it is difficult to suppose that they have themselves anything directly to do with pollen fertility and sterility.

I thank Mrs. P. G. Roberts for assistance, the Department of Physical Metallurgy, University of Birmingham, for the use of the Metrovick microscope, and Prof. O. Lowenstein for making available the Akashi microscope by permission of the Wellcome Foundation, the owners of the instrument.

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REGULATION OF UNESTERIFIED FATTY ACIDS OF THE PLASMA DURING THE ONSET OF STARVATION

By Dr. P. A. MAYES

Royal Veterinary College, London, N.W.1

THE reciprocal relationship between glucose utilization and the release of unesterified fatty acids from adipose tissue is considered to be of crucial importance in caloric homeostasis[1]. Recent work has demonstrated that the rate of production of unesterified fatty acids controls the concentration of such acids in the plasma, which in turn determines their rate of uptake by the tissues[2]. Generally, the concentration of metabolic substrates in the blood is the main factor which determines their rate of utilization. This does not necessarily follow for glucose since other factors, including insulin, have a marked effect on its utilization. Nevertheless, a reciprocal relationship exists between the levels of plasma glucose and of unesterified fatty acids over a wide range of conditions which involve progressive depletion of available body carbohydrate and the need to draw increasingly on fat as a source of calories[1]. During an investigation in this laboratory of the metabolic factors involved in the transition of rats from the fed to the fasting condition, a nearly threefold increase in plasma unesterified fatty acids occurred before there was any significant fall in the blood glucose concentration.

Female albino rats (av. body-wt. 197 gm.) were divided into two groups, a control group provided with the normal stock diet and a group from which food was withdrawn at 10 a.m., designated the 'fasting' group. At intervals of 2 hr., animals from each group were killed by decapitation and blood was obtained for the estimation of plasma unesterified fatty acids[3] and blood glucose[4]. Experimental details of the method used for estimating the liver glycogen content have been described previously[5]. Before the experiment, the rats were handled frequently and the length of daylight, which influences the feeding pattern, was kept constant (6 a.m.–8 p.m.). The environmental temperature was 21° C.

The results obtained from the controls are summarized in Fig. 1(*A*). These show the variations in blood glucose, plasma unesterified fatty acids and liver glycogen which occurred in normal fed rats between 10 a.m. and 6 p.m. The small fluctuations in blood glucose and plasma unesterified fatty acids varied in a reciprocal manner. In the fasting rats (Fig. 1*B*) there was a very regular increase in circulating unesterified fatty acids from 10 a.m. onwards. The rise from 10 a.m. to 2 p.m. was highly significant ($P < 0{\cdot}001$) and was of particular interest as there was no reciprocal change in blood glucose concentration during this period ($P > 0{\cdot}2$). By 4 p.m., however, the glucose-level had fallen significantly

($P < 0{\cdot}001$) and continued to fall until the end of the experiment.

The relationship between the blood glucose and the unesterified fatty acids was examined more closely by plotting the individual results (Fig. 2) and subjecting them to linear regression analysis. A single regression line (A) could be fitted to the data obtained from the fed rats from 10 a.m. to 6 p.m. (P 0·01–0·05) and a similar line (B) could be fitted to the data obtained from the fasting rats from 2 p.m. to 10 p.m. ($P < 0{\cdot}001$). Although the slopes of the lines A and B were not significantly different (P 0·05–0·2), they could not be considered identical because of their distance apart ($P < 0{\cdot}001$). This distance represents the transition from the fed to the fasting condition taking place from 10 a.m. to 2 p.m. (Fig. 1A). During this time the level of blood glucose remained approximately constant, and the liver glycogen content fell by the same amount, in both the fed and the fasting animals. The lack of a definite rise of unesterified fatty acids in the fed animals could be ascribed to the effect of extra glucose absorbed from the gut, but this does not explain why, for a given concentration of blood glucose, the output of unesterified fatty acids is approximately half as much in the fed as in the fasting, post-absorptive animals (Fig. 2). An extra secretion of insulin in the fed animals would increase glucose utilization and could account for this effect. However, if an increase in insulin secretion is caused by hyperglycæmia it is difficult to see what factor would increase insulin secretion significantly from 10 a.m. to 2 p.m. in the fed rats as their blood sugar-levels during this period are virtually identical with those of the fasting rats. At this time the fed rats only occasionally nibble at their food and are forced to draw on their glycogen stores.

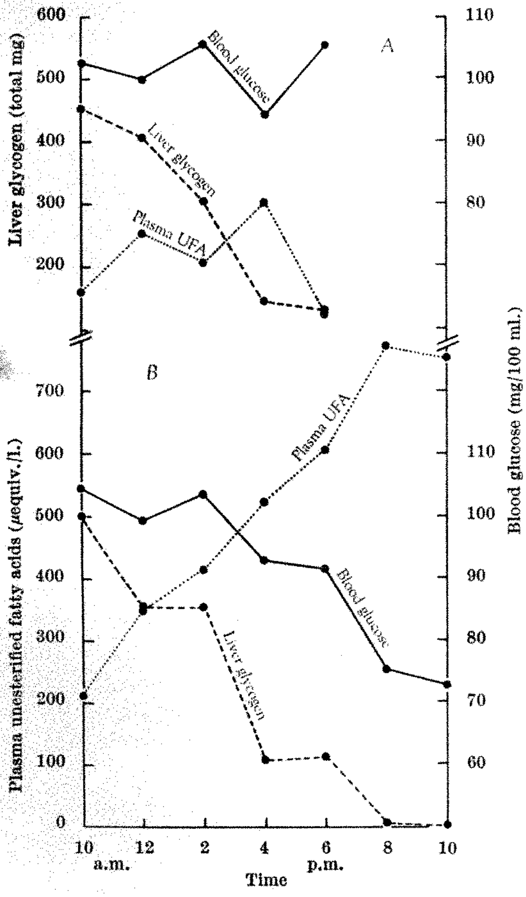

Fig. 1. Changes in blood glucose, liver glycogen and plasma unesterified fatty acid levels; (A) in rats with access to their normal stock diet; and (B) in rats deprived of food from 10 a.m. In (A) each point is the mean from four animals; in (B) each point is the mean from six animals except at 10 a.m., which is the mean from five

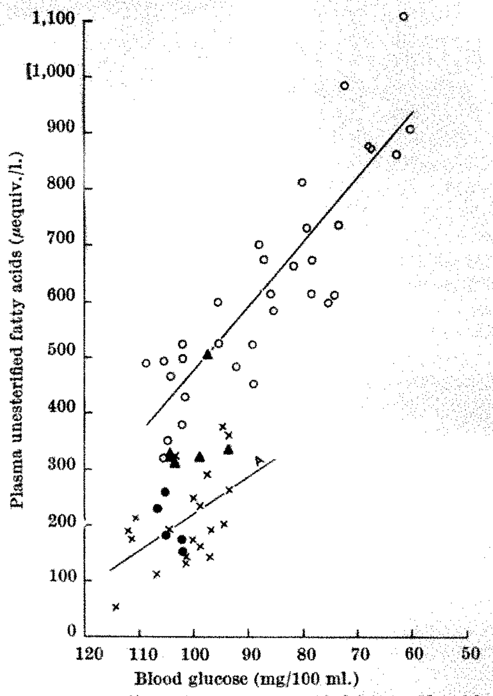

Fig. 2. Regression lines of plasma unesterified fatty acid on blood glucose concentration in (A) fed and (B) fasting rats (2 p.m. to 10 p.m.). ×, fed rats (10 a.m. to 6 p.m.); ●, fasting rats (10 a.m.); ▲, fasting rats (12 noon); ○, fasting rats (2 p.m. to 10 p.m.)

It has been suggested that glucose inhibits the output of unesterified fatty acids from the depots by virtue of it being a ready source of calories[6]. If this is so then other utilizable, calorigenic substrates should act similarly. Amino-acids[6], fat[7] and even acetate in sheep[8] will suppress plasma unesterified fatty acids, and, furthermore, human subjects on carbohydrate-poor diets still show a fall in plasma unesterified fatty acids after meals[9]. It follows that in the present work, the apparently greater effect of glucose in suppressing formation of unesterified fatty acids in the fed animals may at least, in part, be due to the additional effect of the other calorigenic constituents of the diet.

Interpreted in this way, the results are compatible with the hypothesis that the regulation of the output of unesterified fatty acids from the fat depots varies inversely with the total rate of utilization of all calorigenic substrates.

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METABOLICALLY LINKED CHANGES IN FLUORESCENCE EMISSION SPECTRA OF CORTEX OF RAT BRAIN, KIDNEY AND ADRENAL GLAND

By PROF. BRITTON CHANCE, VICTOR LEGALLAIS and BRIGITTE SCHOENER
Johnson Research Foundation, University of Pennsylvania

IN a concurrent paper we have described the development of a microfluorometer suitable for measuring changes in the oxidation-reduction level of the intracellular pyridine nucleotide component in tissues of the rat[1,2].

In the previous method the fluorometer was designed for measuring emission over a fixed wavelength region. Identification of the fluorescing component was based on the assumption of identical fluorescence emission properties in both *in vivo* and the excised tissue. The excised tissues were studied by a specially designed spectrofluorometer which was sensitive to their fluorescence changes. Since it is highly desirable to identify the nature of the fluorescing changes directly, *in vivo*, the microfluorometer apparatus has been modified in order to incorporate a continuous interference filter of a 30μ spectral interval. This interval is shown to be sufficiently narrow for measurement of the fluorescence emission spectrum of mitochondrial pyridine nucleotide and sufficiently broad to allow an adequate measurement accuracy. This article reports fluorescence emission spectra of kidney, brain, and adrenal cortex of anæsthetized rats and shows how the intensity of these fluorescence spectra changes in different metabolic states, such as aerobiosis and anoxia.

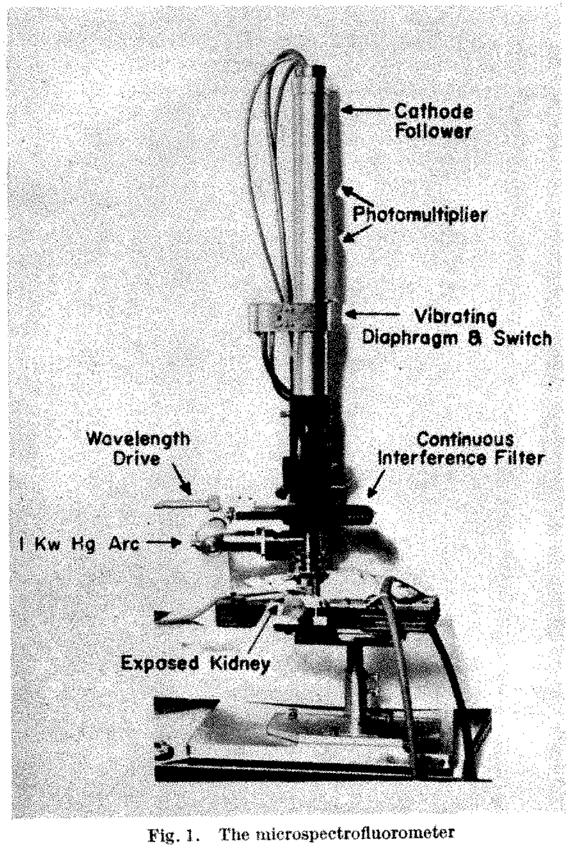

Fig. 1. The microspectrofluorometer

Fig. 1 shows the microspectrofluorometer. In appearance the instrument is identical to that described before[2], except that a continuous interference filter (Schott) is seen as the rectangular object placed near the back aperture of the objective. A flexible shaft connects this filter to the drive of a chart recorder. A counter is used for resetting the wave-length to pre-calibrated points. Since the interference filter gives an approximately linear relationship between displacement and wave-length, the direct gearing between the chart recorder gives the wave-length scale as 10 mμ per division indicated in the figures of this paper. In addition to the interference filter, a Wratten type 2*C* filter is also placed in the back aperture of the objective. The wave-length-range of the interference filter is 400–700 mμ, and the specification on its spectra interval is 30–40 mμ. In order, however, to compensate somewhat for the loss of light intensity, the measuring aperture has been increased from 15–30μ. Other features of the apparatus are briefly summarized: the 1,000-watt high-pressure mercury arc excites the fluorescence of the specimen at 366 mμ by means of an 'Eppendorf' primary filter. Fluorescence excitation and emission pass through the Leitz Ultrapak objective (× 11) and a × 3 ocular. Olson[3] and Loeser and West[4] have described rapid scanning spectrofluorometers which could be suitably modified for these studies.

Fig. 2 indicates the performance of this microspectrofluorometer as compared with that of a macrospectrofluorometer operating with a 10 mμ spectral interval. Two substances are studied, 4-β-methyl umbelliferone (7-hydroxy-coumarin) and a suspension of pigeon heart mitochondria. The half-widths of the emission spectra for pigeon heart mitochondria measured in the two instruments are both 77 mμ. The half-width for umbelliferone is 56 mμ in the macro-instrument and 58 mμ in the micro-instrument. Since the component of tissues in addition to reduced pyridine nucleotide, which might be expected to be observed, is that of flavin, and since the emission band of this substance is even broader than that of reduced pyridine nucleotide, the microspectrofluorometer is considered adequate for the *in vivo* experiments.

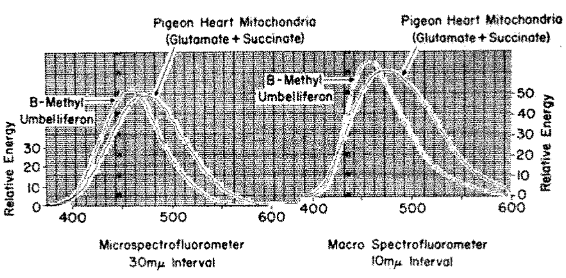

Fig. 2. A comparison of the performance of the microspectrofluorometer with that of an instrument using a grating monochromator (250 mm. focus) set at a 10 mμ interval. The pigeon heart mitochondria were treated with glutamate and succinate in order to give maximal reduction of pyridine nucleotide in the aerobic state

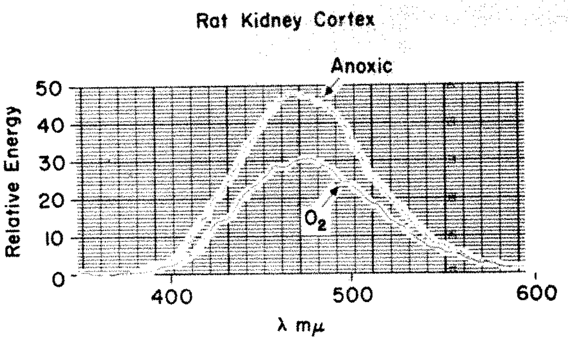

Fig. 3. Fluorescence emission spectra of rat kidney cortex with rat breathing oxygen and rat anoxic

The emission spectra are uncorrected for the energy sensitivity of the cæsium–antimony photosurface. Thus, the peaks of these emission spectra for mitochondrial reduced pyridine nucleotide are at 473 mμ instead of 443 mμ[2]. However, our attention is directed in these studies to the similarity of the emission spectra of isolated mitochondria (Fig. 2) and the intact tissue (see the following figures).

Rat kidney cortex. With the microscope focused on the kidney cortex of the anæsthetized rat, the field appears pale blue and the small blood vessels are seen in dark outlines against the uniform blue background. The kidney is held in a small cup, which reduces considerably motion due to respiratory movements[2]. The microscope is focused on an area of bright fluorescence containing a minimum of blood vessels.

Two types of recording are used, one a time recorder and the other a wave-length recorder. With the monochromator set at 470 mμ, a time recording of several minutes is taken in order to ensure that a stable base-line is obtained with the animal breathing oxygen. Then a spectrum is run through and appears as the lower trace of Fig. 3 (oxygen). The peak of the emission spectrum lies near 475 mμ, and the shape of the emission spectrum suggests that only one component is involved. The inspired gas is changed to nitrogen, and when the time recorder indicates that the fluorescence has reached a plateau (approximately 30 sec. after cessation of breathing)[2], the second spectrum is run (this requires approximately a minute). This time interval is sufficiently short that the animal may be resuscitated by the use of a few manual ventilations following a change of the inspired gas to 100 per cent oxygen. When breathing is resumed, a third spectrum may be run in order to bracket the anoxic spectrum with two aerobic ones (Fig. 4). The fluorescence intensity obtained in anoxia is 60 per cent higher than in the aerobic condition. The peak of the emission spectrum obtained in anoxia is at approximately 470 mμ, and the distribution of energy is maximal at a slightly shorter wave-length than when the animal breathes oxygen. This suggests a greater contribution of bound reduced pyridine nucleotide, presumably that of the mitochondria. The absence of prominent emission bands in the region of 540 mμ indicates that no measurable amount of free flavin is present in the areas under observation in either aerobic or anoxic states.

Adrenal gland. Fig. 4 represents a similar study of exposed cortex of the adrenal gland. The animal was hypophysectomized two days previously and has been treated with 400 milli-units of adrenocorticotrophic hormone. The fluorescence changes described elsewhere[5] have already occurred. With the animal breathing oxygen, the peak of the emission band lies at approximately 475 mμ. If the inspired oxygen concentration is dropped to zero, the emission spectrum rises to a peak nearly identical in wavelength and about 40 per cent higher in intensity. This may be compared with the 60 per cent increase in the case of the rat kidney. If the animal is allowed to breathe oxygen again, the intensity of the emission spectrum diminishes, and the dashed curve is obtained. Here again, the graph suggests the emission spectrum of mitochondrial reduced pyridine nucleotide. The small shift in the anoxic spectrum observed in the kidney is not seen, however, in the adrenal gland.

Brain cortex. Fig. 5 indicates a similar experiment with the microspectrophotometer focused on a small area of the brain near the preoccipital cortex. A trace labelled aerobic is obtained with the animal breathing 20 per cent oxygen and 80 per cent nitrous oxide. The emission spectrum is seen to have a broad maximum at 479 mμ. About a minute after changing the inspired gas to 100 per cent nitrous oxide, the fluorescence increases, and a more distinct peak is now observed at approximately the same wave-length. The similarity of this peak to reduced pyridine nucleotide fluorescence of isolated mitochondria is again to be noted.

In two of the three tissues examined, kidney and brain cortex, emission spectra for excised tissues have been obtained in the perfused and unperfused state and show peaks on a relative energy basis, within several mμ of the wave-lengths observed here (473 mμ). It is of considerable importance to confirm that no artefact has occurred in the use of excised tissues and that the material *in situ* in the anæsthetized animal gives, as a major component of its fluorescence, that which we can attribute to bound reduced pyridine nucleotide. These data show fluorescence increases in the transition from aerobiosis to anoxia in the excised tissues, in the same proportion to those observed *in vivo*. Experiments on excised slices of the cat brain cortex identify increased absorption at 340 mμ to occur in the transition from aerobiosis to anoxia[6].

As in the case of the excised tissues, this type of measurement does not clearly distinguish between the mitochondrial and the cytoplasmic material.

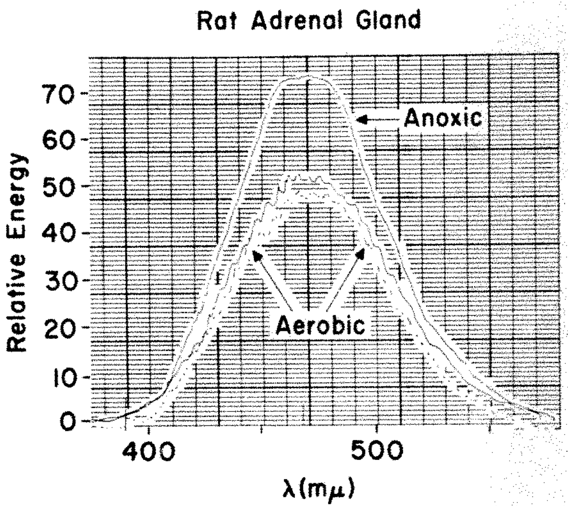

Fig. 4. Fluorescence emission spectra of rat adrenal cortex with rat breathing oxygen and rat anoxic

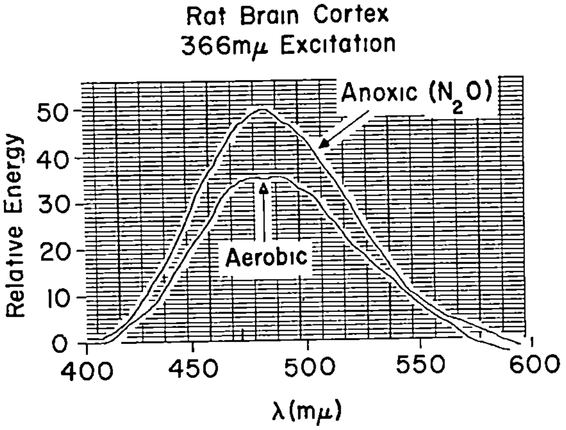

Fig 5 Fluorescence emission spectra of rat brain cortex with rat breathing oxygen and rat anoxic

Experimentation with appropriate inhibitors, such as 'Amytal', however, has already shown that a large part of the change of fluorescence emission occurs in the presence of 'Amytal' and oxygen, suggesting a major contribution to the fluorescence change of the mitochondrial pyridine nucleotide[2]

The positions of the peaks of the emission spectra are sufficiently close to those of the emission spectrum for the reduced pyridine nucleotide component of a suspension of isolated mitochondria (473 mμ) that we identify this material as the major fluorescing component in these spectra

At the present time, insufficient data are available to attach significance to the few millimicrons difference in the position of the peaks of the emission spectra or to the 10–20 per cent difference in the fluorescence increases which occur in this transition from aerobiosis to anoxia in the different organs No doubt further experiments will allow us to attach a greater significance to these quantitative aspects

The lack of evidence for measurable amounts of fluorescent material other than that of reduced pyridine nucleotide is largely a consequence of the preponderance of fluorescence of this material Undoubtedly, in tissues depleted of reduced pyridine nucleotide, other components could be observed The results, however, suggest that with 366 mμ excitation at least, the possibility of measuring intracellular components of these tissues other than reduced pyridine nucleotide is rather small

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SODIUM CARBONATE LAVAS FROM OLDOINYO LENGAI, TANGANYIKA

By J B DAWSON

Geological Survey of Tanganyika, Dodoma

OLDOINYO LENGAI is an active volcano lying some ten miles south of Lake Natron in the eastern or Gregory Rift Valley of northern Tanganyika It is a composite cone consisting mainly of ijolitic and nephelinitic pyroclastics which contain ejected blocks of urtite, ijolite, melteigite, jacupirangite, biotite pyroxenite, wollastonitite, calcium carbonatite (sovite), fenite and various types of lava[1] The interbedded silicate lavas vary with decreasing age from phonolite + nephelinite → nephelinite → melanephelinite, giving evidence of a distinct trend of decrease in the silica content of the extruded rocks during the successive eruptions which built the volcano This trend was further emphasized in 1960 when soda-rich carbonate lavas containing virtually no silica were extruded from the vent of the northern crater in the form of both *pahoehoe* and *aa* lava This is the first time that carbonate lava has been recorded from the volcano although earlier workers[2–5] have noted the presence of soda carbonate ash which has been ejected during earlier eruptions It has been suggested[1] that the volcano has resulted from successive intrusions of carbonatite magma into crystalline rocks, each intrusion being less contaminated with sialic material than its predecessor, and the soda ashes and lavas are regarded as approximating in composition to the uncontaminated parental material

Chemical analyses of two of the soda lavas have been made by the Tanganyika Geological Survey Laboratories (Table 1)

Table 1 ANALYSES OF SODA-RICH CARBONATE LAVAS

	BD 114	*BD* 118
SiO_2	Trace	Trace
TiO_2	0 10	0 08
Al_2O_3	0 08	0 09
Fe_2O_3 (total)	0 26	0 32
MnO	0 04	0 24
*CaO	12 74	12 82
*BaO	0 95	1 05
*SrO	1 24	1 20
MgO	0 49	0 41
Na_2O	29 53	20 70
K_2O	7 58	6 58
P_2O_5	0 83	1 06
H_2O (total)	8 59	8 27
CO_2	31 75	32 40
F	2 69	1 84
Cl	3 86	2 64
SO_3	2 00	2 18
	102 73	100 88
Less O for F and Cl	2 00	1 36
	100 73	99 52

BD 114, *Pahoehoe* lava extruded between October 1 and 8, 1960
BD 118, *Aa* lava extruded at 12 05 p m on October 9, 1960
Analysts, W K L Thomas, G Luena and P Bowden

* Determined spectrographically by P Bowden Tanganyika Geological Survey reference number *X*/7279/2

The analyses are of a *pahoehoe*-type lava and an *aa*-type lava, and it is noticeable that the lava-types are chemically very similar The most significant feature of the chemistry is the virtual absence of silica and the very small amounts of alumina and iron combined with the very high amounts of alkalis and volatiles In particular the extremely high soda content of almost 30 per cent is indicative of the very unusual nature of these lavas The total volatile content of the *pahoehoe* and *aa* lavas is 49 72 and 47 39 per cent respectively

An exhaustive search of the literature has not revealed any other magmatic rock of a similar composition All recorded examples of intrusive carbonate rocks which are believed to be magmatic[6,7] contain high amounts of lime and/or magnesia while some have a high iron content On the other hand, alkalis are usually present in only small amounts and are contained in silicate minerals such as mica and aegerine, not as alkali carbonate The same general characteristics are also exhibited by the only other known extrusive carbonatite—a calcium-rich carbonatitic lava from western Uganda[8] The carbonate lavas from Oldoinyo Lengai, however, are quite different, having a relatively small lime content and little magnesia and iron, whereas soda and potash together comprise between 36 and 38 per cent with unusually high amounts of fluorine chlorine and sulphur trioxide Moreover, although the strontium content is of the same order as in calcitic or dolomitic carbonatites, the barium, substituting for potassium, is present in greater concentrations in the Oldoinyo Lengai lavas

The presence of this soda-rich carbonate lava as the youngest phase in a nephelinitic–ijolitic volcano, beneath which (according to the evidence of the ejected blocks) is a carbonatite complex, has considerable bearing on certain aspects of the genesis of carbonatite complexes At present the hypotheses for the genesis of carbonatites and their associated complexes may be divided into two groups (*a*) Hypotheses which assume a basic or ultrabasic parent which differentiates to carbonatite This parental magma has been interpreted as pyroxenite[9], peridotite[10], kimberlite[11] and alkali–peridotite or nephelinite[12] (*b*) Hypotheses in which carbonatites are derived from primary carbonatite magmas or carbon dioxide of unknown origin[6,8,13–15].

The Oldoinyo Lengai lavas are the first recorded occurrence of magmatic alkali carbonate In all previous cases the residual carbonatite has been calcitic or dolomitic and this factor has undoubtedly influenced the workers who seek to derive the carbonatite from ultrabasic silicate parents The presence of the alkali carbonate on Oldoinyo Lengai strengthens von Eckermann's contention[6] that alkali carbonate has been instrumental in the formation of the alkaline rocks in carbonatite complexes, although the carbonate magma proposed by von Eckermann in the case of Alno Island is dominantly potassic Moreover, with the proved existence of magmatic alkali carbonate there is now a possible indication of the source of the alkalis required for the fenitization process, a long-outstanding problem, and it may be that variations in the potash and soda content of carbonatite magma account for the differences in the nephelinization processes seen at different centres

Mineralogically, the lavas consist of highly birefringent phenocrysts up to 1 mm long set in a microcrystalline matrix of highly birefringent, hydrated sodium carbonate The phenocrysts are of a new mineral which will be described in a later paper on the petrography of the lavas It is probable that a new name will be given to the lavas in view of their unusual chemistry and mineralogy

I thank Mr. W. K L. Thomas, Mr. G Luena and Dr P Bowden for the analyses Mr J W Pallister and Dr D A Harkin kindly read and discussed the manuscript This communication is published by permission of the Commissioner for Geological Survey, Tanganyika

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PROPERTIES OF THE ORGANIC MICROSTRUCTURES OF SOME CARBONACEOUS CHONDRITES

By MICHAEL H. BRIGGS

Department of Geology, Victoria University of Wellington, New Zealand

SEVERAL authors have recently suggested that carbonaceous chondrites contain materials of biological, yet extra-terrestrial origin Conclusive proof of this hypothesis would be of tremendous scientific importance, however, this should not lead to too hasty an acceptance on insufficient experimental evidence To date, two types of evidence have been presented for the presence of biological materials in meteorites First, it has been shown that the distribution of normal and cyclic paraffin hydrocarbons obtained from both the Orgueil and Murray meteorites resembles the distribution of these compounds in crude petroleum, and also in the hydrocarbons of recent marine sediments[1,2] It is argued that this similarity indicates a common origin for the meteorite and terrestrial hydrocarbons As terrestrial petroleum is commonly accepted to be of biological origin, then this indicates a biological origin for the meteorite compounds However, it has been pointed out[3] that while there is some similarity between the hydrocarbon distributions of the meteorites and petroleum, there are differences, and a variety of other objections can be made to drawing a close parallel[3] The argument also rests on the assumed biological origin of crude petroleum While this is certainly a popular hypothesis among Western geologists, an inorganic origin has been supported by several authorities[4] The demonstration of petroleum-like hydrocarbons in meteorites could be used equally as evidence for a non-biological origin for terrestrial petroleum If meteorites are continually adding hydrocarbons to the Earth's surface, petroleum deposits could be accumulations of these compounds

The second argument for the presence of biological materials in meteorites concerns the nature of the 'organized elements' meteorite organic microstructures It has been claimed[4–6] that the microstructures of both the Orgueil and Ivuna meteorites take up biological stains and occur in only a very small number of morphological types There are several difficulties facing the identification of these microstructures First, arguments based on morphology are notoriously subject to personal bias Secondly, contamination of the material under investigation with terrestrial micro-organisms is difficult to eliminate

Examination of the organized elements of the carbonaceous chondrites has now been made independently by several workers and a marked disagreement of opinion has become apparent Thus reports[7,8] from Prof Anders's laboratory at the University of Chicago have stated that attempts to find 'organized elements' resembling those reported by Nagy *et al* [5,6] have largely failed, though terrestrial contaminants and certain mineral and sulphur granules, bearing a close similarity to some of the 'organized elements', were found

It is clear that the nature of the meteorite organic microstructures will be established only by independent examination of samples of the carbonaceous meteorites by more workers in the field In this article a report is presented on the results of an examination of samples of three carbonaceous chondrites

In this laboratory samples of Orgueil, Murray and Mokoia have been examined These meteorites are members respectively of Wiik's classes I, II and III of the carbonaceous chondrites[9] All fragments were carefully examined prior to experimentation to ensure that they were free of any museum markings, fusion crusts and other surface defects To eliminate terrestrial contaminants slides were prepared inside a sterile glove-box The glove-box was thoroughly cleaned and its interior wiped with a dilute solution of phenol The air was gradually removed by suction and replaced with air drawn through glass-wool filters The interior was fitted with an ultra-violet lamp and was irradiated for 2 h prior to the experiments Agar plates were exposed inside the glove-box and, at the completion of the experiments, were removed and incubated No microbial growth was detected on any plate Fractionation of the meteorite samples was conducted also largely within the glove-box using clean, sterile apparatus

As reported by Fitch *et al* [7] a fraction of Orgueil can be obtained that is denser than methylene iodide ($\rho = 3\ 33$) and which on microscopic examination contains numerous approximately spherical particles somewhat resembling the Types I and II 'organized elements' of Claus and Nagy[5] These particles are magnetic, and X-ray diffraction studies indicate the presence of troilite and magnetite Similar fractions were obtained from Murray and Mokoia In the case of these two meteorites the magnetic particles were more irregularly shaped than in Orgueil

Claus and Nagy[5] have reported also the presence of polyhedral 'organized elements' in Orgueil Fitch and Anders[8], however, could find only hexagonal microstructures that they suggested to be goethite pseudomorphs of troilite, together with other hexagonal mineral grains

Examinations of untreated, crushed fragments of the meteorite samples used in this investigation also failed to reveal anything resembling the Type V 'organized elements' of Claus and Nagy[5] However, two distinct types of hexagonal microstructures were detected in Orgueil, though not in Murray or Mokoia The first class were transparent, yellow-brown objects about 12–15μ in diameter They were magnetic Other, rather smaller hexagonal microstructures were opaque and non-magnetic Both classes had a specific gravity greater than 3 33, and were probably troilite grains

In an attempt to verify the report of Nagy *et al* [6] that some of the organized elements can be separated by chloroform extraction, while others can be concentrated with specific gravities between 2 3 and 2 4, the following experiments have been conducted Small samples of each meteorite were treated with chloroform and, after agitation, allowed to stand for two days The chloroform layer was decanted and evaporated in a stream of filtered air The residue was taken up in a small volume of acetone and centrifuged Microscopic examination of the residue revealed no 'organized elements', only a few irregular fragments of a transparent material, some of which were similar to that shown in Fig 2 of a previous paper[10] Similar fragments were obtained from all three meteorites Attempts to concentrate microstructures resembling 'organized elements' from meteorite fragments previously extracted with benzene, by the use of various bromoform–carbon tetrachloride mixtures, were totally unsuccessful However, fragments that had not been previously extracted did yield rounded particles of approximately the specific gravity reported by Nagy *et al* [6] The appearance of these particles, however, was considerably altered on extraction with acetone, then carbon disulphide Consequently, it is probable that they are the sulphur and hydrocarbon droplets previously reported by Fitch *et al* [7].

From the results discussed here it must be concluded that the meteorite samples examined did not contain anything identifiable as the 'organized elements' of Nagy *et al* [5,6] On the other hand, all these meteorites do contain a variety of microstructures which in certain features resemble the 'organized elements' However, it would seem that these microstructures are of several types (*a*) various mineral grains, (*b*) droplets of organic solvent-soluble carbon compounds, possibly associated with elemental sulphur, (*c*) organic solvent-insoluble carbon compounds that perhaps could be high molecular weight hydrocarbon polymers

These conclusions are similar to those of Fitch and Anders[8], who have also presented evidence that some of the 'organized elements' reported by Nagy *et al* [5,6] were contaminating terrestrial pollens and other micro-organisms

At present there is certainly no convincing evidence for the existence of any material within carbonaceous chondrites of extra-terrestrial, biological origin

I thank the following persons for supplying meteorite samples Dr W A Watters of the New Zealand Geological Survey, the Curator of the Wanganui Museum, Dr E P Henderson of the Smithsonian Institute, and Prof Edward Anders of the University of Chicago I also thank Prof R H Clark for advice and assistance with the microscopic examinations

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METAMORPHISM OF A LIGNITE

By Dr. J. ROGERS and Dr. R. P. SUGGATE
Geological Survey
AND
J. O. ELPHICK and Miss J. B. ROSS
Dominion Laboratory, New Zealand Department of Scientific and Industrial Research

MANY questions remain to be answered about the influence of the variables, pressure and temperature and time, on the metamorphism of coal. In the field it is difficult to disentangle the effects of pressure and temperature, both of which increase with depth of burial. There are considerable uncertainties in estimating for most coals the maximum depth of burial and the maximum temperature to which they were subjected. Geological and chemical evidence suggests, however, that temperature is of primary importance in determining the degree of metamorphism of coal.

Attempts at the experimental imitation of the coalification process have been reviewed by van Krevelen[1] and Teichmuller[2] and recently extended by van Krevelen *et al.*[3] Although the effects of temperature on the artificial conversion into coal of starting materials like peat, lignite, wood, cellulose, lignin and waxes are now well established, the pressure variable does not appear to have been carefully studied.

As a contribution to the experimental study of the processes of coal metamorphism, the effects of pressure and temperature on an Oligocene lignite from Waimatua, Southland, New Zealand, have been investigated in the high-pressure laboratory, Department of Chemistry, University of Otago. 20–50-gm samples of lignite, crushed to minus ½ in., were heated under defined conditions of pressure and temperature for times ranging from 1 to 63 days. The pressure vessels, or bombs, were similar to those used in hydrothermal investigations of silicates and the pressure medium was water. The products of these experiments were analysed by the Coal Section, Dominion Laboratory.

An analysis of the Waimatua lignite as used in the experiments is given in Table 1, together with the analysis after air-drying at 70 per cent relative humidity at 20° C., and a calculation to the dry, ash-free basis which is used in presenting the experimental results.

A measure of the metamorphism in the relatively low-rank products resulting from the experiments is the calorific value. Fig. 1 shows the influence of temperature on the changes in this property effected in a given time at various water pressures. At 170° C. and above there is an initial rapid change, whereas up to 100° C. the change in calorific value is slow in terms of the times of these experiments.

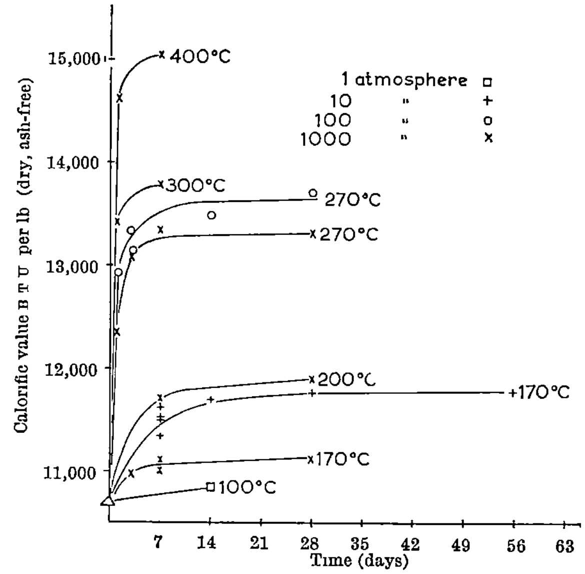

Fig. 1. Changes in calorific value of Waimatua lignite at various temperatures and pressures as a function of time

In numerous experiments at 170° C. there were greater changes in calorific value at 10 atmospheres pressure than at 1,000 atmospheres, as shown in Fig. 1. The equilibrium water vapour pressure at 170° C. is approximately 10 atmospheres. At higher temperatures, greater changes in calorific value were obtained at 100 atmospheres pressure than at 1,000 atmospheres.

Fig. 2 shows that there is a marked point of inflexion at about 170° C. in the calorific value versus temperature

Table 1. ANALYSES OF WAIMATUA LIGNITE

	Moisture (per cent)	Volatile matter (per cent)	Fixed carbon (per cent)	Ash (per cent)	Sulphur (per cent)	Calorific value B.T.U./lb.
As used	58.6	21.1	17.3	3.0	0.1	4,100
Air-dried 70 per cent rel. hum. at 20° C.	22.0	39.8	32.5	5.7	0.2	7,720
Dry, ash-free	—	55.0	45.0	—	0.3	10,680

Table 2. ANALYSES OF GASES FROM WAIMATUA LIGNITE
(Volume per cent calculated to air-free basis)

°C	Days	Atm. press.	Lignite (ml./gm.)	CO_2	C_nH_{2n+2}	n	C_xH_y	CO	H_2	N_2
200	28	1,000	19	95.9	1.1	1.4	n.d.	n.d.	0.5	2.5
270	14	100	37	94.7	4.4	1.48	n.d.	n.f.	trace	0.9
270	28	100	32	95.0	3.2	1.55	0.1	trace	n.f.	1.7
270	28	1,000	38	96.8	1.7	1.5	n.d.	n.d.	0.1	1.4
300	1	1,000	35	94.3	4.7	1.45	trace	trace	0.1	0.9
300	7	1,000	41	90.7	7.6	1.5	0.1	n.f.	n.f.	1.6
400	1	535	89	70.9	28.5	1.55	n.f.	n.f.	0.5	0.1
400	1	1,000	88	71.4	27.6	1.6	trace	0.3	0.2	0.5
400	7	1,000	82	61.5	37.9	1.43	trace	trace	0.3	0.3

n.f., not found; n.d., not determined

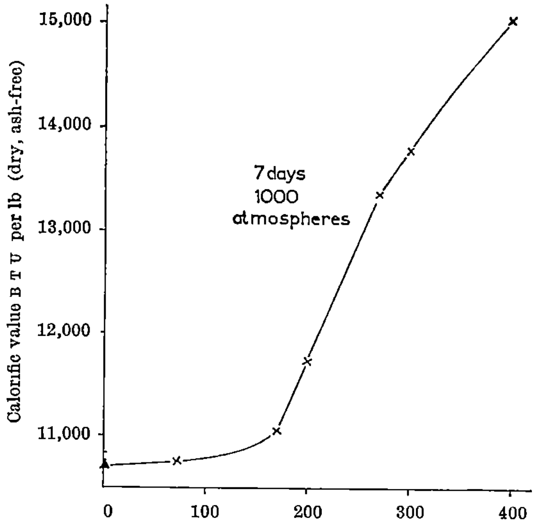

Fig 2 The influence of temperature on the calorific value of the product from heating Waimatua lignite for seven days at 1,000 atmospheres water pressure

curve obtained at 1,000 atmospheres pressure over a period of seven days From the results at 10 atmospheres pressure and seven days, however, the temperature of the point of inflexion is only about 120° C The change in calorific value at a given temperature is less than that shown in Fig. 2 for experimental times shorter than seven days, and more for longer times, but the shape of the curve changes little

The changes in calorific value shown in Figs 1 and 2 are the result of chemical changes in the lignite during the experiments, and the analyses of the gases evolved (Table 2) illustrate, in part, the influence of temperature, pressure and time on the nature and extent of the chemical reactions involved The analyses are expressed as percentages by volume and are calculated to an air-free basis Variable amounts of oxygen and nitrogen, derived from the air in the coal and bomb initially, were always present in the gases

The volume of gas evolved per gram of lignite increases with temperature, especially above 200° C , which is in accord with the greater changes in calorific value above this temperature shown in Figs 1 and 2 At 170° C still smaller amounts of gas were obtained The variables of pressure and time do not influence the volume of gas evolved in the marked and consistent manner of temperature

The composition of the gas evolved also changes with temperature, particularly between 300° C and 400° C where the percentages of carbon dioxide and the saturated hydrocarbons decrease and increase respectively at the same time as the amounts of each gas evolved increase At 300° C and 400° C the percentage of saturated hydrocarbons in the gas increases with time At 270° C the percentage of saturated hydrocarbons produced is greater at 100 atmospheres than at 1,000 atmospheres

Chromatographic analysis of gas from the 400° C samples showed methane to be the major constituent of the saturated hydrocarbon portion, with smaller amounts of ethane, propane, iso- and normal butane, iso- and normal pentane also present At 200° C only methane and ethane were found, whereas at 270° C and 300° C the mixture was methane to the pentanes Small amounts of the unsaturated hydrocarbon, isobutylene, were detected in the chromatograms of some samples, and a trace of propylene in one

In addition to these gases, water is an important product of these experiments and the amount expelled from the lignite increases rapidly above 170° C

In Fig 3, the calorific value and volatile matter of the products of the experiments at the various temperatures and pressures are compared with those of the starting material and with the New Zealand coal band[4]—the path of natural metamorphism of normal-type New Zealand coals It is clear that the products of the experiments above 200° C differ from normal-type New Zealand coals in that their percentage of volatile matter is low In this respect they resemble many coals known to have been metamorphosed by igneous intrusions On the other hand, the progressive decrease of moisture-holding capacity with increase of calorific value is similar to that of normal-type New Zealand coals

A tentative interpretation of the changes of volatile matter and calorific value (expressed on a dry ash-free basis) in Fig 3 is as follows If the lignite is heated to 400° C in less than one day under water pressure, its changing calorific value and volatile matter can be represented, as the temperature increases, by the dotted line bordering the lower volatile edge of the band of experimental results shown in Fig 3 The distance moved along the line is a function of temperature but is also inversely affected by the pressure applied If the lignite sample is held at a constant temperature of 270° C or higher, then the path of metamorphism diverges from the line of rapid heating in a direction more nearly parallel to the natural path The distance along the constant temperature line is a function of time

Although it is not possible to draw any conclusions from these experiments as to the temperature of metamorphism of normal coals, Figs 1 and 2 do indicate that coals will increase only slowly in calorific

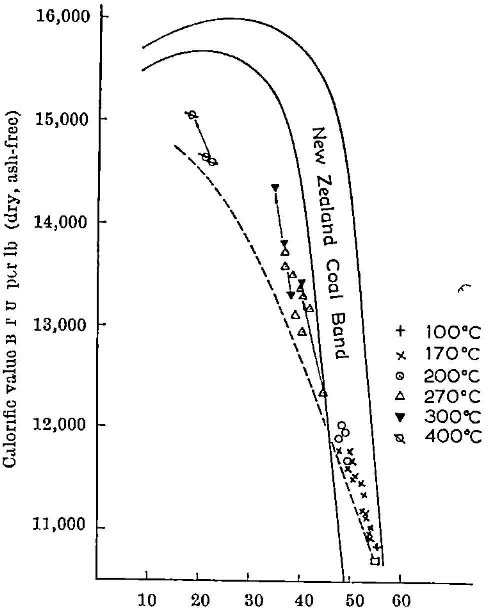

Fig 3 Comparison of the artificial metamorphism of Waimatua lignite at various temperatures with the New Zealand coal band—the path of metamorphism of normal-type New Zealand coals ---, Path of metamorphism during rapid heating, ←, path of metamorphism at constant temperatures

value and rank with temperature until a particular temperature is attained, after which rank increases more rapidly with temperature. The higher the pressure, for $P_{H_2O} = P_{total}$, the higher this threshold temperature will be Although water pressure appears to affect the initial rate of increase of rank the question remains whether the total change over geological periods of time is a function of temperature only

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ORIENTATION DEPENDENCE OF STRUCTURE IN PYROLYTIC GRAPHITE

By ROBERT H. BRAGG and CHARLES M. PACKER

Lockheed Missiles and Space Co , Sunnyvale, California

PYROLYTIC graphite is made by the pyrolytic decomposition of hydrocarbons on a mandrel heated to temperatures above 1,500° C X-ray investigations have shown that it consists of an oriented aggregate of turbostratic crystallites having unit cell parameters similar to those of carbon blacks at the onset of graphitization[1] The crystallite layer planes tend to lie parallel to the deposition surface, and heat treatment above 2,000° C produces a general increase in this preferred orientation, in crystallite size, and in layer stacking order. After being heated at temperatures above 3,000° C , the material is almost completely graphitized[2,3] A systematic X-ray investigation of singularly nucleated pyrolytic graphite was conducted in which particular emphasis was given to measurements of preferred orientation and the orientation dependence of the structure of individual crystallites following heat treatments The measurements were made on cylindrical rods of circular cross-section 0 15–0 35 cm. in diameter cut from the central portion of 1 0-cm thick plates of material which had been deposited in the temperature-range 1,850°–2,100° C All specimens were heat treated in an argon atmosphere to temperatures in the range 2,200°–3,000° C for periods up to 1,000 min

The X-ray measurements were made at room temperature with the rods mounted in such a way that it was possible to obtain the diffraction profiles for those crystallites in the rod having layer planes tilted at an angle α away from the original deposition surface The integrated intensity, centre of gravity, and the integral breadth were calculated from these results The centre of gravity of the basal reflexion profiles was used to determine the interlayer spacings, and accurate c-axis lattice parameters were obtained by extrapolation using the estimates from the 0002, 0004, 0006 and 0008 reflexions in a Taylor–Sinclair extrapolation[4]

Because there was no preferred orientation in the deposition plane of the deposited material, the observed integrated intensity for the 0002 reflexions I(α) obtained for a rod rotation of α is proportional to the volume of material in the specimen having (0001) normals oriented in the solid angle dω situated in the angular range α to α + dα relative to the original deposition surface

Typical preferred orientation measurements are shown in Fig 1 where the integrated intensity has been normalized at α = 0 The peak intensities actually ranged from 2,000° for the 1,950° C deposition material to about 8,000° for a specimen heated 25 min. at 3,000° C The linearity of the two upper curves shows that in the deposited material the orientation distribution is Gaussian, that is, has the form

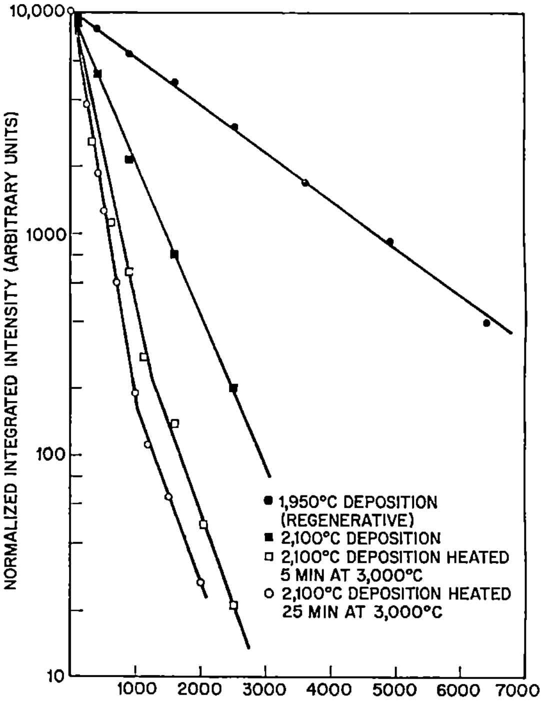

Fig. 1 Preferred orientation in rods of pyrolytic graphite α is the angle of inclination of crystallite layer planes relative to deposition surface in degrees

$$I(\alpha) = I_0 \exp (\alpha^2/2\sigma^2)$$

where σ is the variance of the distribution Increasing the deposition temperature decreases σ, that is, produces a sharper but still Gaussian orientation distribution The lower curves show that the texture is made even sharper by heat treatment at 3,000° C , and it remains approximately Gaussian Similar distributions were noted at all intermediate temperatures, the texture becoming sharper as the temperature rose, and most of the ultimate change at a given temperature occurred within the first hour

The basal texture can be characterized by a mean square crystallite tilt parameter $<\alpha^2>_{AV}$, where

$$<\alpha^2>_{AV} = \int \alpha^2 I(\alpha)\, d\alpha / \int I(\alpha)\, d\alpha$$

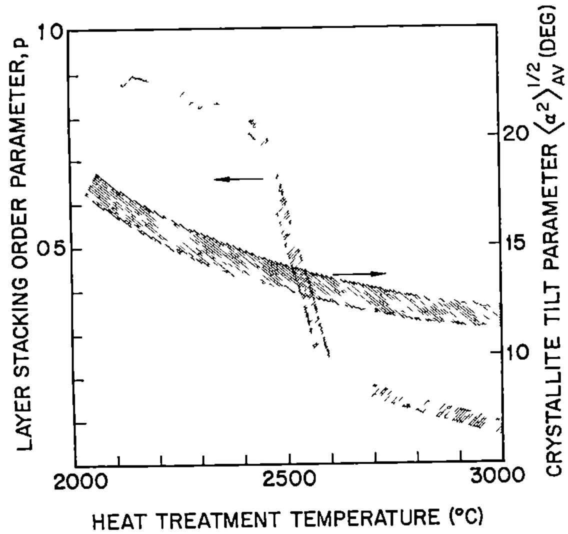

Fig 2 Effect of heat treatment on layer stacking order and crystallite tilt parameter

The layer stacking order attained for a given heat treatment can be characterized by an order parameter, p, which can be deduced from the mean crystallite interlayer spacing[5–7]. Here, p is the proportion of layers within a crystallite which possess the random stacking of turbostratic carbons. Both the crystallite tilt parameter $< \alpha^2 >_{AV}$ and the order parameter p obtained at $\alpha = 0$ are shown in Fig 2. The scatter bands cover the data obtained at 10-, 100- and 1,000-min heat treatments. In contrast to the sharp change in layer stacking order, in the temperature range 2,400°–2,600° C there is no well-defined temperature range in which the crystallite orientation is strongly affected.

The order parameters shown in Fig 2 were obtained at $\alpha = 0$. It was found that with a given specimen the interplanar spacing varies with crystallite orientation angle α. Such a variation could result from residual strains in the massive material, but other studies have shown that heat treatment relieves the strains in pyrolytic graphite[8]. Thus, the observed angular variation in interplanar spacing is due to variations in layer stacking order. Careful measurements show that there is a slight orientation dependence of line width as well, the width being greater for the larger tilt angles.

A typical orientation dependence of layer stacking order is shown in Fig 3 for material heated 100 min at 2,800° C. Similar variations were observed at all temperatures. Fig 3 shows that even in the untreated material, as deposited, the crystallites tilted at large angles from the deposition surface are more disordered than those misoriented by small amounts. While heat treatment sharpens the basal texture and produces a general ordering, it is less effective on the highly misoriented material. In fact, comparison of the two curves in Fig 3 shows that the total range of p is actually increased by heating. The extreme tails of the lower curves of Fig 1 depart from linearity, indicating a small amount of material which has not been strongly affected by the heat treatment. Thus, the present results show that some crystallites retain a large misorientation, grow very little, and remain highly disordered even after heating at 3,000° C.

A sharp change in layer stacking order in some temperature range above 2,000° C, such as that shown in Fig 2, is commonly observed in all graphitization processes. Franklin[9] has suggested that crystallites in graphitizing carbons grow by displacement of whole layer planes or even groups of layers and that graphitization occurs because of high internal thermal stresses set up during thermal treatment. The latter concept has been developed in some detail by Tsuzuku[10], Mizushima[11] and Mrozowski[12]. Tsuzuku showed that in spherical or cylindrical structures temperatures around 2,500° C can produce stress concentrations sufficient to buckle the crystal lattice. Stress relief can occur by the generation of dislocations or rearrangement of the turbostratic crystallites into the three-dimensional graphite lattice. Tsuzuku's theory predicts a minimum crystallite size of about 200 Å, below which thermal stresses cannot be relieved by generation of dislocations, but cause fracture instead.

The present work shows that orientation-dependent changes of layer stacking order during heat treatment are an integral part of the graphitization process in pyrolytic graphite. A stress relief mechanism seems most consistent with the observed behaviour. Stresses are developed because individual crystallites have a large anisotropy in their thermal expansion coefficients, and experience an average strain field which is also anisotropic. No estimates of the resulting stresses or strains are available, but an orientation dependence is clearly implied. It has already been demonstrated in other experiments that tensile straining parallel to the deposition surface enhances the ordering process[13]. Consequently, it seems likely that in heated pyrolytic graphite the resulting local stress distributions are such that the largest stresses parallel to layer planes are experienced by those crystallites tilted least away from the original

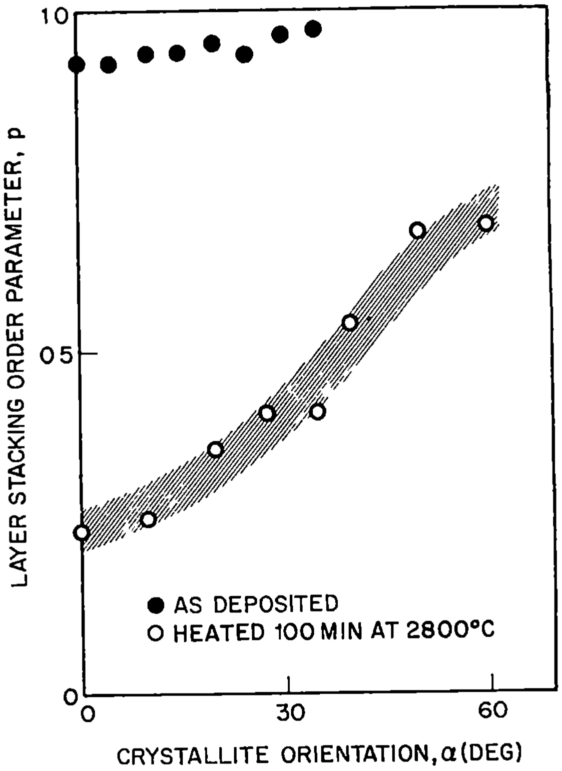

Fig 3 Variation of layer stacking order parameter with crystallite orientation

deposition surface The need for more quantitative experiments relative to this orientation dependence and an analysis of the local stress distribution in this situation is clearly indicated

The variation of order parameter with crystallite tilt angle shown in Fig 3 has the important consequence that the usual method of X-ray residual stress determinations cannot be applied to pyrolytic graphite The method depends on interpreting a variation of interplanar spacings with orientation as being due to strains alone The residual stresses are inferred from these strains by an equation which involves the observed strains and Poisson's ratio Since in pyrolytic graphite the variation of interplanar spacings with orientation can be an indication of ordering as well as strain, the usual method of residual stress determinations cannot be used This does not preclude use of an empirical correlation between X-ray line shifts and applied loads in studies of incremental stress-strain behaviour of a particular specimen, but such results could not be used in determining strains in other specimens

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FLUCTUATIONS OF GROUND-WATER LEVELS DUE TO ATMOSPHERIC PRESSURE CHANGES FROM NUCLEAR EXPLOSIONS

By Dr J INESON

Water Division, Geological Survey of Great Britain, London

THE effects of the nuclear explosions by the U S S R at Novaya Zemlya in October 1961 on atmospheric pressure disturbances in Great Britain[1-3] and in New Zealand[4] have already been reported

Automatic float-operated water-level recorders have been installed at a number of wells sunk into a variety of aquifers in England[5] They are used primarily for the investigation of seasonal and long-term variations in ground-water levels The recording-rate does not usually exceed 1·2 in /day and the water-level fluctuations are transmitted through a system of reduction gears ranging from 1 1 to 1 20 The hydrographs (traces of the fluctuations of ground-water levels) are investigated for macro- and micro-fluctuations

An examination of the hydrographs from certain wells sunk into Chalk, Lower Greensand and Jurassic limestones in southern England has confirmed rapid responses of ground-water levels to atmospheric pressure changes arising from the nuclear explosions of October 23 and 30, 1961 The maximum recorded fluctuation in ground-water level was about 0 02 ft in a Chalk well, although fluctuations of less than 0 01 ft are more common The hydrograph from a Chalk well at Wested, Kent (*TQ* 518668), shows a rapid fluctuation of ground-water level of about 0 015 ft just before noon on October 30, part of this hydrograph from October 30 to November 2, 1961 (inclusive), is reproduced in Fig 1*a* This fluctuation corresponds to the arrival of the direct pressure wave, the maximum peak to trough amplitude recorded on the microbarogram at Kew Observatory being about 900–1,000 microbars Successive reflexions of the pressure waves at the antipodes of Novaya Zemlya and at Novaya Zemlya itself were recorded at Foulness[2], Edinburgh[3] and at Kew Observatory (Fig 1*a*) The changes in pressure due to these reflexions affected ground-water levels up to November 2, being particularly well developed on the Wested hydrograph for October 31 and November 1 All wells recording the effect of the direct wave did not necessarily show every reflexion No positive evidence of reflexions after November 2 has been obtained

One automatic water-level recorder was installed on a Chalk well at Wheathampstead, Hertfordshire (*TL* 172145), with a recording time-scale of 0 4 in /h At about 1150 G M T , October 30, 1961, the hydrograph was interrupted by a clearly defined anomalous fluctuation in ground-water level comprising a fall, then a rise, succeeded by a fall in water-level to about the same level as before the anomaly The maximum trough to peak amplitude was about 0 02 ft This pattern is the mirror image of the initial stage of the pressure changes recorded on the microbarogram

The effect of the nuclear explosion on October 23, 1961, is less pronounced due to the smaller magnitude of the micropressure disturbances Fig 1*b* shows the hydrograph for the Wested well during the period October 22–25, 1961 (inclusive), the fluctuations in ground-water level just before noon on October 23 correspond to the arrival of the direct wave The maximum peak to trough amplitude at Kew Observatory was about 350 microbars Ground-water fluctuations due to reflexions are also present on October 24 and 26

The effect of changes in atmospheric pressure on ground-water levels is well demonstrated during this same period, as illustrated by Fig 1c, which is part of the corresponding barogram obtained at South Kensington, London, the reciprocal nature of atmospheric pressure variations and ground-water fluctuations is apparent In addition to the microfluctuations of ground-water levels resulting from the nuclear explosion on October 23, an anomaly occurred just before midnight October 22–23, 1961, a period associated with marked climatic changes, for the microbarogram at Kew Observatory shows a pronounced angular rise in atmospheric pressure (about

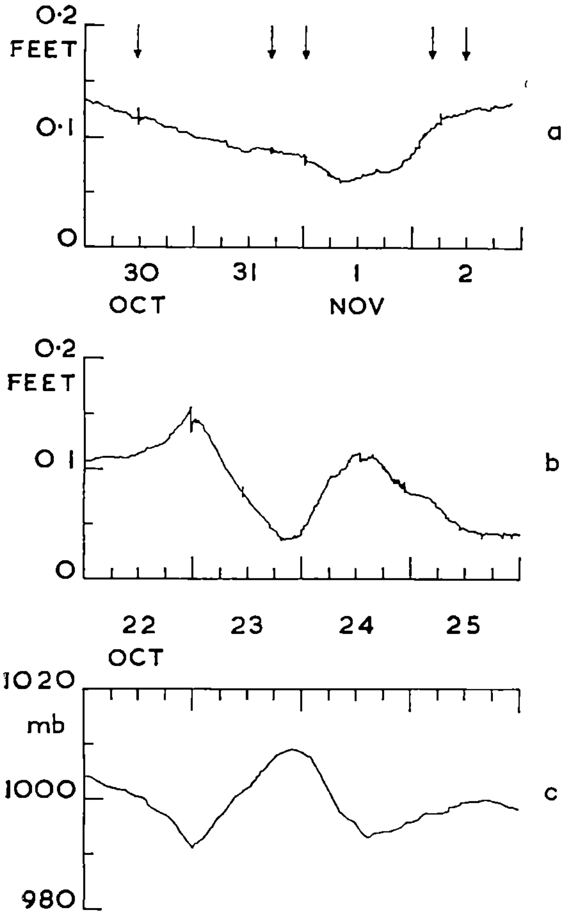

Fig 1a and b Ground-water fluctuations in Chalk well at Wested, Kent, a, between October 30 and November 2, the arrows indicate the onset of the direct atmospheric pressure wave and its reflexions, arising from the nuclear explosion by the U S.S.R, as recorded at Kew Observatory, b, between October 22 and 25, 1961 Fig 1c Barogram from South Kensington, London, between October 22 and 25, 1961

1,300 microbars) at about 2337 G M T, October 22, 1961 The pressure later declined gradually over the succeeding 1½ h· This pattern is faithfully reproduced in the ground-water fluctuation, again confirming the effect of microbarometric pressure changes

No positive evidence has been obtained of the effect of other nuclear explosions on ground-water levels, probably due to their lower range

The relationship between gradual changes in atmospheric pressure and fluctuations in ground-water levels can be determined over a period of time For the Wested well (Fig 1) an approximate value of 0 008 ft fluctuation in water-level is caused by a change in one millibar of atmospheric pressure The fluctuations of ground-water levels discussed here, due to rapid changes in atmospheric pressure, suggest a somewhat higher ratio (about double) This may be due either to the type of instrument used (a horizontal rotating drum to record water-level changes) or to a real effect with an almost instantaneous pressure-differential being created

Changes in barometric pressure influence the elastic state of a confined aquifer The magnitude of the effect depends on the pressure differential, the elastic properties of the aquifer and the degree to which confined conditions of ground-water flow exist in that aquifer As the aquifer responds elastically to these external pressure variations, the porosity and intrinsic permeability of the water-bearing material change with the movement of ground water into or out of the effective pore spaces, associated with fluctuations in water-levels In addition to ground-water fluctuations in confined aquifers due to this series of nuclear explosions, similar anomalies have been identified in so-called 'water-table' aquifers, as, for example, the Wested well in Chalk referred to here Two conclusions can be drawn, such aquifers are in fact confined or consist of a series of semi-confined aquifers, or unconfined aquifers do respond to changes in atmospheric pressure The former is probably applicable to the examples studied More detailed investigations are necessary to consider the second possibility

Microfluctuations of ground-water levels due to earthquake waves have been recorded in Great Britain[6], confirming the elastic state of an aquifer The arrival time of seismic waves arising from the nuclear explosion of October 30, 1961, would be about 0840 G M T in southern England[1] So far as is known, no record of these waves has been identified on ground-water hydrographs in Great Britain

Although the present use of automatic water-level recorders is generally restricted to relatively long-period traverses, rapid time traverses (up to 4 8 m / min) have been adopted for particular problems The form of ground-water fluctuations (corresponding to changes in the elastic state of an aquifer) due to seismic waves and atmospheric micropressure changes from nuclear explosions and other causes could be established, but the precise time of onset of these fluctuations cannot be catered for with the present equipment Microfluctuations of ground-water levels could be measured continuously by pressure transducers and other instruments recording electronically on magnetic tape, the form of the fluctuations being analysed afterwards The structural properties of aquifers can be elucidated more accurately by an analysis of ground-water fluctuations Since the elastic properties of an aquifer are implicit in all confined ground-water flow conditions, more intensive investigations by means of continuously recording equipment, suitably located, should contribute to a better understanding of aquifer properties and to the study of the availability of ground-water resources

Note added in proof Ground-water fluctuations arising from the atmospheric pressure waves created by the nuclear explosion on August 5, 1962, by the U S S R have been recorded over a wider area than after previous explosions The maximum recorded change in ground-water level was 0 045 ft in a Chalk well, this is double that in October 1961 The maximum peak to trough amplitude recorded on the microbarograph at Kew Observatory was about 1,300 microbars No reflexions of the atmospheric pressure waves were identified on ground-water hydrographs on August 6 and 7 This may be due to the subdued nature of the pressure changes and to a pronounced rise in ground-water levels on August 6, 1962, the result of a general reduction in barometric pressure

Information provided by the Meteorological Office, the Metropolitan Water Board and the Science Museum is gratefully acknowledged

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LETTERS TO THE EDITOR

RADIOPHYSICS

Faraday Rotation Effects associated with the Radio Source Centaurus *A*

Recent observations[1,2] with the 210-ft. telescope of the Australian National Radio Astronomy Observatory have revealed the existence of linearly polarized radiation from the source Centaurus *A* at frequencies of 3,000 Mc/s and 1,410 Mc/s.

This communication reports investigation of Faraday rotation effects associated with this radiation. Measurements have been carried out at frequencies ranging from 970 Mc/s to 3,000 Mc/s at a number of points in the source. The points, the coordinates of which are given in Table 1, are located in the bright central component and in the northern and southern extensions of the source. The observing methods were essentially those of Gardner and Whiteoak[2].

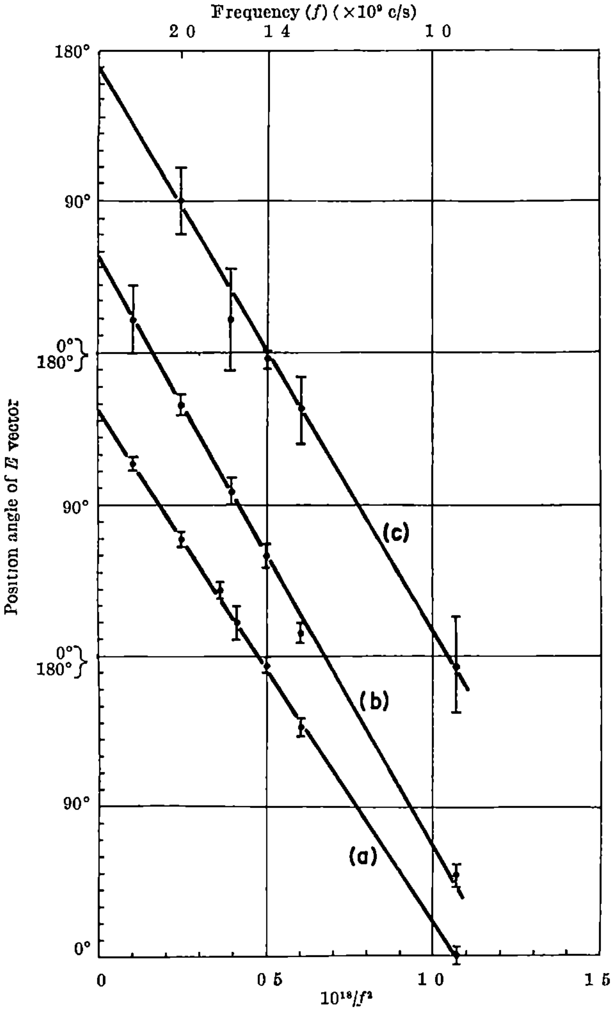

Fig. 1. Frequency dependence of the polarization position angle at three points in Centaurus *A*. *f*, Frequency in c/s. *a*, R.A. 13h 23m 15s, declination −42° 48.5′; *b*, R.A. 13h 24m 00s, declination −42° 30′; *c*, R.A. 13h 20m 30s, declination −44° 25′. Curve *c* has been arbitrarily displaced upwards by 180° relative to *a* and *b*.

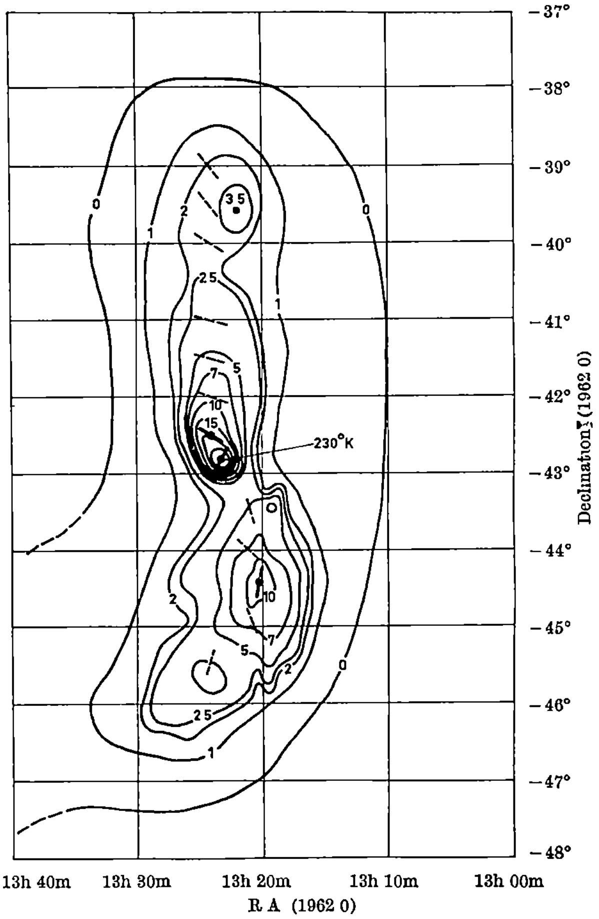

Fig. 2. Isophotes of Centaurus *A* at 1,410 Mc/s in deg. K. The short solid lines show the direction of the electric vector corrected for Faraday rotation at the three points quoted in Fig. 1. Also shown dotted are directions of the electric vector at several other points as observed at 1,410 Mc/s. Aerial beam-width is 13.5′ to half-power points.

Table 1 lists the observing frequencies and the position angle of the electric vector observed at the various points. The percentage of linear polarization is also given.

In Fig. 1 the position angle of the electric vector is plotted against a frequency variable $10^{18}/f^2$ for the three quoted points in the source. It is seen that, within the experimental error, the position angle has a $1/f^2$ dependence, as expected for the Faraday effect, the agreement being particularly good for the central component. Thus the position angle corresponding to $f = \infty$ should be the true polarization angle at the source. It is also to be noted that the rotation is in the same sense at all three observed points and the slopes of the three curves do not differ greatly from each other. Curve *a* exhibits a total rotation from 1,000 Mc/s to infinite frequency of 306°, curve *b*, 350°, and curve *c*, 344°.

Table 1 FARADAY ROTATION

Frequency (Mc/s)	Beam-width (min of arc)	R A 13h 20m 30s Dec −44° 25′		Central component R A 13h 23m 15s Dec −42° 48 5′		R A 13h 24m 00s Dec −42° 30′		R A 13h 24m 00s Dec −42° 10′	
		Position angle	Per cent	Position angle	Per cent	Position angle	Per cent	Position angle	Per cent
3,000	6 7			115° ± 3°	15 ± 2	20° ± 20°	45 ± 15		
2,000	10 0	90° ± 20°	28 ± 5	70° ± 3°	8 ± 4	150° ± 3°	45 ± 5		
1,650				40° ± 4°	9 ± 4				
1,575		20° ± 30°	27 ± 5			98° ± 7°	30 ± 3		
1,550				20° ± 10°	6 ± 4				
1,410	13 5	175° ± 5°	15 ± 5	175° ± 3°	7 ± 2	60° ± 5°	30 ± 5	71° ± 5°	30 ± 5
1,280		147° ± 20°	20 ± 5	138° ± 4°	6 ± 3	15° ± 3°	24 ± 4		
970	20 0	174° ± 30°	18 ± 10	180° ± 5°	3 ± 1	49° ± 3° (at −42° 20′)	19 ± 3	50° ± 5°	17 ± 4

At 970 Mc/s a receiver was used which passed two sidebands separated by 60 Mc/s It can be deduced from Fig 1 that the differential rotation between these sidebands amounted to about 45°, so that the true degree of linear polarization was approximately 10 per cent higher than that observed It is interesting to note that receiver band-width needs to be severely restricted from polarization observations of Centaurus *A* at lower frequencies since the band-width depolarization effect goes as $1/f^3$

A complete survey of the source has now been made at 1,410 Mc/s with a sensitive parametric receiver and analysis of the observations is in progress Fig 2 is a preliminary sketch of the isophotes with a superimposed indication of the direction of polarization at the three observed points, corrected for Faraday rotation It has been known for some time that the central component consists of two small sources lying on a line in position angle ~ 45° and separated by 7 minutes of arc, and as shown by Bracewell, Cooper and Cousins[1] only the north-east source is polarized The present determination of 147° for the polarization position angle at the north-east source places its electric vector roughly at right angles to the line joining the two sources

At the two points examined in the extended source the polarization runs approximately in the direction of the extension, but this orientation may not be too well maintained over the whole extended source Although linear polarization has been detected at 1,410 Mc/s over the whole area covered by the source, the Faraday rotation measurements have been made only at the three quoted points where a satisfactory signal-to-noise ratio could be obtained with simple receivers tunable over a wide frequency-range

The observed Faraday rotation is nearly two orders of magnitude greater than can be ascribed to ionospheric effects. Evans[3], for example, has measured a one-way Faraday rotation in the ionosphere above Jodrell Bank of approximately 720° at 120 Mc/s at 08 h local time If similar ionospheric conditions apply to the present measurements the ionospheric Faraday rotation can be no more than 10° at 1,000 Mc/s In the present measurements no significant variation of the polarization angle has been observed between the afternoon hours and midnight, as would be expected if the ionosphere were playing a considerable part Hence the majority of the rotation must be occurring either within our own Galaxy or in the outer regions of Centaurus *A*

The amount of Faraday rotation to be expected from a cloud of electrons of concentration N_e (cm^{-3}) situated in a magnetic field H (gauss), which is uniformly oriented over a path-length L cm at an angle α to the line of sight, is given by

$$\psi = 2\ 36 \times 10^4 N_e H L f^{-2} \cos \alpha \text{ radians}$$

where f is the frequency of the radiation in cycles per second

For the observed amount of rotation a value of the product $N_e HL \cos \alpha$ of $\sim 2\ 5 \times 10^{14}$ is required Since the galactic co-ordinates of Centaurus *A* are $l^{\rm II} \approx 320°$, $b^{\rm II} \approx + 20°$ the radiation from the source travels for a considerable distance through the galactic corona If the rotation is to occur in this region we may put $L \approx 10$ kiloparsecs, $H \approx 10^{-5}$ gauss and for the highly favourable case of $\alpha = 0$ over the whole 10-kiloparsec distance we need an electron density of 7×10^{-4} cm^{-3} This value is feasible, but the requirement of uniformity of the magnetic field over such a great distance raises serious doubt that the rotation can be occurring in this region

On the other hand, if the rotation is occurring in the outer regions of Centaurus *A* the effect is, again, remarkably uniform over great distances At an assumed distance of 4 megaparsecs the three regions investigated cover a distance of 160 kiloparsecs transverse to the line of sight However, the 1,410 Mc/s survey referred to earlier is revealing that the polarization angle is itself fairly constant over some quite large distances in the source, so that the possibility of a substantially constant Faraday rotation in the outer regions of the source cannot be ruled out entirely

Investigation of Faraday rotation effects in other polarized extra-galactic sources may shed more light on the problem and will be awaited with much interest

We thank our colleagues whose joint efforts have made these observations possible, and in particular Mr J G Bolton and Dr F J Kerr for helpful advice and criticism One of us (R M P) is the recipient of a Fulbright Fellowship

B F C COOPER
R M PRICE

Division of Radiophysics,
C S I R O,
University Grounds, Sydney

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Cyclotron Theory of Very-Low-Frequency Discrete Emissions

It was recently shown[1] that the Doppler shifted cyclotron radiation from receding electrons would give the observed frequency-time shape of discrete very-low-frequency emissions A further development enables information about these electrons to be deduced from this shape. Thus, electron energy (E), initial helical pitch angle (ψ_0), geomagnetic field line of occurrence (λ), and the exosphere electron density parameter[2], 'scale frequency' (f_a), are obtained from four parameters (two frequencies and two times) scaled from a spectrogram of the emission. Full details of this process will be published elsewhere

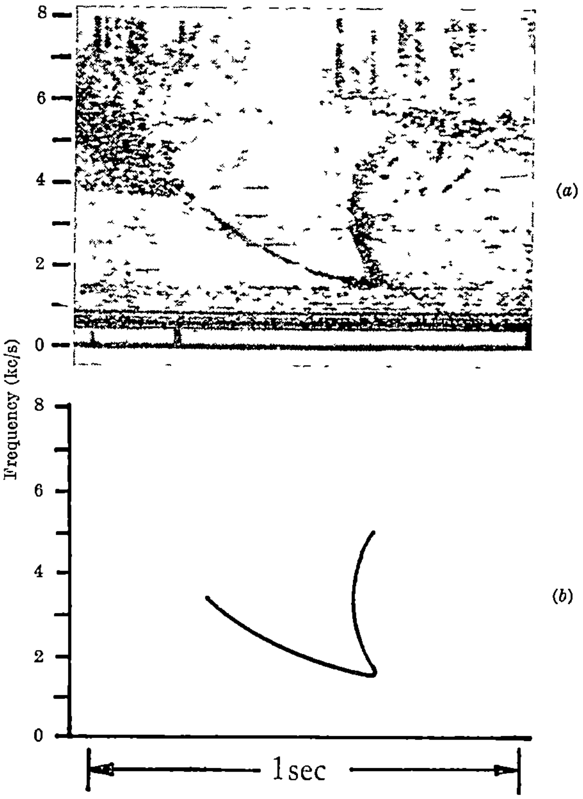

Fig 1 *a*, 'Hook' recorded at Seattle, on September 23, 1957, at 2035 28 U T (after Helliwell and Carpenter, ref 6), *b*, as calculated from the theory (ref 1) for E = 150 keV, ψ_0 = 68 6°, λ = 61 4° and f_a = 527 kc/s

As a demonstration, consider the 'hook' shown in Fig 1*a* The location of three points in the frequency-time plane unambiguously requires E = 150 keV, ψ_0 = 68 6°, λ = 61 4° and f_a = 527 kc/s To test the theory, 25 points were calculated from first principles using this information The curve through these points is shown in Fig 1*b* Comparison shows excellent agreement

The theory[1] explains other types of discrete emissions as special cases of 'hooks' In particular, the theory predicted a repetitive type of emission not previously recognized This occurs when the electron bunch survives several hemisphere to hemisphere traverses or 'bounces' between magnetic mirror points The emission appears as a succession of similar hooks separated by the bounce period of the electrons It is readily distinguished from whistler mode echoes of a single hook since successive hooks are not progressively more dispersed according to their order in the sequence Since the observable radiation is unidirectional the hooks are received in opposite hemispheres alternately, that is, the sequence observed in one hemisphere is displaced half a bounce period from that observed in the other This effect distinguishes the predicted emission from some other effect such as periodic injection of electrons

Observation of this predicted emission showing a bounce period consistent with E, ψ_0, and λ scaled from the shape would be a crucial test of the theory both qualitatively and quantitatively

A sequence which was probably of this predicted type was recently discussed[3] It appeared as two bursts of 5–6 kc/s noise (presumably parts of a single hook) seen three times It was observed in both hemispheres, not simultaneously, but alternately as explained here Since only a narrow frequency band was observed the progressive dispersion criterion could not be applied However, the recurrence period was consistent with a bounce period for electrons of typical energies as deduced from other hooks, but only a half to a third of typical whistler mode echo, delay times at this frequency On the other hand, in a reply[4], Helliwell pointed out that whistler delay times can be sufficiently small during magnetic storms so that in this case the echo possibility cannot be ruled out

I have since noticed four clear-cut examples published by Gallet[5] In each of these there was a striking lack of progressive dispersion despite the considerable frequency extent of the hooks and the relatively large number of separate hooks (six, in one sequence) Gallet[5] remarked on this at the time Table 1 shows E, ψ_0 and λ deduced from the shape of one of the hooks for each sequence The expected bounce periods calculated from E, ψ_0 and λ are shown as P_c and the observed periods (as measured by Gallet) are shown as P_0 The spectrograms as published[5] are rather small for accurate scaling so that the probable error in P_c is rather large However, the general agreement is quite good

Table 1 COMPARISON OF OBSERVED RECURRENCE PERIOD (P_0) WITH THAT CALCULATED (P_c) FROM E, ψ_0 AND λ DEDUCED FROM THE FREQUENCY-TIME SHAPE

Sequence No	No of hooks in each sequence	Energy (E) (keV)	Helical pitch ψ_0	Geomagnetic latitude λ	Calculated period P_c (sec)	Observed period P_0 (sec)
1	3	14	70°	59°	1 2	1 6
2	6	8	62°	59°	1 7	1 6
3	2	15	72°	59°	1 1	1 6
4	3	5	48°	59°	2 1	1 6

The electron cyclotron theory gives excellent agreement with the observed frequency-time shape of discrete emissions In addition, the predicted periodic emission is shown to occur and with periods reasonably close to those predicted Verification of the theory not only clears up a mystery of long standing but also justifies the use of spectrograms of emissions for finding information about the electrons which produce them Large numbers of such spectrograms were recorded during the International Geophysical Year

I thank Prof G R A Ellis for advice

R L DOWDEN

Ionospheric Prediction Service,
University of Tasmania,
Hobart, Tasmania

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PHYSICS

Stark Effect Broadening of the Krypton Lines and Emission of the Kr_2^+ Molecule

IN the investigation of the asymmetrical broadening of atomic lines of inert gases, one usually neglects other perturbations such as the formation of unstable molecules from excited and normal atoms Most molecules bound by Van der Waals forces produce bands on the shorter wave-length side of the line,

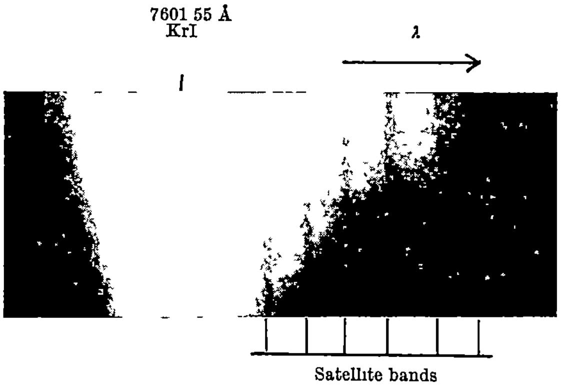

Fig 1

while the external electric field shifts the line towards the longer wave-lengths

There are numerous observations of absorption satellite bands appearing in the presence of foreign gases on the red side of the principal series of the alkali metals[1] Their intensity increases with the polarizability of the foreign gas In emission, complex bands appear on both sides of some mercury lines in the presence of inert gases at high pressure[2]

So far as inert gas molecules are concerned, only He_2 spectra are well known For other inert gases continuous bands are emitted either in the positive column or in the arc It has been assumed that diatomic molecules like Kr_2 were responsible for these intensity fluctuations[3] However, no spectroscopic analysis is available to support this interpretation

So far as we know, the molecular emission analysed in this communication is the first to verify by spectroscopic means the existence of Kr_2 or Kr_2^+ molecules

The discharge lamp used to excite the Kr_2 and Kr_2^+ spectra was made of a thick glass bulb of 2 cm diameter having two 2-mm diameter tungsten electrodes separated by 5 mm This bulb was thoroughly pumped out, and then filled with pure commercial krypton supplied in glass containers by the Liquid Air Co This gas was first liquefied under reduced pressure at liquid nitrogen temperature, and then warmed up until the pressure reached 10 atmospheres The discharge current of a few m amp was supplied by a 4,000-V d -c generator and the emission photographed with a Jaco-Ebert 3 4-m spectrograph having a dispersion of 3 24 Å per mm at 7500 Å

Fig 1 shows an enlarged print of the broadened KrI λ7601 55 Å ($1s_5$–$2p_6$) line Like most of the other lines, it is shaded to the red Its intensity decreases very rapidly with the wave-length It is not easy to decide whether the observed structure is due to re-absorption or to emission An attempt to find an absorption spectrum using a second continuous emission lamp failed Therefore, we will assume that the intensity fluctuations are true emission bands From rough wave-length measurements of the intensity minima the successive band intervals are (in cm^{-1}) 1 89, 1 98, 1 95, 2 08, 2 39, 2 06, 2 38, 2 32 Secondary minima may also be seen showing frequency intervals of 1–1 25 cm^{-1}

If the emitting molecule were diatomic one would have a choice between the following interpretations

(1) The bands are due to an unstable Van der Waals-like molecule The spacing between the most intense bands, of the order of 2 cm^{-1}, may then be explained as originating from a transition between a fixed upper vibrational level and a variable vibrational level of the lower electronic state The vibrational frequency of 2 cm^{-1} would then correspond to a dissociation energy of about 0 005 eV, which is unusually small

(2) The bands are a portion of a sequence located near the turning point In this case, the frequency difference could be represented by

$$\Delta\nu = 2(\omega_e' x_e' v' - \omega_e'' x_e'' v'')$$

Here the observed frequency difference (2 cm^{-1}) does not give any precise information on the molecular stability

In the first approximation the lines are simply shaded to the red It is natural, first of all, to think in terms of asymmetrical broadening by the ions and electrons through the quadratic Stark effect There may be no fundamental difference between the emission of a weakly bound excited Kr_2^+ molecule and the emission of a line broadened by intermolecular Stark effect Since the effect of the electric field is larger for the $2p_6$ excited state than for the $1s_5$ metastable state, the band system corresponding to the transition

$$[Kr\,(2p_6) + Kr^+\,(4s^2\,4p^5)] \rightarrow [Kr\,(1s_5) + Kr^+\,(4s^2\,4p^5)]$$

then would lay-out on the long wave-length side of the non-perturbed line, just as the atomic line is asymmetrically broadened by electric fields

The Kr_2^+ molecule has been found in its fundamental state in mass spectrometer investigations of a partially ionized plasma[4] The Kr_3^+ and Kr_4^+ complex ions have also been observed From an analysis of ionic mobilities it is found that the ionic equilibrium is shifted towards the ions with a larger number of atoms Thus the complicated structure of the emission bands may be due to a polyatomic molecule However, such a molecule is usually less stable in an excited state than is the normal one, contrary to what is observed here Indeed, the shading towards the red of the bands corresponds to a higher stability of the upper excited state The attraction force between the excited Kr ($2p_6$) atom and the Kr^+ ion can easily be estimated from the quadratic Stark effect constants, and is much larger than the Van der Waals force between the same atoms It is difficult to build up a spectroscopically detectable number of Kr_2^+ molecules because for small intermolecular distances they dissociate through the interaction between the potential energy curves, while, on the other hand, when the electric field intensity is sufficiently high or the distance between the ion and the excited atom small enough, pre-ionization may occur The atomic state vanishes and so does the corresponding molecular state

Margenau and Klein[5] have attempted to interpret some of the emission satellite bands At low inert gas pressures, one usually observes violet bands which are generally the consequence of the transition from an upper vibrational level formed by collision (for example, between Kr and Kr^+) located near the dissociation limit to different vibrational levels of the lower electronic state As the pressure increases, collisions will build up the population of the lower vibrational levels of the excited electronic state, and shift the emission towards longer wave-lengths Thus, one is brought back to the hypothesis of a molecular transition between high vibrational levels In the present case, the absence of violet bands would indicate that we are concerned with Kr_2^+ and not Kr_2 molecules since, in the case of metallic lines emitted in presence of inert gases, one observes intense violet bands Such bands may be due to neutral Van der Waals molecules the polarizability of which is smaller for the more excited state For the 7601 55 Å line with which we are concerned here, the violet bands are practically absent This is in agreement with the fact that the Stark effect constant is larger for the more excited state.

To sum up, in usual discharges at high ion densities, as in arcs or sparks, the temperature of the gas is so high that most of the neutral or ionized molecules are dissociated But when the ionic concentration is high, at low gas temperatures, the contribution of molecular ion emission may be detectable

L HERMAN
R HERMAN

Observatoire de Paris,
Meudon,
Seine-et-Oise

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Interpretation of Quantum Physics

ATTENTION has been directed to persistent questions of interpretation in quantum physics by the recent exchange of views[1] on the subject between Dr H V. Stopes-Roe and Prof A Landé To Prof Landé's picture[2] of the particle as permanently localized phenomenon, influenced through action-at-a-distance by the extended structure of a measuring instrument, Dr Stopes-Roe understandably objects that a necessarily detailed and 'suitable interpretation of the collective action of the apparatus' has not been provided Prof Landé's failure to furnish such an interpretation is equally understandable, since any interpretational superstructure in physics that lacks roots in the underlying mathematical formalism (cf the luminiferous ether) tends sooner or later to be viewed as excess ideological ballast

Those who believe, however, that all ballast of this kind has been jettisoned from the present most widely accepted version of quantum mechanics should reflect on the absence of a necessary basis[3] in the existing quantum mechanical formalism for the conceptual device known as 'reduction of the wave packet', by which device most physicists reconcile their natural prejudice that events 'really happen' with the fact that the equations of quantum mechanics contain no parameters descriptive of events (as such) and hence rigorously deny respectability to the concept of 'event'

Perhaps the most harrowing questions, then, that plague contemporary quantum physics are not those concerning the 'wave-particle dualism', which bear on the loss of causality (trajectory definability) in any system described by a wave function, but those concerning objectivity (event definability) and the proper severing-point of the 'von Neumann chain'[4] connecting the thing observed with the mind of the observer Several deep-going paradoxes[5], which strike to the foundation of physics as a non-solipcist discipline, arise if (as is now customarily done) the non-mechanical element 'mind', which appears in none of the equations, is adduced as necessary ballast in mechanical description

It is the purpose of this communication to point out that there is an altogether different approach to interpretational problems and paradoxes in quantum physics, by which one directly attacks the mathematical formalism itself, rather than the words that are said about it By this approach one can take advantage of the epistemological leverage exerted by the formalism, whereby the gentlest modification of the mathematics characteristically engenders the most drastic changes in the interpretation

Until recently the formalism of quantum mechanics was considered proof against all modifications except such as would spoil its excellent agreement with experiment However, it has now been shown[6] that a perfecting of the formal correspondence (with Hamilton-Jacobi mechanics) by which the quantum-mechanical equations of motion are derived leads to a generalized form of the latter The solutions of the generalized equations corresponding to ordinary quantum mechanical states are identical with the familiar Schrodinger or Dirac solutions except for the attachment to the wave function of a constant phase factor, for example, of the form $\exp[-(i/\hbar)\Sigma_k P_k X_k]$ The X_k and P_k are constants, formal analogues of the 'initial conditions' constants that appear in the classical theory (as parameters descriptive of the event by which a classical state of motion is entered or produced)

The constancy of the phase factor implies its general unobservability (absorption into the wave-function normalization factor) Thus, the description of stationary states and non-contingent events is the same in the generalized theory as in the customary one However, the appearance of new parameters that may be considered to describe the event (or world point) by which a system enters a new state, or changes its state, puts an altogether new complexion on many matters of interpretation Not only is the 'event' concept, which is virtually indispensable to relativity theory, rendered for the first time respectable in quantum theory, but the hitherto nebulous and controversial idea of 'quantum jump' is given an explicit formal-descriptive characterization, as reflecting a discontinuous jump in the value of the phase factor (a jump that 'reduces' not the wave packet but the wave function itself) Only the fact that changes in the X_k, P_k occur is physically significant, not the magnitudes of these parameters The von Neumann chain is severed wherever and whenever an event, or localized particulate interaction (as distinguished from non-local interactions with extended structures), occurs That individual-particulate events seem to occur physically (for example, scintillations of a fluorescent screen, decay of single radioactive atoms) and to occur independently of cognition has always been undeniable Now, if we like, we can aver that appearances are not deceptive

With the re-entry into the formalism of parameters specifically descriptive of events, objectivity is restored, and 'mind' ceases to play a non-classical role Observation, as in classical mechanics, produces no disturbance incompatible with equations of motion, and the latter now suffice for physical description without need or place for non-mechanical additives On the other hand, the fact that the development of a system in time is described by a wave function implies non-restoration of causality The basic facts of Bohr's 'complementarity' remain unaltered World points and objectivity are re-established, world lines and causal pictorializations of trajectories are not This variety of formal 'completion' of quantum mechanics leaves God (in Einstein's figure of speech) playing dice with the world, but the fall of dice no longer depends for consummation on the merciful presence of an observing mind

In summary, it would appear permissible to view the world as a lattice of discrete space-time event points, existing independently of observation, as was true in classical physics But the classical option of causally connecting the lattice world points by continuous world lines appears fruitless to pursue, and this fruitlessness remains the hall-mark of quantum physics Even the limited return to classical precepts envisioned here seems unattainable within the existing formalism of ordinary quantum mechanics Rather, the formalism itself must be modified, in a way that affects none of the simpler agreements with experiment, but that drastically affects the theory of measurement (that is, theory of contingent events) The question of possible comparison with experiment of an altered theory of measurement has been touched on elsewhere[7] The matter will bear further study

T E Phipps

U S Naval Ordnance Test Station,
China Lake, California

[1] *Nature*, **193**, 1276 (1962)

[2] Landé, A , *From Dualism to Unity in Quantum Physics* (Cambridge Univ Press, 1960)

[3] Dirac, P A M , *The Principles of Quantum Mechanics*, third ed , 110 (Oxford, 1947) The author states that "a disturbance caused by a process of observation" is not "compatible with equations of motion'

[4] *Observation and Interpretation*, edit by Koerner, S (Academic Press, 1957)

[5] The classic example is that adduced by Einstein, A , Podolsky, B and Rosen, N , *Phys Rev* , **47**, 777 (1935) Still more poignant is the 'cat paradox' of Schroedinger, E , *Naturwiss* , **23**, 812 (1935), an interesting reformulation of which has been given by D Bohm on p 138 of Koerner's book (loc cit)

[6] Phipps, T E , *Phys Rev* , **118**, 1653 (1960)

[7] ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D C , *Document No 6257*

In a previous communication[1] I asked Prof Landé to fill out certain details of his interpretation of quantum physics, namely, the mode of action of a certain piece of apparatus I described Dr Phipps now suggests that I was demanding that Prof Landé give some sort of ether type model This was not at all my intention—I was asking him to describe the apparatus in whatever terms were appropriate to his approach I could not see how this could consistently be achieved, and I therefore asked for enlightenment

Prof Landé's reply[2] to my question does not meet the problem He appeals to the fact that collective action 'is always taken for granted' in contemporary quantum theory, but the general acceptance of collective action rests on the general acceptance of non-simply-localized 'particles'—which Prof Landé specifically rejects

The spread mode of action between 'particle' and environment is an important experimental feature, and it must be given explicit recognition Broadly there are three ways of approaching a treatment (apart from anything radically new, as for example some of Bohm's recent ideas)

(1) The 'particle' has a spread quality, (2) The environment acts collectively, (3) Action at a distance between the two, by an intervening medium, by a field

The first (with complementarity) is the classical quantum view, the second indicates Landé's approach, the third is illustrated by Bohm in his earlier period But 1, 2 and 3 characterize programmes for theory construction, not theories What is it for the three bits of metal I described to 'act collectively' in the very special way required ? Talk of a crystal or a gas makes the given problem look simpler by adding irrelevant features which serve only to confuse The difficulty is not simply the relativistic one of instantaneity in general, nor simply the mechanical one of perfect rigidity, this will be recognized if the example is examined in detail

Dr Phipps, in the major part of his communication, leaves the problem of localization and asks 'What constitutes an event ?' I will not follow him now

H V Stopes-Roe

Department of Extra-Mural Studies,
University of Birmingham

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[2] Landé, A , *Nature*, **193**, 1277 (1962)

Effect of Adsorbent Temperature on the Brunauer–Emmett–Teller Surface Area determined by Krypton Adsorption at 77° K

In a recent paper on the use of krypton adsorption at low temperatures as a method of determining Brunauer–Emmett–Teller (B E T) surface areas, Haynes[1] has shown that the monolayer volume, v_m is dependent on the value chosen for the saturation vapour pressure of krypton, p_0, and has pointed out that if a 'wrong' value of p_0 is used a B E T plot which is curved in the monolayer region will result In particular, the use of the vapour pressure of solid krypton in the monolayer region can lead to curved B E T plots, though this difficulty can usually be largely, if not completely, overcome by using instead the extrapolated liquid vapour pressure as has been recommended by Beebe, Beckwith and Honig[2]

There is, however, another factor which may cause a 'wrong' vapour pressure to be used, so giving B E T plots which are curved within the monolayer region Moreau[3] has reported that in determinations of B E T surface areas by nitrogen adsorption at 77° K the adsorbent may be at a higher temperature than the surrounding refrigerant Temperatures, measured in three samples 20 min after nitrogen had been admitted to the apparatus at a pressure of 100 mm mercury, were between 1 8 and 2 3° K higher than that of the surrounding liquid nitrogen If the saturation vapour pressure of nitrogen corresponding to the liquid nitrogen temperature is used in the B E T calculation, it will be lower than that corresponding to the adsorbent temperature by 20–25 per cent and this will in turn influence the value of v_m and hence the surface area calculated from it Depending on the adsorbent, the B E T plot may also then show curvature, which in this case will be convex to the relative pressure axis, within the monolayer region

The effect of similar temperature differences on the extrapolated liquid vapour pressure of krypton at 77° K will be more marked than in the case of nitrogen on account of the higher heat of vaporization of the former as can be seen from Table 1, in which the vapour pressures have been calculated from the equation proposed by Meihuizen and Crommelin[4]

The change in temperature from 77 4° K to 79 4° K increases the liquid vapour pressure by about 55 per cent and hence the effect on the B E T plot of a 2° K

Table 1 VARIATION OF EXTRAPOLATED LIQUID VAPOUR PRESSURE OF KRYPTON WITH TEMPERATURE

T ° K	77 4	77 8	78 2	78 6	79 0	79 4
p_0 mm mercury	2 514	2 748	3 001	3 274	3 567	3 883

uncertainty in the adsorbent temperature will be at least as great as that caused by using the solid rather than the extrapolated liquid vapour pressure

To show the effect of such changes in vapour pressure, determinations of the surface area of a uranium trioxide and of a uranium dioxide powder were recalculated taking in turn 3 000, 3 500 and 4 000 mm mercury as the vapour pressure of liquid krypton at the adsorbent temperature Both adsorbents gave B E T plots which were linear in the monolayer region when the liquid vapour pressure corresponding to liquid nitrogen temperature was used in the calculations For the uranium trioxide, deviation from linearity was barely perceptible with a vapour pressure of 3 000 mm mercury, but at the two higher pressures there was obvious curvature, concave to the relative pressure axis In the case of the uranium dioxide which has a lower *c* value, 30 as against 150 for the trioxide, curvature was apparent at all three pressures The temperatures corresponding to these vapour pressures, the monolayer volumes calculated by drawing tangents to the B E T curves, and the corresponding B E T surface areas are shown in Table 2

Table 2 CHANGE OF B E T SURFACE AREA WITH KRYPTON VAPOUR PRESSURE

p_0 (mm mercury)	Corresponding temperature (° K)	v_m (ml)	Surface area (m²/g)
		Uranium trioxide (200 mg)	
2 553	77 5	0 416	10 8
3 000	78 2	0 432	11 2
3 500	78 9	0 445	11 6
4 000	79 5	0 463	12 0
		Uranium dioxide (406 mg)	
2 558	77 5	0 185	2 37
3 000	78 2	0 190	2 44
3 500	78 9	0 195	2 50
4 000	79 5	0 199	2 55

Though the curvature is more pronounced for the dioxide the greater change in surface area occurs with the trioxide

One of the advantages of krypton adsorption in determining surface areas is the possibility of using relatively small volumes of adsorbent so that the development of significant temperature differences between absorbent and refrigerant will not be favoured Such differences in temperature are, however, a possible source of error which should be considered, and they may explain some of the curved B E T plots which have been reported In particular, they may be significant where very low specific surfaces are being measured and where, as a result, it is necessary to use a large volume of adsorbent

I thank Mr R V Moore, managing director, and Dr H Kronberger, deputy managing director, of the Reactor Group, U K Atomic Energy Authority for permission to publish this communication

J B AINSCOUGH

U K Atomic Energy Authority,
Reactor Group,
Reactor Fuel Element Laboratories,
Springfields, Salwick,
Preston, Lancs

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CRYSTALLOGRAPHY

Hydrogen Bonding in Calcium Orthophosphates

RECENT discussion[1,2] about the general formula of synthetic hydroxyapatites deficient in calcium has involved the nature of hydrogen bonding not only in the low calcium/phosphorus ratio defect apatites themselves but also in related stoichiometric calcium orthophosphates In particular, the relationship between OH stretching frequency and the length of the oxygen–oxygen bridge connecting orthophosphate groups has been considered The assignment by Stutman, Posner, and Lippincott[1] of the infra-red absorption band at 3,400 cm^{-1} in synthetic phosphocalcic apatites to O–H O stretching between hydrogen-bonded phosphate groups has been disputed by Winand and Dallemagne[2], who attribute this band to adsorbed water We have recently re-examined three calcium orthophosphates crystallographically and from the P–O lengths and O O separations have attempted to infer which oxygen atoms carry hydrogen atoms These more detailed results point to some difficulties in the interpretation made by Stutman *et al* [1], for example in their Table 2, which includes a comparison of a single O O distance (derived from infra-red data) with unit-cell X-ray data for various orthophosphates

In dicalcium *bis*(dihydrogen phosphate) monohydrate[3], $Ca(H_2PO_4)_2\ H_2O$, we find that the distances between oxygens in phosphate groups which we believe to be hydrogen-bonded cover the range from 2 48 ± 0 03 Å to 2 87 ± 0 05 Å with infra-red peaks at 2,320, 2,430, 2,670, 2,920, 3,160, and 3,450 cm^{-1} In monetite[3], $CaHPO_4$, the range of distances is from 2 44 ± 0 03 to 2 66 ± 0 02 Å with infra-red peaks at 2,400, 2,780 and 3,010 cm^{-1} In each structure one hydrogen bond, O_7 $O_{7'}$, in monetite and O_3 O_5 in dicalcium *bis*(dihydrogen phosphate) monohydrate, is short enough to be symmetrical For brushite[4], $CaHPO_4\ 2H_2O$, which has infra-red peaks at 2,350 and 2,900 cm^{-1}, as well as doublets ascribed to adsorbed water[5] at around 3,200 and 3,500 cm^{-1}, the distances are uncertain as the crystal structure has a lower symmetry than had been assumed in the original X-ray analysis[6] (Indeed, our experience with monetite suggests that further X-ray data would be inadequate to locate the hydrogen atoms, for which neutron diffraction, preferably at low temperatures, would be required) In the crystal structures of monetite and dicalcium *bis*(dihydrogen phosphate) monohydrate there are two phosphate groups in the asymmetric unit of the crystallographic cell and the O–H O bonds are expected to be roughly linear Since the O O distances in these structures spread over several tenths of an Ångstrom and some of the distances are, we consider, only partly occupied by hydrogen atoms, and since each structure exhibits a range of frequencies, some probably the result of combinations[7], it would be a simplification to expect one O–H frequency to typify each structure It does not seem advisable, therefore, in these orthophosphates to correlate a representative frequency with one O O distance or with Ca/P ratio Although the mean volume occupied per phosphate group would appear to decrease slightly with increasing acidity, in accordance with Table 2 of reference 1, the shortest O O distance obtained from the X-ray refinements did not alter significantly when the Ca/P ratio was changed from 1 0 to 0 5

If hydrogen bonding within a phosphate group is rejected as we have suggested in brushite[8], the shortest interphosphate O O distance of 2 9 Å in hydroxyapatite itself, given by Stutman *et al* [1] from a recent refinement[9] of the crystal structure in space-group $P\,6_3/m$, is larger than the 2 50–2 60 Å generally found between fully hydrogen-bonded phosphate groups in a range of both organic[10] and inorganic[11] hydrogen phosphates In brushite, an unusually close apparent O O distance of 2 63 Å without a hydrogen bond has been attributed[4] to a reduction in crystal symmetry In monetite and calcium *bis*(dihydrogen phosphate) monohydrate, rather long interphosphate distances of 2 66 and 2 67 Å respectively have been explained[3] on the basis that they are not always occupied by hydrogen atoms, that is, the structural framework of the heavier atoms is sufficiently flexible to enable hydrogen atoms to occupy different sites It is possible that one or other of these factors may help to resolve the apparent inconsistency between the X-ray diffraction patterns of defect hydroxyapatites and some of the infra-red measurements reported

D W JONES
Department of Chemical Technology,
Institute of Technology,
Bradford

J A S SMITH
School of Chemistry,
University of Leeds

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Velocity of Twin Propagation in Crystals

It is generally believed that twin lamellæ in deformed crystals propagate rapidly, but direct measurements of this velocity do not appear to have been made Recent experiments on calcite (rhombohedral calcium carbonate) have shown that, in this material, twins may propagate with the modest speed of about 2 m/sec

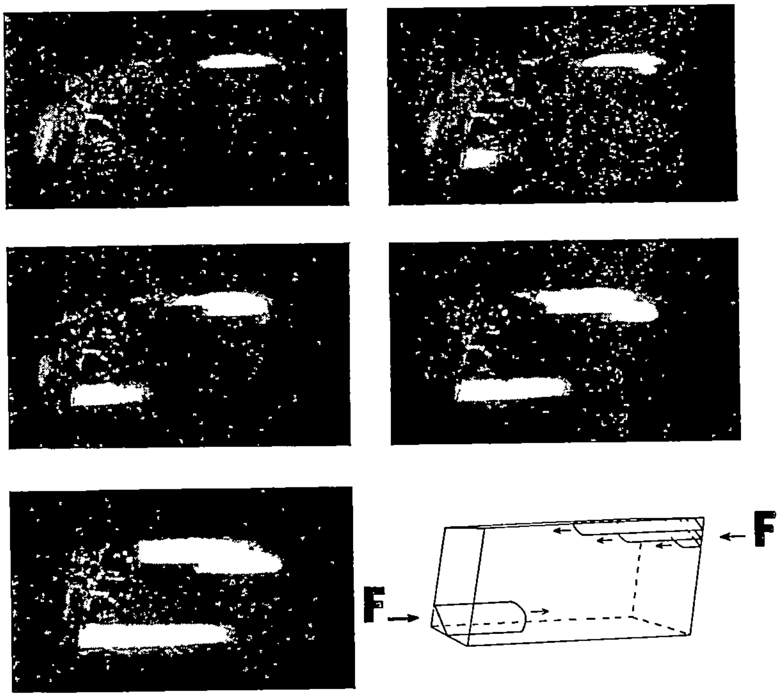

Fig 1 A series of high-speed photographs of twins propagating across a stressed calcite rhomb Interval between frames 0 6 msec, exposure time 0 05 msec

High-speed photographic methods were used to observe a cleavage rhomb of calcite to which was applied a shear stress Crossed polaroids in the optical system cause the twin lamellæ to show up as bright areas while the parent crystal remains dark (apart from surface scratches) Stopped twins—twins which have been accommodated by the lattice at elevated temperatures and stop part way across the specimen—have been etched to reveal their true extent using etching methods which detect individual dislocations[1] It is found that stopped twins extend only about 0 05 mm beyond the point at which they are too thin to be detectable by polarized light This supports the view that it is the actual propagation of the twin rather than the lateral growth which is being observed The discrepancy is of the same order as the accuracy of measurement of twin lengths from high-speed photographs and is negligible for path lengths of 6 mm or more Fig 1 shows a typical sequence of photographs, and Fig 2 shows the information which can be obtained from such observations It was not possible to investigate a wide stress range because at higher stresses a considerable amount of fracture occurred during the twinning process This diminished the path-lengths over which twins

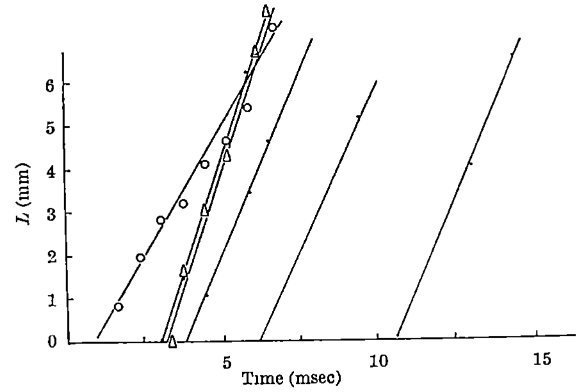

Fig 2 Measurements made from the event shown in Fig 1 The growth of six separate twins is recorded The velocity of each individual twin is reasonably constant during growth The individual velocities lie between 1 3 and 2 1 m/sec

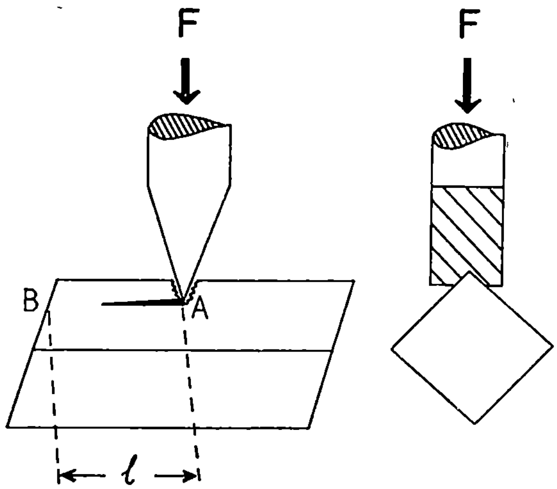

Fig. 3. Method of applying a localized transient stress used for indirect measurement of twin velocities

could be observed and reduced the accuracy of measurement. Over the range studied there was a small increase in propagation velocity with increasing shear stress.

Indirect velocity measurements were also made at various temperatures between 20° C and 340° C by applying localized stress pulses of various durations, as shown in Fig. 3. It was found experimentally[2] that if a twin starts from point *A* and, under the influence of the stress pulse *F*, reaches a length of approximately 2/3 *l*, it will become unstable and will propagate fully across the crystal to point *B* to become permanent, even if the external stress is removed during this unstable stage. If the stress is removed before the twin reaches this critical length it will retreat under the influence of unbalanced elastic stresses in the surrounding parent lattice. For a stress pulse of given duration there will therefore be a value of *l* below which a permanent twin will occur and above which no permanent twin will be formed.

Experiments were carried out to determine the critical value of *l* at various temperatures and from these values the average velocity of propagation of a twin lamella was calculated as a function of temperature in the range 20° to 340° C. The 20° C value agreed well with the value obtained by direct observation, being 2·2 (± 0·1) m/sec. The velocity of propagation increased with temperature so as to correspond to a thermally activated process with an activation energy of about 1·6 kcal/mole.

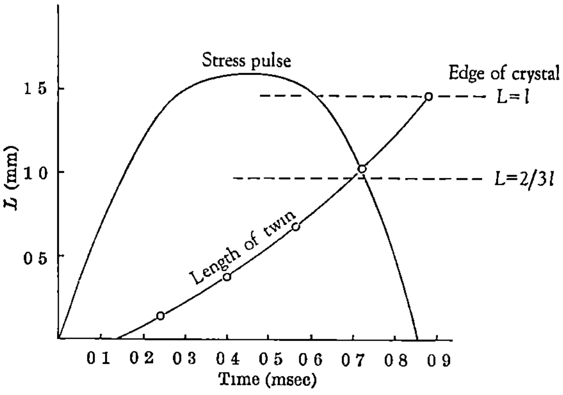

Fig. 4. A typical transient stress experiment showing length of twin lamella and stress as functions of time

High-speed photographs were taken of the twinning process under the latter experimental conditions where stress pulses were used, and Fig. 4 shows the information obtained. It is clear that the twin continues to propagate after the stress has ceased, but by this time it is more than two-thirds of the way across the specimen ($L > 2/3\, l$).

From the work of Jillson[3] it is possible to say that the velocity of propagation of twin lamellæ in zinc must be greater than about 30 m/sec when they are propagating freely. Dr. J. H. Brunton (private communication) of this laboratory has recently obtained high speed cine films of the propagation of twins in zinc and has found a range of velocities up to a maximum of about 300 m/sec. Explosive loading was used for these experiments, and the stresses and rates of strain were very high so that the maximum velocities observed are probably near the maximum possible for this material.

This velocity of twin propagation for zinc is about one-seventh of the velocity with which the shear stress would propagate in the metal. It is thus comparable with the expected maximum velocity of dislocation movement[4]. The observed velocity of twin propagation for calcite is, however, remarkably low. This may perhaps be associated with the brittle nature of the crystal.

The kinetic mechanism of the twinning process is far from clear, and further work is necessary. Such studies may give us a fuller understanding of the behaviour of crystalline materials under high rates of strain and under impact conditions.

We thank Prof. B. A. Bilby for helpful discussion.

F. P. Bowden
R. E. Cooper

Physics and Chemistry of Solids,
Cavendish Laboratory,
University of Cambridge

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CHEMISTRY

Failures in Stressed Nylons

Ensanian[1] has observed that concentrated hydrochloric acid alone, or a mixture of hydrochloric acid, alcohol and zinc dust, caused rapid failure of a stressed nylon strip. In this connexion the results of some work we have been doing independently should be of interest.

Several nylon mouldings had been observed to crack and delaminate when in contact with electrolyte from discharged dry cells. Investigation of a number of possible causes of this cracking has shown that aqueous zinc chloride solutions, varying in concentration from 20 to 80 per cent, are effective in producing the failures observed (Fig. 1).

Mouldings that have been suitably stress-relieved are not affected by 80 per cent zinc chloride solution. The types of failure shown in Fig. 1 *B* occur with nylon 6, nylon 66, and nylon 11 under stress, and nylon components made by different techniques such as plunger and screw pre-plasticized injection moulding or by extrusion moulding can exhibit this stress cracking.

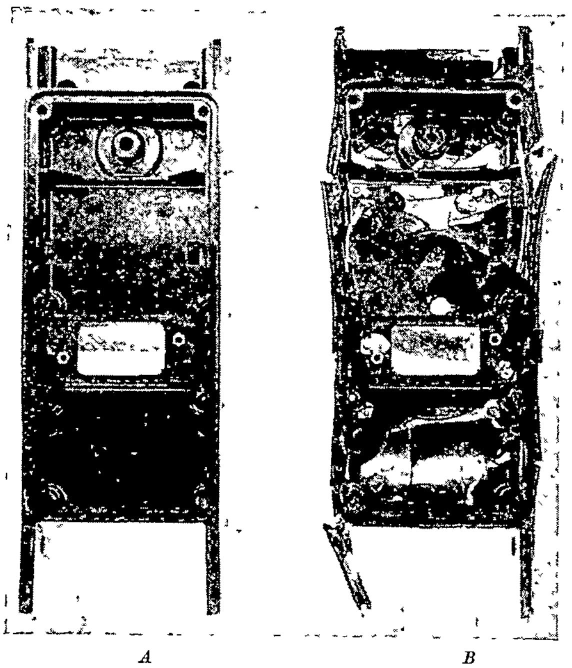

Fig 1 Nylon 6 mouldings (length, 23 cm) *A*, Untreated, *B*, treated with 80 per cent zinc chloride solution (one drop of solution at each of eight points for 24 hr at room temperature) After cracking the moulding in Fig 1 *B* had increased in length by 2 per cent

Saturated aqueous solutions of other chlorides and zinc salts have been examined and shown to be ineffective on stressed nylon mouldings These include ammonium chloride, aluminium chloride, cupric chloride, barium chloride, calcium chloride, magnesium chloride, manganese chloride, nickel chloride, ferric chloride, zinc nitrate, zinc acetate, zinc permanganate, and zinc sulphate

Zinc chloride solutions are acidic, but they are much more effective than hydrochloric acid alone or the mixture used by Ensanian[1] in causing failure The effect of single drops of 80 per cent zinc chloride solution when placed on a stressed nylon moulding is dramatic This effect is very sensitive to temperature, mouldings that crack in 3–4 hr at room temperature (20° C), crack in 30 min at 35° C and in 5 min at 50° C

When a single drop of 80 per cent zinc chloride solution is applied to a stressed nylon moulding, cracking can occur at points up to 15 cm from the drop Chemical examination of cracked areas remote from the drop shows the presence of zinc chloride This suggests that the zinc chloride moves rapidly, by capillary action, along the cracks as they grow

Results indicate that aqueous zinc chloride solutions could be used to test for the presence or absence of stresses in nylon mouldings and also for the effectiveness of any stress relieving process used on a moulded component Further work is being done to enable the most suitable test conditions to be selected

Stressed polymethyl methacrylate in contact with 80 per cent zinc chloride solution exhibits severe surface crazing before cracking and breaking The rate of attack, however, is much slower than with nylon Stressed samples of low- and high-density polyethylene, polypropylene, rigid polyvinyl chloride high-impact polystyrene and polyacrylonitrile-butadiene-styrene were not affected by 80 per cent zinc chloride solutions at 20° C

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P. Dunn
A J C Hall
T. Norris

Department of Supply,
Australian Defence Scientific Service,
Defence Standards Laboratories,
Maribyrnong, Victoria,
Australia

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Infra-Red Extinction Coefficients for Amorphous and Crystalline Nylon

In a recent study of the NHCO group in polyamides and proteins[1] it was found that the peak extinction value of the infra-red fundamental NH absorption band at 3,300 cm^{-1} for nylon 6 depended on the mode of preparation of the specimen film under examination Films cast from dry formic acid by rapid evaporation of the acid gave lower extinction values referred to the CH absorption band than films subjected to heat annealing

It was found that these results could be explained on the assumption that the films prepared from formic acid were less crystalline than those subjected to heat treatment and that the peak molar extinction coefficient of amorphous nylon is about one-sixth that for crystalline nylon[1]

Further results have now been obtained which support this hypothesis by recording the change in peak extinction of the NH absorption band during the melting of nylon 6 A film was cast on a microscope slide and clamped between the two halves of a high-temperature cell similar to that described by Richards and Thompson[2], but with the cell windows covered by microscope cover-slips to maintain a uniform temperature The extinction-temperature relation obtained is shown in Fig 1 A sharp drop in

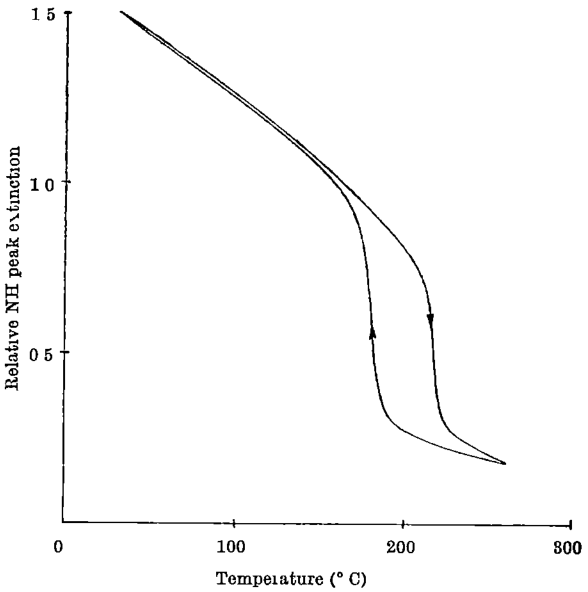

Fig 1 Change in peak extinction with temperature for the infra-red fundamental NH absorption band at 3,300 cm^{-1} for nylon 6

extinction occurs at about the melting point of nylon 6 (~ 215° C) to a value about one-third that just above the melting point Now measurements on similar samples of nylon give values of crystallinity of between 50 and 60 per cent as originally prepared, so the peak extinction of the crystalline nylon must be about five times as great as that for molten nylon at the temperature of the melting point, in rough agreement with the estimates based on crystallinity measurements[1]

A relation similar to that shown in Fig 1 has also been obtained for nylon 11, the rapid fall in extinction taking place at a lower temperature consistent with the melting point of nylon 11 (~ 185° C)

These results appear to conflict with those reported by Holliday[3] for nylon 66, who found a fall in extinction of the NH absorption band at a temperature about 50° C higher than the melting point (~ 265° C) Attempts to repeat his results failed because of the tendency of the sample to scorch even in an inert atmosphere

In conclusion, it should be mentioned that small shifts in the frequency of the NH absorption band to higher values during the melting process were recorded in agreement with Holliday's results

G KING
F WOOD

Wool Industries Research Association,
Headingley Lane,
Leeds, 6

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Reaction of CCl_2F_2 and C_2H_6 with Iron Films

THE stability of CCl_2F_2 ('Freon 12', E I du Pont de Nemours and Co trademark) at moderately high temperatures (300° C) in the presence of metals and saturated hydrocarbons is of importance because of the wide application of this substance as a refrigerating fluid A number of workers have investigated its stability Norton[1] observed the thermal decomposition of pure gaseous CCl_2F_2 to occur at a significant rate (1 per cent decomposition/h) at a temperature of about 520° C He observed that the decomposition was strongly catalysed by Fe_2O_3 but not by bulk iron Spauschus and Doderer[2] reported that at 175°–200° C, CCl_2F_2 reacts with a highly refined mineral oil to produce $CHClF_2$ ('Freon 22') and a carbonaceous residue The exact nature of this reaction is unknown, but it is influenced by the presence of bulk iron and other metals

Clean metal surfaces, such as those generated in an ultra-high vacuum or during a wear process, are often observed to be highly reactive chemically[3,4]

The interaction of CCl_2F_2 and C_2H_6 and a clean iron film was investigated The iron film was prepared by evaporating in ultra-high vacuum ($p = 5 \times 10^{-9}$ torr) a small coil of 99 99 per cent pure iron on to the surface of a 4 in diameter 'Pyrex' reaction bulb The bulb was heated to the desired temperature with a heating mantle or an oil bath The CCl_2F_2 was 99 5 per cent pure and the C_2H_6 was 99 99 per cent pure After the iron film was deposited, the gas or gas mixture was admitted to the reaction bulb At the completion of an experiment, the gases were removed from the bulb and analysed on a mass spectrometer Ultra-high vacuum procedures and measurement techniques used in these experiments are similar to those published in detail elsewhere[4]

A summary of the experimental conditions and results for the experiments performed are listed in Table 1 No gaseous decomposition products were observed when CCl_2F_2 or $CCl_2F_2 + C_2H_6$ remained in contact with an initially clean iron film at 200° C, however, some adsorption did occur At an iron film temperature of 350° C, a dose of 2 18 × 10^{18} molecules of CCl_2F_2 was completely adsorbed and a small amount of carbon monoxide appeared in the gas phase This probably was due to a reaction of an adsorbed species with the glass substrate Ten per cent of an initial dose of 1 76 × 10^{18} molecules of C_2H_6 was adsorbed on an iron film at 350° C and a small amount of H_2 appeared in the gas phase When a mixture of CCl_2F_2 and C_2H_6 was in contact with an iron film at 350° C, a small amount of H_2 and CO appeared in the gas phase, there was no evidence for the presence of $CHClF_2$

These experiments indicate that while CCl_2F_2 and C_2H_6 adsorb on a clean iron film at 200° C and 350° C, there is no conversion of CCl_2F_2 into $CHClF_2$

I thank Miss Nancy Keary for assistance with the experiments

RICHARD W ROBERTS

General Electric Research Laboratory,
Schenectady,
New York

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Table 1 SUMMARY OF EXPERIMENTAL DATA FOR THE REACTION OF CCl_2F_2 AND C_2H_6 WITH CLEAN IRON FILMS

Gas	Initial amount of gas (× 10^{-18} molecules)	Iron temp (°C)	Iron thickness (Å)	Reaction time (min)	Gaseous products	Comments
CCl_2F_2	1 52	200	127	113	CCl_2F_2	~1 × 10^{18} mol CCl_2F_2 adsorbed
CCl_2F_2 C_2H_6	1 55 1 93	200	165	1,069	CCl_2F_2 C_2H_6	~1 4 × 10^{18} mol ($CCl_2F_2 + C_2H_6$) adsorbed
CCl_2F_2	2 18	350	1919	115	CO (small amount)*	All CCl_2F_2 adsorbed
CCl_2F_2 C_2H_6	2 06 1 76	350	864	187	C_2H_6 CO } (small amount)* H_2	All CCl_2F_2 adsorbed
C_2H_6	1 68	350	648	198	C_2H_6 H_2 (small amount)*	10% C_2H_6 adsorbed

*Small amount ≅1 × 10^{17} molecules

Elasticity of Soap Films : an Amendment

IN a recent communication[1], I suggested that the Gibbs theory of surface elasticity provided a satisfactory interpretation of the experimental elasticities of films of sodium dodecyl sulphate solution which had been reported by Mysels, Cox and Skewis[2] However, Prof K J Mysels[3] has kindly pointed out that my equation 3 implies depletion of the surface-active solute by adsorption while the soap film is being formed, whereas, in fact, there is a good deal of evidence against this

It is well known, for example, that the surface tension of soap films does not change appreciably as they undergo drainage, indeed, a film could not sustain a measurable gradient of tension Secondly, the recent work of Mysels and Cox[4] has fully substantiated Frankel's equation for film thickness[5], which is based on the assumption that a soap film is drawn off the surface of the bulk solution without significant stretching, carrying unchanged substrate solution with it Thirdly, direct evidence for this model of soap films has been provided by Corkhill, Goodman, Haisman and Harrold[6], who determined the composition of draining soap films by radio-tracer methods

Consequently, a more realistic starting point for estimating the contribution of the Gibbs effect to film elasticity—in place of equation (3)—would be

$$C = 10^3 (2\Gamma_0/L_0 - 2\Gamma/L + 10^3 C_0) \qquad (3)$$

in which C_0 and Γ_0 refer to the original solution from which a film (thickness L_0) was prepared, while C and Γ apply to this film after it had been subjected to sudden, deliberate stretching Clearly, lower values of elasticity will be predicted on this basis Examples to show the order of magnitude with two initial concentrations of sodium dodecyl sulphate are given in Table 1, extensions of 10 per cent having been assumed

Table 1

Initial film thickness (L_0, μ)	Calculated Gibbs elasticity, E (dyne cm^{-1}) C_0 = 0 008 M	C_0 = 0 01 M
2	9	0 8
1	14	1 2
0 2	37	33

Mysels *et al* recorded elasticities in the range 8–20 dynes cm^{-1} for films of 0 01 M solution of thickness 0 25–2 5μ (ref 2) For the thinnest films the theoretical and experimental values might be considered in agreement, but for the thicker films the discrepancy is beyond the limits of uncertainty, especially as no significant variation of elasticity with film thickness could be noticed in the experiments The explanation of the results therefore becomes problematical

One possibility is that a small degree of stretching does occur in the formation of the film, sufficient to reduce the concentration to the critical micelle concentration, 0 008 M an excess surface tension of only 1 dyne cm^{-1} during the drawing of the film would suffice The calculated Gibbs elasticity just below the critical micelle concentration is seen to be of about the correct order of magnitude

Another possibility is that attainment of equilibrium between surface and surfactant solutions is not as rapid as is generally supposed Experimental data for sodium dodecyl sulphate solutions[7] show that the tension falls quickly during the first 30 msec, but by no means reaches equilibrium The graphs suggest that more than 0 1 sec is required Results are lacking for times in the range 0 1–1 sec for want of a suitable method of determination Consequently, the possibility cannot be excluded that the observed elasticities are partly due to the Marangoni effect Yet another possibility, as suggested by the authors, is that the surfactant was not pure enough to justify calculations appropriate to a two-component system

Unfortunately, therefore, the relevance of the Gibbs theory of elasticity in soap films must be considered still uncertain, as it has been since 1878

J A KITCHENER

Department of Mining and Mineral Technology,
Royal School of Mines,
London, S W 7

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Reaction of Boron Trichloride with Ribonucleic Acids

THE effect of acids on ribonucleic acids (RNA) is markedly different from their effect on deoxyribonucleic acids (DNA) In the latter there is first a liberation of the purines to give apurinic acid[1], and then a slower elimination reaction to give pyrimidine-containing oligonucleotides[2] Advantage has been taken of this sequence of reactions to develop methods for the determination of the distribution of the pyrimidine nucleotides in DNA[3–5] With RNA, however, there are indications that mild acid treatment causes non-specific fission of phosphodiester linkages to give oligonucleotides[6], so that it appears that in this case the phosphodiester linkages are more acid-labile than the purine glycosidic linkages This can be attributed to the participation of the 2′-hydroxyl group in the formation of a cyclic intermediate with the phosphate group We have studied the action of various acid reagents, that is, hydrochloric acid under a variety of conditions, diphenylamine and 67 per cent formic acid[5], diphenylamine and absolute formic acid, methanolic hydrogen chloride, orthophosphoric acid, and polymetaphosphoric acid, and in all cases the phosphodiester linkages were broken either faster or at about the same rate as the purine glycosidic linkages When RNA was treated with boron trichloride, however, a difference was found, and this was studied in detail

The insoluble nature of RNA and its cetyltrimethylammonium salt in solvents which were not attacked by boron trichloride necessitated the reactions being carried out in suspension After the reaction the boron trichloride was removed by evaporation, the residues were dissolved in sodium bicarbonate at *p*H 7 and the solution chromatographed on Whatman No 1 paper in propan-2-ol/water/ammonia (sp gr 0 880) (35 15 3) In cases where reaction occurred, ultra-violet-absorbing components corresponding to adenine and guanine were detected and determined spectrophotometrically In addition, ultra-violet-absorbing zones were detected on the origin and as a streak from the origin to a point of R_F 0 06

The reaction of boron trichloride with yeast microsomal RNA[7] was studied at various temperatures At 12° reaction occurred with moist RNA (water content, about 15 per cent), but not with RNA which had been dried at 110° *in vacuo* In the former case, 25 per cent of the purines were liberated after 12 h No pyrimidine mononucleotides or the corresponding cyclic phos-

Table 1 REACTION OF BORON TRICHLORIDE WITH YEAST MICROSOMAL RNA

Temperature (deg)	22	22	22	22	50	50	50	95	*95	†95	105	*105	†105	‡105	110
Total time of reaction (h)	26	91	144	172	13	25	56	15	23	33	10	20	30	40	12
Per cent of total purine liberated	5 2	10 4	20 6	21 6	14 6	24 7	31 0	25 0	52 0	63 0	34 5	44 0	60 0	104	100

*, †, ‡, 1st, 2nd and 3rd treatments of the material indicated in the previous column

phates were detected The role of the water was obscure When dried RNA was used and extra water added, either directly or as wet methylene dichloride, the RNA was degraded to give pyrimidine mononucleotides All reactions at higher temperatures, therefore, were carried out with dried RNA as follows

Freeze-dried yeast microsomal RNA (sodium salt, 100 mg) was dried at 110° for 3 h *in vacuo* and heated in a sealed tube with boron trichloride (2 ml) The boron trichloride was removed by distillation and the residue dissolved in sodium bicarbonate at *p*H 7 and freeze dried In some cases the freeze-dried material was again dried at 110° and resubmitted to treatment with boron trichloride The extent of the reaction was estimated as described here

The results (Table 1) showed that complete liberation of the purines was achieved at 110° in 12 h When the 2′-(3′)-phosphates of cytidine, uridine, adenosine, and guanosine were treated similarly, no reaction was detected with the pyrimidine nucleotides, but the purine nucleotides gave the free base and there was considerable charring of the material

The product from the reaction with RNA at 110° was dissolved at *p*H 7 and fractionated on a column (20 cm × 2 cm^2) of 'Dowex-1-chloride' (2 per cent cross-linked, 200–400 mesh), elution being carried out with dilute hydrochloric acid (0 001–0 01 N) and then with 0 01 N hydrochloric acid containing lithium chloride (0 025–0 2 N) In addition to adenine and guanine about 24 ultra-violet-absorbing components were eluted The total ultra-violet absorption corresponded to 92 per cent of that of the original RNA, but, as some of this was due to the absorption of brown material which had been produced by the reaction, it was concluded that some pyrimidine residues had been destroyed Two major components (*A* and *B*) which were eluted with 0 01 N hydrochloric acid containing 0 1 N lithium chloride were subjected to paper chromatography in propan-2-ol/10 N hydrochloric acid/water (39 11 10) Each gave three components with one (*A′* and *B′*, each of R_F 0 97) predominating Both *A′* and *B′* had R_F 0 41 in propan-2-ol/water/ammonia (sp gr 0 880) (35 15 3) and on paper electrophoresis at *p*H 3 5 they migrated 1 5 times the distance of uridine-2′(3′)-phosphate They both had ultra-violet absorption spectra characteristic of uridine (λ_{max} 260 mμ) except that *B′* had a somewhat higher absorption at 220 mμ This was probably due to impurities eluted from the paper There were present 1 87 and 2 02 g atoms of phosphorus per mole of uridine in *A′* and *B′* respectively It appeared, therefore, that they were isomeric uridine diphosphates In a reaction carried out at 130°, decompositions of the pyrimidines was considerable and many of the fractions eluted from the 'Dowex-1' column contained non-pyrimidine and non-purine nitrogen The only pyrimidine-containing component which could be identified with certainty in this case was a cytidine monophosphate

These results show that the action of boron trichloride on RNA is first to liberate the purines and then to cause fission of the phosphodiester linkages It seems probable that the reason for the difference between this reaction and that of dilute hydrochloric or formic acids is that the boron trichloride initially combines with the 2′-hydroxyl groups of the RNA, so preventing the formation of a cyclic phosphate intermediate and subsequent breakage of the internucleotide phosphodiester linkages In support of this it was noted that after removal of the boron trichloride *in vacuo* at the end of the reaction, the dry product had a weight which was 24 per cent greater than the original RNA, and that it dissolved in water to give a strongly acid solution Further action of the boron trichloride or the acid produced in the reaction would result in the liberation of the purines, and then the phosphodiester linkages would be broken by an elimination reaction, similar to that which occurs in the case of the acid degradation of DNA[2] This would explain the formation of the uridine diphosphates In the case of RNA two isomers, namely, uridine-3′,5′-diphosphate and uridine-2′,5′-diphosphate, might arise due to the treatment with acid on the ion exchange columns

So far the extreme conditions which were necessary to effect complete liberation of the purines have resulted in some degradation of the pyrimidines to non-ultra-violet-absorbing nitrogenous groups and also to the formation of pyrimidine mononucleotides The latter may have arisen by non-specific degradation of interpyrimidine nucleotide linkages or to removal of a phosphate group from pyrimidine nucleoside diphosphates It may be possible, by repeatedly treating the RNA with boron trichloride at lower temperatures, to eliminate these non-specific effects and to obtain pyrimidine oligo-nucleotides

We thank Prof M Stacey for his interest and the British Empire Cancer Campaign (Birmingham Branch) for financial assistance

M E BRAMWELL
A S JONES

Department of Chemistry,
University of Birmingham

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RADIATION CHEMISTRY

Effects of Ionizing Radiation and Peroxides on Hæmocyanin

IT has been postulated[1] that part of the radiobiological action of ionizing radiation involves an attack on the copper component of 4-electron transfer oxidases Accordingly, we utilized as a mode the oxygen-carrying protein hæmocyanin[2,3], which contains copper as the 'active centre'

From previous irradiation studies on hæmocyanin it was concluded that doses of about 200,000–600,000 r were required to modify the electrophoretic pattern or rupture the copper-protein bond[4-6] A more sensitive criterion of radiation damage is the ability of hæmocyanin to combine reversibly with molecular oxygen From measurements of the optical absorption peak at 340 mμ, which disappears on deoxygenation or destruction of the oxygen-carrying ability, Felsenfeld[7,8] demonstrated that one equivalent of peroxide per mole of copper is sufficient to destroy most of the function of hæmocyanin by oxidizing the cuprous ion of deoxygenated hæmocyanin to the cupric state

In our experiments hæmocyanin was used as it exists naturally in the serum from the horse-shoe crab, *Limulus polyphemus* The serum was obtained and processed in the manner described by Redfield[9] Dilutions of the hæmolymph were made with a 0 05 M potassium dihydrogen phosphate buffer, $pH = 7\ 0$ The radiation source was cobalt-60 γ-rays delivered at a dose-rate of 1,000 r /min (1 r = 0 91 rad) The solutions were irradiated at room temperature (22° C) while in 60-ml serum bottles fitted with rubber diaphragm stoppers Peroxide assays were made by the triiodide method of Ghormley[10]

Deoxygenation of the hæmocyanin solutions was accomplished by bubbling purified, oxygen-free, water-saturated helium gas through the solutions for 2 h The entry and exit of the helium was made by means of hypodermic needles inserted through the rubber stoppers The solutions were reoxygenated by a stream of water-saturated air Optical density measurements were made on solutions usually diluted 1 20 with buffer Appropriate controls were used throughout

The results of a large number of experiments are summarized here (1) When oxygenated hæmocyanin is resistant to attack by ionizing radiation, no change is observed in the optical density, even after storage for several days following doses as high as 40,000 r

(2) When solutions of *oxygenated* hæmocyanin are irradiated, *deoxygenated* and *reoxygenated*, measurable oxidation of the copper is observed (Fig 1) Measurements of the optical density of solutions of oxygenated hæmocyanin irradiated under different dilutions gives an initial yield[11] value of $G_0(\mathrm{Cu}^{++}) \simeq 2$ Thus, about 2 micromoles of cuprous copper are oxidized per 1,000 rads

(3) The action of ionizing radiation on solutions of hæmocyanin is due to the formation of hydrogen and organic peroxides, for example (*a*) Irradiation of *deoxygenated* hæmocyanin produces no peroxides, hence reoxygenation caused no change in oxygen-carrying capacity (*b*) Hæmocyanin is damaged in the same manner when the buffer is irradiated separately and added to unirradiated hæmocyanin (*c*) Addition of high-purity catalase to buffer diluted hæmocyanin solutions can prevent 80–90 per cent the damaging action of irradiation (*d*) Radiation damage to hæmocyanin decreases with time and has been found to be a function of the residual concentrations of peroxide (*e*) Direct addition of H_2O_2 or organic peroxides such as methyl ethyl ketone peroxide produces changes similar to that produced by irradiation

(4) The resistance of oxygenated hæmocyanin to irradiation is utilized to measure the life-time of the radiation-produced peroxides and radicals When undiluted hæmolymph is irradiated with 40,000 r the absorption peak decreases with a half-time of 24 h, but irradiation of a 1 20 dilution gives a half-time of 70 h The catalase-like action of hæmocyanin[12] makes it possible to distinguish the 'after-effects' caused by organic peroxides resistant to catalase relative to those caused by hydrogen peroxide

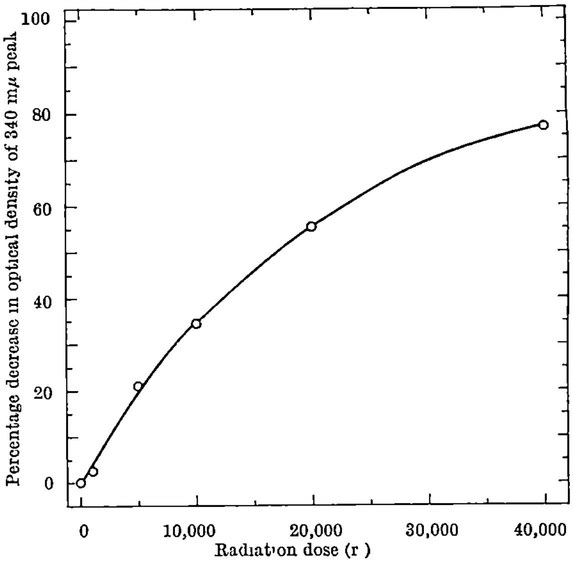

Fig 1 Effect of cobalt-60 γ-radiation on hæmocyanin The hæmocyanin was present in its natural state in the serum from *Limulus polyphemus* The serum, after dilution 1 20 with KH_2PO_4 buffer, 0 05 M, $pH = 7\ 0$, had a copper concentration of 8 55 × 10^{-5} M

This work, which will be described in more detail elsewhere, was performed under the auspices of the U S Atomic Energy Commission

Jack Schubert*
William M Westfall

Division of Biological and Medical Research,
Argonne National Laboratory,
Lemont, Illinois

* Present address Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

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BIOCHEMISTRY

Separation of Free Iodides from Iodotyrosines of Serum and Thyroidal Extracts by Means of Modified Charcoal

Blau's modification[1] of the Leland and Foster[2] method for separation of free iodides and iodotyrosines from thyroxine in *n*-butanol extracts has constituted the cornerstone of blood butanol-extractable iodine methods[3,4] and facilitates the quantitative analysis of thyroxine in thyroid extracts[1] The complete fractionation of iodinated compounds, however, requires the use of lengthy techniques of column or paper chromatography, the latter is not always easy to quantitate and is subject to considerable experimental error Block *et al* [6] indicated that activated charcoal can quantitatively separate iodinated amino-acids from inorganic iodide, but fortunately no experimental details were given We consequently worked out a method whereby Blau's alkaline reagent, containing iodide and iodotyrosines washed out of butanol extracts, is treated with modified charcoal to quantitatively adsorb the iodotyrosines and thus separate them from free iodide The method is simple and is applicable to both chemical as well as to radioisotope studies

Activated charcoal (E Merck A G Darmstadt) was de-activated with purified stearic acid as described by Synge and Tiselius[7] Two gm of stearic acid were dissolved in 200 ml absolute ethyl alcohol and added to 50 gm of activated charcoal in a 2-l flask After standing for 1 hr with vigorous shaking every few minutes, the deactivated charcoal was washed 10 times with 2-l portions of distilled water, and dried over sulphuric acid in a vacuum desiccator Five grams of dried deactivated charcoal were treated with 1 gm of DL-tyrosine dissolved in 40 ml of Blau's alkaline reagent (4 N sodium hydroxide in 5 per cent sodium carbonate), the mixture was thoroughly shaken and allowed to stand for 1 hr This constituted the 'charcoal reagent'

Standard solutions containing iodine-131 labelled free iodide, 3-monoiodotyrosine (MIT), 3,5-diiodotyrosine (DIT), 3,5,5′-triiodothyronine (T_3) and thyroxine (T_4) in 0 02 N sodium hydroxide were prepared with final concentration of 1 µgm /µl and specific activities of 1 µgm /mgm (All labelled substances were obtained from Abbott Laboratories, Oak Ridge) Samples of 0 1 ml of each of these solutions were dissolved in various solvents and treated with charcoal The solutions were centrifuged, the supernatants decanted, and the radioactivity of each charcoal sample counted in a deep-well scintillation counter Only in strongly alkaline aqueous solutions could we effect a quantitative adsorption of the iodinated amino-acids with no adsorption of free iodide By two treatments of Blau's alkaline reagent with deactivated charcoal, about 98 per cent of the iodotyrosines and only 2–3 per cent of free iodide were adsorbed

The iodotyrosines were held tenaciously by the charcoal Stearic-acid-deactivated-charcoal was therefore treated with DL-tyrosine as described here No change in the adsorbing capacity of the modified charcoal was noticed On the other hand, the iodotyrosines could be eluted from the charcoal with three washings of 10 ml 0 05 N NaOH After the addition of NaOH, the tubes were thoroughly shaken and following centrifugation the supernatant-eluent was poured off In this way, up to 98 per cent of the adsorbed iodotyrosines were eluted from the stearic acid-deactivated and DL-tyrosine-treated charcoal

Rats were injected with 25 µc of carrier free sodium iodide-131 per rat intraperitoneally At 42 hr after injection, total blood was collected from the abdominal aorta through polythene cannulas under ether anæsthesia The thyroid glands were removed, the two lobes separated and each placed immediately in a tube containing 4 N sodium hydroxide The tubes were partially evacuated of air, flame sealed and incubated at 110° C for 6 hr At the end of the hydrolysis, the digests were cooled and transferred to extraction tubes, the hydrolysis tubes were washed 4 times with 0·5 ml portions of water After neutralizing with 10 per cent v/v sulphuric acid using neutral red as an indicator, 0 2 ml acid was added in excess and the digests extracted three times with 5 ml n-butanol The combined extracts were made up to 25 ml in a volumetric flask with n-butanol Up to 96 per cent of the total thyroidal activity was thus extracted Five-ml aliquots of the n-butanol extracts were washed three times with equal volumes of Blau's alkaline reagent[4] Great care was taken to eliminate any traces of butanol from the aqueous layer The washings were combined in a 25-ml volumetric flask, made up to volume with alkaline reagent Two-ml aliquots diluted to 10-ml with alkaline reagent were treated in centrifuge tubes with 12 drops of well-mixed freshly prepared 'charcoal reagent' After centrifugation, the supernatants were decanted into another set of test tubes and were again treated with the charcoal reagent Where necessary, this treatment was repeated one or more times until no more countable activity was found in the charcoal Usually two such treatments were adequate, unless traces of butanol were carried over with the alkaline reagent The various fractions were counted in a deep-well scintillation counter When standard solutions added to 1 ml of 4 N sodium hydroxide, hydrolysed for 12 hr and then analysed under the same conditions as the thyroid extracts, free iodide was produced as follows MIT, 7 3, DIT, 6 6, T_3, 9 0 and T_4, 14 5 percentage of total organic activity in each original sample This would indicate that the largest fraction of free iodide produced during the hydrolysis came from the iodothyronines

Table 1 RADIOACTIVE IODINE DISTRIBUTION IN VARIOUS FRACTIONS OF n-BUTANOL EXTRACTS OF RAT SERUM IN PERCENTAGE OF TOTAL ACTIVITY

Rat No	Thyroid uptake (percentage dose)	Percentage of dose/l of serum	Iodo-thyronines	Free iodide	Iodo-tyrosines
1	6 1	21 1	37 2	62 8	0 0
2	5 4	17 9	38 1	61 9	0 0
3	5 3	20 9	35 5	64 5	0 0
4	4 6	28 6	38 4	61 6	0 0
5	3 9	13 7	35 7	64 3	0 0
6	5 1	17 0	20 0	80 0	0 0

Serum studies in normal rats at 42 hr after iodine-131 injections are represented in Table 1 In these investigations 1 ml of serum was extracted with 5 2 and 2 ml n-butanol respectively after acidifying with 0 2 ml of 10 per cent v/v sulphuric acid The butanol extracts were combined and made up to a total volume of 10 ml, 5 ml of which was washed three times with equal volume of Blau's reagent[4] The alkali wash was treated with charcoal as above No traces of iodotyrosines were found in the alkaline wash of the normal rats' serum extracts, in line with findings of other workers[5,9]

The alkaline hydrolysis of thyroid glands of 8 normal rats yielded the following results iodothyronines 25 3 ± 2 9 per cent, iodotyrosines 68 6 ± 2 7 per cent, free iodide 5 6 ± 0 9 per cent of total activity of the thyroid extracts which are in fair agreement with literature values[8–10] The low values for free iodide concur with the view that the unhydrolysed thyroid contains not more than traces of iodide in the free form[10,11] Our values for iodothyronines exceed those usually reported in the literature During enzymatic hydrolysis iodothyronines are deiodinated[9] to a larger extent than by the hydrolysis used here Some deiodination also takes place during paper chromatography, where as much as 15 per cent of total activity is often encountered at the origin, indicating incomplete enzymatic hydrolysis

The development of methods for chemical analysis of the various fractions separated by the present technique are now in progress

We thank Dr Karl Gaede of the Instituto Venezolano de Investigaciones Científicas, for supplying us with the radioisotopes used in this work

ISRAEL POSNER
ENRIQUE PIMENTEL

Cátedra de Patología General y Fisiopatología,
Instituto de Medicina Experimental,
Universidad Central de Venezuela,
Caracas

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Free Amino-acids of Isolated Calf Thymus Nuclei

CALF thymus nuclei prepared by the sucrose–calcium chloride method of Allfrey *et al* [1] are remarkably biochemically active, as judged by the incorporation of labelled amino-acids into the nuclear proteins[1,2] Such nuclei apparently lose little nuclear material during the process of isolation since they still retain their acid-soluble nucleotides[3] Furthermore, as will be shown in this communication, the sucrose–calcium chloride nuclei also contain a free amino-acid pool which can be released by ethanol extraction of the nuclei

During the course of examining the soluble proteins of the isolated thymus nuclei[2], it was observed that the total protein and nucleic acid could not account for the total nitrogen content of the whole saline extract Either 10 per cent trichloroacetic acid or 70 per cent ethanol precipitation of the saline extract gave only partial nitrogen recovery and the acid or alcohol supernatant was found to be ninhydrin-positive Direct alcohol extraction of the whole nuclei gave an intensity of ninhydrin colour comparable to that of the alcohol supernatant made from the saline extract It appeared obvious that the thymus nuclei prepared by the sucrose–calcium chloride method retained their free amino-acids, which can be recovered from the saline extract Two-dimensional paper chromatography of such an alcohol extract was carried out, utilizing the phenol–lutidine solvent system[4], and showed, among some unidentified peptides, the following constituents alanine, β-alanine, arginine, aspartic acid, cystine (as cysteic acid), glutamic acid, glutamine, glutathione, glycine, histidine, leucine and isoleucine, lysine, methionine (as methionine sulphone), phenylalanine, proline, serine, taurine, threonine, tyrosine, and valine Of these, the glutamic acid spot was the most intense Other amino-acids found in relatively significant concentration were serine, glycine, aspartic acid, lysine, threonine and taurine

Because of the great difference in concentration among the amino-acids, they were examined quantitatively by column chromatography using a Spinco model 120 amino-acid analyser, the results of which are shown in Table 1 It may be noted that the total amino-acid content is quite low when estimated on the basis of total nitrogen content of the ethanol supernatant This is because only part of the free amino-acids are included in the quantitative analysis, and the presence of small peptides is excluded Moreover, not all the nitrogen of the alcohol supernatant is due to amino-acids Osawa *et al* [3] have reported that more than 80 per cent of ultra-violet light absorbing material present in the acid-soluble fraction of the calf thymus nuclei is due to free nucleotides The present result, showing that only a small portion of the ethanol extract is due to amino-acids, thus indirectly confirms their findings

Table 1 FREE AMINO-ACIDS OF CALF THYMUS NUCLEI

Amino-acid	% of total nitrogen of ethanol supernatant	Relative weight ratio (glutamic acid = 100)
Alanine	0 54	2 3
Arginine	0 80	12 5
Aspartic acid	1 3	3 3
Cystine	0 29	4 5
Glutamic acid	8 5	—
Glycine	2 6	7 9
Histidine	0 21	0 91
Leucine	0 16	1 5
Isoleucine	0 07	0 65
Lysine	1 3	7 5
Methionine	0 05	0 59
Phenylalanine	0 06	0 89
Proline	0 48	3 4
Serine	2 1	12 6
Threonine	1 1	0 84
Tyrosine	0 06	1 1
Valine	0 15	1 1
Total	19 77	

The characteristic feature in the pattern of the free amino-acids of the isolated thymus nuclei is the high concentrations of taurine and glutamic acid High level of taurine in extracts of normal and neoplastic mouse tissues has also been reported by Roberts and Frankel[4], although the significance of its presence is unknown The high concentration of glutamic acid in the nuclear extract seems to be a common feature among many tissue extracts[4-6]. Apart from the predominance of glutamic acid and taurine, however, the quantitative pattern of free amino-acids of the thymus nuclei is different from that of other normal tissues, such as muscle, liver and epidermis[4], and appears to be, as pointed out by Roberts and Frankel[4], characteristic of the particular tissue used

The free amino-acids of the calf thymus cell nuclei seem to contain almost all the protein-bound amino-acids However, the relative concentrations of the individual amino-acids show little relationship to the amino-acid composition of calf thymus nuclear proteins[5,7] The significance of the nuclear free amino-acids cannot yet be assessed from the present quantitative estimation

This work was supported by grants from the U S National Science Foundation (*RG*-7923) and the Public Health Service (*G*-19573)

TUNG-YUE WANG

Henry Shaw School of Botany and
The Adolphus Busch III Laboratory
of Molecular Biology,
Washington University,
St Louis, Missouri

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Sub-cellular Distribution of 5-Hydroxytryptamine in Guinea Pig Brain

Inouye, Kataoka and Shinagawa[1] have recently criticized, by implication, my work on the sub-cellular distribution of 5-hydroxytryptamine (HT) in guinea pig brain[2] I do not agree that my assays were significantly interfered with by substance *P* The reasons for this are as follows

(1) The rat fundus strip was found to be relatively insensitive to substance *P* (1 μg HT was equivalent to 2,500, instead of 570 U substance *P*) and the response was qualitatively different substance *P* gave a rapid contraction followed by a fall in tonus during the 30-sec period of application of the drug, while HT gave a slower contraction which was not complete in 30 sec (cf Vane[3]) The contraction produced by the brain extracts resembled that of HT More recent work by Miss J Cleugh[4] shows that homogenates of the whole brain of guinea pigs contain 50–100 U substance *P*/g tissue when assayed on the fowl rectal cæcum[5] in the presence of blocking agents for HT and other interfering substances The HT content of my guinea pig brain homogenates was 442 ± 187 ng/g in 11 experiments This compares with Correale's[6] figures of 200–500 ng/g for guinea pig brain Amin, Crawford and Gaddum's figures[7], referred to by Inouye *et al*, are here irrelevant, being for the dog

Thus, taking the best and worst situations (lowest substance *P* content with highest HT content, and vice versa) 3–16 per cent of my total HT activity might theoretically have been due to substance *P* In practice, in the doses used for assay, the amount of substance *P* present would have been below threshold

(2) Although HT was usually assayed on brain preparations heated at 100° and *p*H 4 for 10 min to release bound HT, the results were checked against the acetone extraction procedure of Baker[8] modified from that of Amin *et al*[7] The results agreed with the simpler acid extraction method within the error of the assay method

(3) The HT contents of the brain homogenate and fractions were very low when prepared from animals pre-treated with reserpine

(4) Baker[9], using the rat fundus strip, found essentially the same distribution of HT in brain fractions as I did, this is in contrast to her own earlier results with duodenum[10] Michaelson and I[11] have checked the earlier results using the spectrofluorophotometric method and have obtained essentially the same results, with, however, less variation from one experiment to another We found the same distribution in untreated animals and animals treated with iproniazid, a monamine-oxidase inhibitor, in the latter, the absolute concentration of HT in the various fractions, including the nerve-ending fraction, was, as would be expected, more than twice as high as that in the untreated animals A detailed account of this work is in preparation Potter and Axelrod[12] have confirmed the localization of hydroxytryptamine in the presynaptic-vesicle-containing nerve-ending fraction[13] using the fluorophotometric method

Regarding the stability of endogenous and added HT, my results showed[2] that both endogenous and added HT were stable in the presence of intact brain particles but were rapidly destroyed when the preparation was treated with ether This destruction was inhibited by iproniazid (10^{-4}–10^{-3} M) The brain particle preparation in question contained some microsomes

Apart from possible species differences, it is difficult to give a rational explanation of the discrepancy between my findings and those of Inouye *et al*, however, in the light of subsequent results obtained in my laboratory and elsewhere, I do not feel inclined to modify my conclusion that HT in guinea pig brain is localized in the pre-synaptic vesicle-containing nerve-ending fraction, and that the dense type of storage particle found in duodenum does not occur in this brain

V P WHITTAKER

Biochemistry Department.
Agricultural Research Council
Institute of Animal Physiology,
Babraham,
Cambridge

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Metabolism of Rose Bengal

ROSE bengal has been used for chromo-excretion liver tests usually without a more accurate investigation of the composition of the dye From the results of Stowe *et al*[1], it appears that only highly halogenated derivatives of fluorescein are selectively excreted by the liver It is important to use a pure preparation which does not contain less-halogenated derivatives Recently, a number of authors[2-4] directed attention to the heterogeneous character of commercial preparations, but only Bocci[4] isolated pure rose bengal (tetrachlorotetraiodofluorescein) by paper chromatography and used it for investigations in metabolism Recent publications[3-5] indicate that rose bengal is not metabolized by the organism and is not transformed into other derivatives which can be detected by paper chromatography

Since it is difficult to use paper chromatography for obtaining pure substance on a large scale, we used adsorption chromatography on alumina[6] Commercial rose bengal (Geigy), dissolved in 96 per cent ethanol, was applied, and for the elution the same solvent was used, whereby a separation into ten dyes was obtained Moreover, we investigated the dye which was eluted as the second from the column It had an absorption maximum at 550 mμ, a molar extinction coefficient of 99,120 and elementary analysis corresponded to tetrachlorotetraiodofluorescein Labelling with iodine-131 as described by Liebster and Andrysek was adopted, with a 70 per cent radioactivity yield and labelled rose bengal was purified by removal of the traces of inorganic iodide iodine-131 by repeated precipitation

This preparation was administered intravenously to rats in amounts of 1–20 μc per animal In the contents of the small intestine and in liver homogenates we were unable to detect a substance which differed from that administered by paper chromatography in systems 25 per cent ethanol – 5 per cent ammonia (1 : 1) and n-butanol–ammonia–water–ethanol (100 : 44 : 1 : 20). Investigating the distribution of radioactive rose bengal in the organs, urine and fæces of rats, we found a considerable amount of radioactivity in the urine (as much as 8 per cent of the administered activity) although the urine was not coloured. The thyroid gland had also a higher activity (about 0·08 per cent of the administered activity) than the other organs (about 0·001 per cent). When the same amount of preparation was administered orally, the activity in the thyroid gland was substantially lower (about 0·015). The rats were killed 2, 4 and 6 days after administration, which did not influence the level of activity in the thyroid gland and urine. From this it appears that substantial metabolic changes occurred within 48 hr. after the administration.

From these results it appears that after the intravenous administration of rose bengal a partial loss of iodine occurs or at least an exchange of iodine-131 for inactive iodine. Probably an active metabolic process in the organism is involved as suggested by the different activities of thyroid glands, depending on the route of administration. We also can rule out a simple exchange reaction of iodine-131 for inactive iodine in the organism, as under physiological conditions (temperature 37°, pH 7) this reaction does not occur *in vitro*.

M. JIRSA

Research Laboratory for
Hæmatology and Liver Diseases,
First Medical Clinic,
Prague.

P. RABAN

Biophysical Institute,
Charles University,
Prague.

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Coumarin-like Substances from Australian Termites

HÆMOLYMPH from several Australian termites of the genus *Nasutitermes* (family Termitidae) contains, in appreciable quantities, three closely allied, highly fluorescent substances of unknown metabolic function. These substances, for which the names nasutin-*A*, -*B* and -*C* are proposed, are readily detectable by means of paper chromatography. With water-saturated butanolic ammonia as mobile phase, the nasutins give yellow spots (with characteristic R_F values), fading to colourless on exposure to air, but thereupon brilliantly blue-fluorescent under ultra-violet light.

Nasutins have been detected in *Nasutitermes exitiosus* Hill, *N. fumigatus* Brauer, *N. magnus* Froggatt and *N. walkeri* Hill, but they appear to be absent from *N. dixoni* Hill and from all species of other genera (*Porotermes*, *Heterotermes*, *Hamitermes* and *Drepanotermes*) so far examined. With *N. exitiosus*, the species most thoroughly investigated so far, nasutins appear to be present at all stages of development and in all castes other than alates, but they exist in greatest quantities in mature workers and soldiers. As would be expected of components of hæmolymph, they are to be found in all major segments of the insects; they occur also in fresh fæces but are not detectable in the saliva. Recent large-scale work has resulted in the isolation of the three nasutins in crystalline condition, one of them being obtained in quantities sufficient for preliminary chemical characterization.

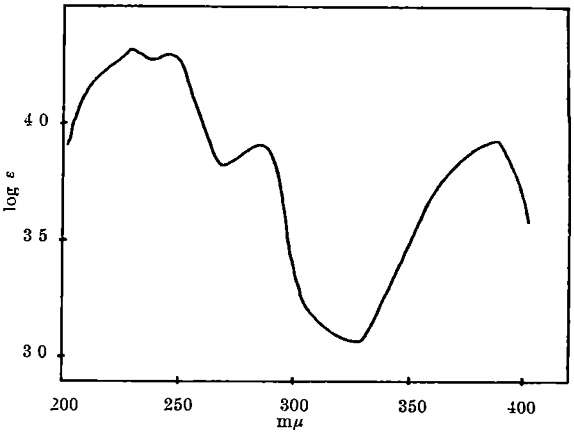

Fig. 1. Absorption spectrum of nasutin-*A* in ethanol

Nasutitermes exitiosus, the common mound-building termite of south-eastern Australia, proved to be a convenient source of nasutins. Some 15 kg of these insects were homogenized twice in methanol, the extract being separated by centrifugation and then freed from organic solvent *in vacuo*. The residual aqueous liquor was brought to pH 8·5 with ammonia, filtered, then acidified with hydrochloric acid, saturated with sodium chloride and extracted exhaustively with ether. The ethereal extract was shaken with successive portions of N aqueous ammonia until no more coloured material was removed, then discarded. The aqueous washings were acidified with hydrochloric acid and extracted exhaustively with ether. Material from the dried ethereal extract was triturated with methanol and allowed to stand. The dark brown solid (1·4 g) which separated was collected, dried, and extracted to exhaustion with acetone in a Soxhlet apparatus. Evaporation of the acetone extract left the crude nasutins as a light brown powder (660 mg). This material was recrystallized twice from large volumes of acetone to give 350 mg of pure nasutin-*A*. A portion of the material from the mother-liquors was resolved into its components by means of chromatography on Whatman 3*MM* paper with, as ascending mobile phase, butanol which had previously been equilibriated with 1 per cent aqueous ammonia. Three major bands, of R_F 0·04, 0·09 and 0·33, were obtained and these, when cut out and eluted separately with methanolic ammonia, afforded nasutin-*C*, -*A* and -*B*, respectively. Nasutin-*A* formed yellow platelets which sublimed without melting at about 300°; nasutin-*B* formed nearly colourless sheaves, melting-point 298°; and nasutin-*C*, colourless needles of undetermined melting-point.

According to analytical figures obtained from the free substance and from its dimethyl- and diacetylderivatives, nasutin-*A* has the constitution $C_{16}H_8O_7$, with no alkoxyl group present The ultra-violet absorption spectrum of the nasutin in ethanol (Fig 1) reveals the existence of an extended chromophore and the infra-red spectrum (potassium bromide disk) shows peaks at 3,300 and 3,290 cm^{-1} (2 × phenolic OH), 3,076 cm^{-1} (aromatic CH), 1,735 and 1,705 cm^{-1} (2 × αβ-unsaturated ester or lactone) Nasutin-*A* dissolves in mild alkali (*p*H 8 5) to form a deep yellow solution from which it is reprecipitated by acid With stronger alkali, however, the colour is rapidly discharged and acidification then affords the colourless nasutic acid (λ_{max} 223 and 313 mμ in ethanolic sodium hydroxide), which slowly reforms the nasutin on standing in contact with mineral acid (rapidly on being heated) Nasutin-*A* would therefore appear to have a coumarin-like structure with, probably, two phenolic groups and two lactone rings No information is yet available concerning the nature of the remaining oxygen atom

B P Moore

C S I R O , Division of Entomology,
P O Box 109, City,
Canberra.

Inhibition of Antibiotic Formation by Bromthymol Blue and Other Indicators in *Streptomyces rimosus*

Antibiotic production can be altered either by the induction of stable genotypic changes through mutation of a culture or by the addition of compounds which modify the biosynthesis of the antibiotic Alikhanian *et al* [1] described the antagonistic effects of high inorganic phosphate-levels on oxytetracycline formation and the isolation of mutants which required the addition of increased phosphate for maximal antibiotic production Ševčík, Musílek and Komersová[2] examined the effect of various inhibitors of terminal oxidases on production of oxytetracycline and oxidation of glucose by washed *S rimosus* mycelia, and found that such compounds as potassium cyanide, sodium azide and quinacrine markedly inhibited synthesis of oxytetracycline Arsenite has also been found to inhibit production of chlortetracycline[3] Gadó, Horváth and Magyar[4] reported on the reversal of the inhibitory effect of iron on formation of oxytetracycline by methylene blue in *S rimosus* There is only limited information available, however, with the previously described compounds as to the specificity with which they inhibit tetracycline formation as opposed to antagonizing growth processes in general

In this communication are summarized several observations on the inhibition of antibiotic formation by a variety of indicators—compounds which have not previously been reported to inhibit biosynthesis of oxytetracycline Recently it has also been shown that DL-ethionine[5] is a potent inhibitor specifically of antibiotic synthesis in *S rimosus* without affecting cell growth

The procedures used for all shake flask fermentations, preparation of inocula, antibiotic assays and determinations of growth were similar to those previously described[6] Composition of the basal medium follows (g per l) glucose 10 0 (autoclaved separately), β-alanine 10 0, NaCl 5 0, K_2HPO_4 2 0, $MgSO_4$ $7H_2O$ 1 0, $CaCl_2$ $2H_2O$ 0 4, $(NH_4)_2HPO_4$ 0 2, $FeSO_4$ $7H_2O$ 0 02 and $ZnSO_4$ $7H_2O$ 0 01

When bromthymol blue was used as an acid-base indicator in a fermentation medium with *S rimosus* strain N R R L 2234, it was found that antibiotic formation was markedly inhibited This initial observation was confirmed and extended in further experiments Several other sulphonphthalein indicators, bromcresol green, bromphenol blue and bromcresol purple, were also tested Of these, bromcresol green was also a potent antagonist of antibiotic formation Other types of indicators found to be potent inhibitors of antibiotic production were methylene blue and phenolphthalein Phenylsulphonphthalein, triphenyltetrazolium chloride and litmus were only slightly active as inhibitors (Table 1)

Table 1 Summary of the 'Indicator-Dye Type' Chemical Compounds screened for Possible Inhibition of Antibiotic Formation with *Streptomyces rimosus*

Test compound*	Percentage inhibition of antibiotic yield	Percentage of inhibition of cell mass
Bromthymol blue	95	15
Bromcresol green	80	0
Bromphenol blue	21	3
Bromcresol purple	5	8
Methylene blue	89	33
Phenolphthalein	95	26
Phenolsulphonphthalein	17	0
Triphenyltetrazolium chloride	31	12
Litmus	15	0

* All test compounds were added at a level of 20 μgm /ml

It is difficult to explain the inhibitory activity of certain compounds in the sulphonphthalein series, namely, bromthymol blue and bromcresol green, and the inability of related analogues to decrease markedly antibiotic production When *p*K values are compared, one finds values of 7 0 for bromthymol blue, 4 7 for bromcresol green, 4 0 for bromphenol blue and 6 3 for bromcresol purple No apparent correlation appears to exist between the *p*K values and antagonistic activity

The recent work of Miller *et al* [7], in which a new co-factor, cosynthetic factor I, has been discovered in *S aureofaciens* which acts as a hydrogenating catalyst in reducing 7-chloro-5*a*(11*a*)-dehydrotetracycline to chlorotetracycline, would suggest that hydrogen acceptors such as methylene blue could interfere with reactions of this type Whereas methylene blue and triphenyltetrazolium chloride are both active hydrogen acceptors, one finds that the former compound is about three times as potent in inhibiting antibiotic formation as the latter compound on an approximately equimolar basis

These results show that bromthymol blue and several other indicators can act as highly specific inhibitors of tetracycline formation by *S rimosus* without adversely affecting growth

W A Zygmunt

Department of Nutritional
and Biochemical Research,
Mead Johnson Research Center,
Mead Johnson and Co ,
Evansville, Indiana

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Isolation of Tetramethylpyrazine from Culture of *Bacillus natto*, and Biosynthetic Pathways of Tetramethylpyrazine

As has been shown in the previous communication[1], we have obtained tetramethylpyrazine from cultures of a strain of *Bacillus subtilis* and it had the characteristic smell of fermented soybean. The fermented soybean is the favourite food as 'natto' among Japanese people. The isolation of tetramethylpyrazine from 'natto' has been described[2]. Since *Bacillus natto* is a bacterium which ferments soybean to produce 'natto', we made an attempt to isolate tetramethylpyrazine from cultures of this bacterium.

After cultivating the bacterium for a few weeks in a medium containing glucose, peptone and meat extract, the whole cultures were subjected to flash evaporation. Water was evaporated out and the distillate extracted with ether. By addition of suitable amounts of picric acid to the ether extract a picrate was obtained. It was confirmed that the picrate is identical with an authentic sample of tetramethylpyrazine picrate by mixed melting points and comparisons of their infra-red spectra. This proved that *Bacillus natto* produces tetramethylpyrazine as does *Bacillus subtilis*.

The biosynthetic pathways of tetramethylpyrazine are of interest, since it was the second pyrazine to be discovered as a natural product and we supposed that the biosynthetic pathways differ somewhat from the pathways of the first-discovered pyrazine, aspergillic acid.

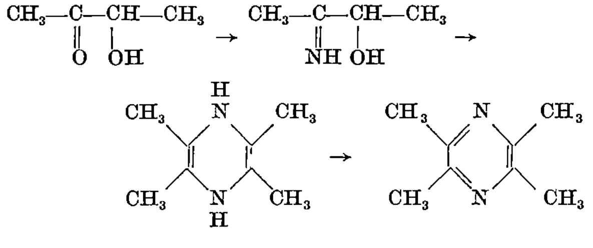
Baxter and Spring[3] have reported that aspergillic acid is biosynthesized from isoleucine via the diketopiperazine. But it is difficult to propose a plausible mechanism for the pathways from amino-acid to tetramethylpyrazine, since the hydroxy group should remain on the pyrazine nucleus instead of the methyl if it was biosynthesized from amino-acids.

In our previous communication[1], it was reported that tetramethylpyrazine was obtained after cultivation of the bacterium in media with asparagine as a source of nitrogen. The same compound was obtained when the bacillus cultivated in medias with arginine, aspartic acid, alanine, glycine, glutamic acid and even ammonium chloride, respectively, as nitrogen source.

The results might suggest that those amino-acids would be utilized as only amine parts of pyrazine and carbon parts might be converted from another source, possibly sugars.

In some micro-organisms acetoine is synthesized from sugars via pyruvic acid, and it was found by means of the Voges-Proskauer test that both *Bacillus subtilis* and *B. natto* also produced acetoine in detectable amounts.

It is thought from the chemical point of view that tetramethylpyrazine might be biosynthesized from acetoine and the amino part of amino-acids. A plausible scheme for the biosynthetic pathways is given thus:



Further proof for the mechanism is needed and we are working on this.

Takuo Kosuge
Tahei Adachi
Hiroko Kamiya

Shizuoka College of Pharmacy,
Shizuoka,
Japan

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PHYSIOLOGY

Percutaneous Absorption of Nicotinic Acid and Ethyl Nicotinate in Human Skin

This communication concerns the vast difference in percutaneous absorption through human skin of two related substances, nicotinic acid and ethyl nicotinate.

Human skin removed surgically from breasts, legs, and arms was used within 4 h of removal. Great care was exercised in handling the specimen, and the subcutaneous fat was carefully cut away with scissors. The skin was draped over a funnel and secured with elastic bands, the corium being directed to the inside of the funnel and the epidermis to the outside. The substance to be measured was applied to the epidermis, and the funnel with the epidermis downward was placed on wooden strips in a Petri dish. When using ethyl nicotinate, because of its volatility, aluminium foil was used to cover the epidermis and the cut sides of the skin were occluded with adhesive tape. Saline was injected into the funnel so that it bathed the corium and the narrow opening of the funnel was covered. Aliquots of saline were withdrawn periodically for quantitative determinations.

Both nicotinic acid and ethyl nicotinate were labelled with carbon-14 in the carboxyl groups. The specific activity of the nicotinic acid samples was 9.2 mc./mmole and 6.8 mc./mmole, the sample of ethyl nicotinate was 6.77 mc./mmole. 0.1 c.c. nicotinic acid and 0.01 c.c. ethyl nicotinate were pipetted on to the epidermis. With nicotinic acid the excess fluid was dried off the skin surface before inverting the funnel into a Petri dish; with ethyl nicotinate the skin was covered with aluminium foil before inversion. At regular intervals an aliquot was removed from the saline bathing the corium and placed in a planchet for counting with a Nuclear-Chicago gas-flow thin-window Geiger counter. Ethyl nicotinate was converted to ethyl nicotinate hydrochloride by previously adding two drops of 1 N hydrochloric acid to the planchet. The percentage of substance recovered was determined by measuring the counts obtained in the saline bathing the corium and comparing this with the number of counts applied to the epidermal surface. In order to determine the rate of penetration of skin without epidermis, the epidermis was removed by heating the skin to 63° C on a hot plate for 2 min, after which the epidermis peels away from the corium with ease. The radioactive compounds were then applied directly to the surface of the dermis and in the same way aliquots were taken at regular intervals. Percentage penetration through skin without epidermis was thus calculated for both compounds.

Twenty-four pieces of skin from 12 different specimens were used for nicotinic acid, and 14 pieces from

Table 1 Percutaneous Absorption of Nicotinic Acid and Ethyl Nicotinate in 4 H

	Penetration of nicotinic acid			Penetration of ethyl nicotinate		
	No of observations	Range (per cent)	Average (per cent)	No of observations	Range (per cent)	Average (per cent)
Epidermis intact	24	0 0003–0 008	0 001	14	26 0–49 0	37 0
Epidermis removed	3	24 0–33 0	28 0	4	49 0–80 0	59 0

9 specimens were used for ethyl nicotinate in order to determine the penetration of human skin with epidermis intact

It is evident from Table 1 that ethyl nicotinate penetrates excised human skin at a rate 37,000 times that of nicotinic acid It is also evident that this difference in penetration is a function of the epidermis

The explanation of the remarkable difference between the penetration of ethyl nicotinate and nicotinic acid is not entirely clear Previous *in vivo* studies of penetration of these compounds through human skin revealed a large difference but not as large as that found in this *in vitro* study[1] It was shown that the absolute solubility of nicotinic acid in water and ether was less than that of ethyl nicotinate and that the water/ether partition coefficient of ethyl nicotinate was 0 136 as compared with 9 25 for nicotinic acid Although ether and water solubility may play some part in penetration[2], this work also suggests the possibility that volatility of a substance may be an important factor in the difference observed Using disappearance-rates of radioactive nicotinic acid and ethyl nicotinate from standard-size planchets, we determined that ethyl nicotinate is about 10,000 times more volatile than nicotinic acid

Etain Cronin
Richard B Stoughton

Western Reserve University,
Cleveland, Ohio

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Relationship of Plasma Salicylate Concentration to Urinary Salicylate Excretion-Rate

A number of workers have determined the plasma salicylate-levels in man at various time intervals after oral doses of aspirin, while others have studied salicylate excretion[1-3] However, there appear to be few reports of the concurrent determination of plasma and urine salicylate after dosage with aspirin In this laboratory, the plasma salicylate concentrations and the urinary salicylate excretion-rates have been studied simultaneously in two groups of eight men who received a single oral dose of either aspirin B P or aloxiprin (The latter is the Approved Name selected by the British Pharmacopœia Commission to describe a "polymeric condensation product of aluminium oxide and aspirin")

An examination of the urinary excretion (Fig 1) shows that both aspirin B P and aloxiprin give rise to excretion-rates which are almost identical at their respective maxima, although in the case of aloxiprin the maximum is reached later It is suggested that this delay in the achievement of a comparable excretion-rate by aloxiprin can be attributed to slow absorption from the stomach This may be related to a lower rate of solution under acidic conditions, for it has been observed that the aspirin of aloxiprin dissolves more slowly than aspirin B P in buffer solutions over the range of *p*H 2–8, the difference being most marked at *p*H 2 (ref 4)

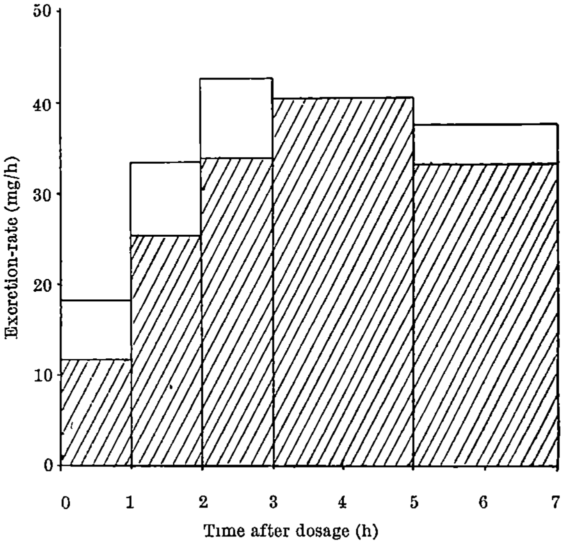

Fig 1 Mean rates of salicylate excretion in urine after aspirin B P (white), and aloxiprin (hatched)

In view of the finding that the maximum rates of salicylate excretion after aspirin and aloxiprin were very similar, it was anticipated that the maximum plasma salicylate-levels would be in approximately the same ratio This was not found to be so In fact, aspirin gave maximum plasma salicylate concentrations within the range of 3 7–6 8 mg/100 ml (mean 5 1 mg/100 ml), whereas aloxiprin gave only 2 3–3 9 mg/100 ml (mean 3 0 mg/100 ml) Also, the areas under the curves of the respective plots of plasma salicylate concentration against time seemed at first to indicate that not all the aspirin of aloxiprin was absorbed (Fig 2)

However, no appreciable difference was found between aspirin and aloxiprin with respect to the

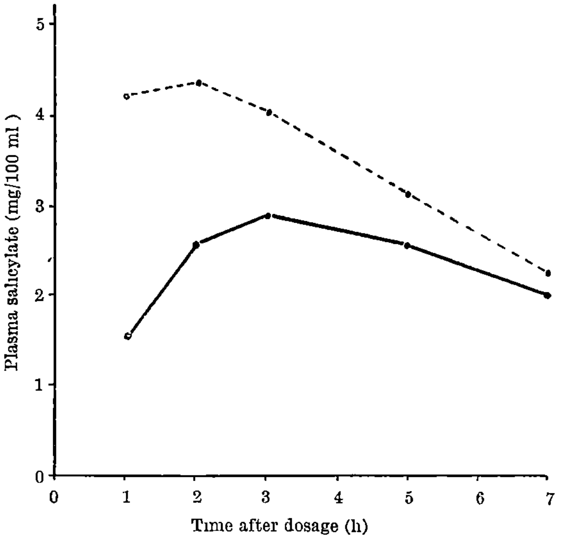

Fig 2 Mean plasma salicylate concentrations after aspirin B P , (– – –) and aloxiprin (——)

24-hour-recovery of total salicylate in the urine Also, two groups of subjects who received 1 g of aspirin or the equivalent of aloxiprin every 6 h for 36 h, excreted almost identical amounts of salicylate during a 5-h period at the end of the trial The salicylate content of aloxiprin would therefore seem to be completely available and an alternative explanation was sought for the lower blood-levels obtained

It was observed that after aspirin B P most individuals exhibited a maximum plasma salicylate at about 1 h. but the maximum rate of salicylate excretion in the urine was not attained until the third hour or later (Fig 3) Similar results are discernible in the published reports of others, but to our knowledge this fact has received no special comment

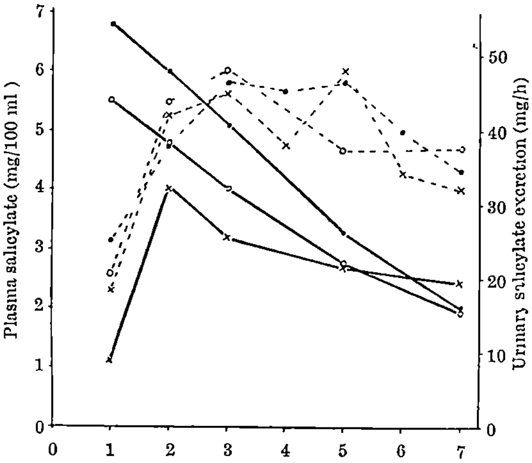

Fig 3 Examples of plasma salicylate concentrations (——) and corresponding urinary excretion-rates (- - -) in three subjects after aspirin B P

By far the greater proportion of salicylate excreted in the urine is in the form of metabolites, and it is considered that this metabolism takes place in the tissues, as distinct from the blood Thus, before being excreted, the greater part of the salicylate must pass from the blood into the tissues concerned with this metabolism The later attainment of the maximum urinary excretion rate suggests that the rate of transfer of salicylate from the blood to these tissues—and presumably to tissues generally—is lower than its rate of absorption from the gut

It is considered that after its oral administration, aspirin B P is absorbed and enters the plasma at a rate which is much faster than that at which aspirin or salicylate moves from the plasma to the tissues and therefore the serum salicylate concentration rises to a disproportionately high level The absorption from aloxiprin in the gut proceeds less rapidly, and the rates at which salicylate enters and leaves the plasma seem to be more comparable Thus the plasma and tissue salicylate concentrations always show a greater correspondence and a lower maximum plasma level is obtained

This interpretation of the experimental results implies that the relative speed of absorption of aspirin formulations cannot be accurately assessed by a comparison of the respective plasma salicylate concentrations Any difference in the relative absorption-rates will be exaggerated in that the more rapidly absorbed formulations will appear to be absorbed faster, and the less rapidly absorbed, slower, than is actually the case

The concentration of a drug in the blood is a function not only of its rate of entry but also of its rate of removal from the blood That is

$$\begin{pmatrix}\text{amount in}\\ \text{blood}\end{pmatrix} = \begin{pmatrix}\text{amount which has}\\ \text{entered blood}\end{pmatrix} - \begin{pmatrix}\text{amount which}\\ \text{has left blood}\end{pmatrix}$$

For the rate of change in the blood concentration to provide a measure of the rate of absorption of a drug, the rate of removal of the drug from the blood must either be directly proportional to its blood concentration or must have a negligible value

The experimental results presented here suggest that in the first few hours after the oral administration of a single dose of salicylate the relationship between the plasma concentration and the rate of removal of salicylate from the plasma is not always a simple proportionate one and that some other factors exercise an effect Protein-binding of the salicylate could be one such factor

A J CUMMINGS
B K MARTIN

Nicholas Research Institute, Ltd ,
Slough, Bucks

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Acetylcholine : a Central Transmitter?

ALTHOUGH many factors need to be considered before a chemical compound can be identified as a synaptic transmitter substance within the nervous system[1], it is essential that, when administered to the appropriate subsynaptic receptors, it evokes the same response of the particular neurones as does the synaptically released transmitter The demonstration of such a postsynaptic action is, however, insufficient by itself to warrant the assumption that an agent is a transmitter Thus the conclusion that acetylcholine is an excitatory transmitter of Renshaw cells is based not only on the excitatory action of this substance but also on the very close similarity between the pharmacology of this excitation, the pharmacology of the synaptic excitation of these neurones by volleys in motor axon collaterals and the pharmacology of the mammalian neuromuscular junction[2,3] Recently, the sensitivity of neurones in the cerebral cortex to electrophoretically administered acetylcholine[4,5] has led to the suggestion that such cells may be activated synaptically by cholinergic fibres[5] However, no details have been given of the effect of pharmacological agents on the synaptic excitation of these neurones, moreover, the reported pharmacology of the excitation by acetylcholine differs in certain respects from that of the excitation of Renshaw cells by this choline ester

This communication is concerned with the excitatory effect of acetylcholine on neurones in the ventrobasal complex of the thalamus of cats anæsthetized with sodium pentobarbital Extracellular action potentials (0 2–1 0 mV) were recorded by means of the central barrel of five-barrel micropipettes, the four outer barrels of which contained aqueous solutions of the various compounds which could be ejected electrophoretically[3] The neurones were excited synaptically by electrical stimulation of the contralateral median, ulnar, superficial radial, sural, tibial or peroneal nerves

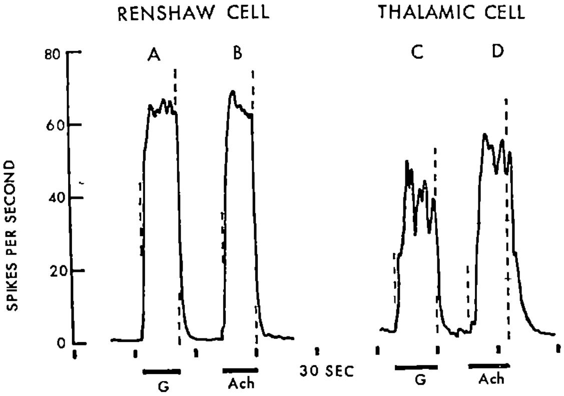

Fig 1 Frequencies of firing of a Renshaw cell (*A*, *B*) and a thalamic neurone (*C*, *D*) plotted by means of a paper recorder which was driven by a rate meter of time constant 1 sec The lower bars and vertical lines indicate the time of electrophoretic ejections of L-glutamate ion (*G*) and acetylcholine (*Ach*) using currents of 70 (*A*), 30 (*B*), 60 (*C*) and 40 (*D*) × 10^{-9} amp respectively Time, 30 sec

Almost without exception thalamic neurones were excited by acetylcholine, and this sensitivity was not restricted to spontaneously active cells In many cases, particularly when neurones were fired synaptically by an afferent volley in only one peripheral nerve, the excitation by acetylcholine was of sufficient intensity to fire the cells and the sensitivity to this ester appeared to be similar to that of Renshaw cells On the other hand, particularly with more superficially located cells which were fired synaptically by volleys in two or more afferent nerves, the excitation remained subthreshold, although electrophoretic currents of up to 150 n amp were used Nevertheless, this excitation was readily revealed as a facilitation of the excitatory action of L-glutamate ion The excitatory action of acetylcholine on both types of neurone was of delayed onset and continued for much longer after the termination of the electrophoretic current than has been observed with Renshaw cells This is illustrated in Fig 1, where the frequencies of firing of a Renshaw cell (*A*, *B*) and a thalamic neurone (*C*, *D*) are plotted with respect to time These frequencies were plotted by an ink recorder and a rate meter of time constant 1 sec Consequently, short intervals could not be indicated accurately, but were measured from simultaneously filmed records Firing was evoked by L-glutamate ion (*A*, *C*) and acetylcholine (*B*, *D*), and the times of electrophoretic ejection are indicated beneath the tracings With both neurones the times of onset and offset of the amino-acid-induced firing were less than 500 msec Acetylcholine began to fire the Renshaw cell within 500 msec of the beginning of the ejection and the firing ceased within 1 sec of its termination In contrast, there was a delay of approximately 4 sec before the thalamic neurone responded to acetylcholine (*D*) and the cell continued to fire for 16 sec after the electrophoretic current was terminated

The relative potencies of various cholinomimetics was estimated by comparing the effects of injections made with electrophoretic currents of the same intensity[6] Carbamoylcholine was more potent than acetylcholine, whereas acetylhomocholine, arecoline and nicotine were less active Prostigmine was an excitant and produced little or no prolongation of the repetitive responses evoked synaptically Dihydro-β-erythroidine, a powerful depressant of both the acetylcholine and the motor-axon collateral-synaptic activation of Renshaw cells[2,3], was without action on the synaptic responses of thalamic neurones but readily blocked the excitation produced by acetylcholine In addition, dihydro-β-erythroidine had an excitatory action, an effect which has been observed with Renshaw cells[3] D-tubocurarine affected neither synaptic nor acetylcholine-evoked responses and was a weak excitant, while gallamine and tetraethylammonium also excited the neurones Atropine and atropine-like compounds had a similar action on thalamic neurones to that which has been observed on Renshaw cells[7] The depression of the excitatory effect of acetylcholine was more powerful and prolonged than the depression of the spikes which were evoked by L-glutamate ion, but synaptically produced spikes were particularly resistant to the action of these compounds

These observations are similar to those made on lateral geniculate nucleus neurones[6] and cortical neurones[4,5], and lead to the suggestion that the receptor on thalamic neurones, with which acetylcholine and structurally related excitants interact, differs from the cholinoceptive receptor of Renshaw cells Furthermore, it is unlikely that the synaptic excitation which was examined was produced by acetylcholine It is considered that the results cannot at present be interpreted as indicating a transmitter function for acetylcholine in the ventrobasal complex of the thalamus Although this compound may indeed be a transmitter, with receptor sites which differ from those of the 'nicotinic-type' of receptor on Renshaw cells, the findings raise the alternative possibility that a compound structurally related to acetylcholine may be an important transmitter within the mammalian central nervous system

D R CURTIS
P ANDERSEN

Department of Physiology,
Institute of Advanced Studies,
Australian National University,
Canberra

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Vibrio fetus Endotoxin

Vibrio fetus is recognized to-day as an important pathogen affecting reproduction in cattle and sheep *V fetus* hypersensitivity in the bovine has not been previously reported so far as we can determine

Dennis[1] has reported the isolation of a toxic lipopolysaccharide from *V fetus* The endotoxin was reported as being toxic, pyrogenic and antigenic in rabbits and mice Winter and Dunne[2], in an antigenic analysis of *V fetus*, found that an endotoxin could be extracted from the cells that was lethal for mice. This communication concerns hypersensitivity in the bovine to an endotoxin-like substance from *V fetus*

A catalase-positive, hydrogen sulphide-positive strain of *V fetus* was grown at 37° C in broth in an atmosphere containing 85 per cent nitrogen, 10 per cent carbon dioxide, and 5 per cent oxygen[3] A broth culture and the supernatant fluid from a broth culture were inoculated intravenously into cattle

Twelve calves, aged 1–6 months, and 6 adult cows, aged 3–12 years, showed clinical symptoms of anaphylactic shock varying from mild to lethal depending on the size of the inoculum. One ml per 50 lb of body-weight of the supernatant fluid from a broth culture of *V fetus* proved lethal for two calves weighing about 300 lb. Calves were refractory to a second dose of supernatant fluid for at least 2 days after the first inoculation. The same *V fetus* supernatant fluid inoculated intradermally into cervical skin reacted positively in 36 cows, calves, and young adult cattle of a college-owned herd. The tests were read at 1–2 h after injection. These experiments, and others which will be reported later, have demonstrated a marked hypersensitivity in the bovine to *V fetus* broth culture and its supernatant fluid.

Clinical symptoms, gross pathological and histopathological changes following intravenous inoculation of *V fetus* endotoxin-like substances are very similar to those reported for other Gram-negative bacterial endotoxins.

It is suggested that the bovine becomes hypersensitive either passively during prenatal development or by contact during the early post-natal period with vibrios or other micro-organisms with antigens common to the vibrios. Abortion may be directly or indirectly associated with the hypersensitive state of the bovine to antigens of *V fetus*.

J Clark Osborne
R M Smibert

Department of Veterinary Science,
Virginia Polytechnic Institute,
Blacksburg, Virginia

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Whole-Body Skeletal Metabolic Response on Local Skin Burns

Burns, depending on their degree and extent and other complications, such as shock and infection, may lead to serious local and systemic changes in the whole body, including disorders in metabolism of water, electrolytes, proteins, etc. Some of these effects have been recorded by several authors[1,2], but still too little is known about them and about the mechanisms which control them. Practically nothing is known about the metabolic disorders in the skeleton of the burned patients. In only few reports[3–5] mention has been made of structural changes in bone seen on roentgenograms in burned patients, such as osteoporosis, periostitis, bone necrosis, osteomyelitis and periarticular calcifications and ossifications.

We are investigating these as well as other related problems connected with the influence of some physical traumatic agents (ionizing radiation, burns, frost-bite, electric shock, ultrasonic effects) on the mineral metabolism in bones. One of the numerous problems encountered during this work is that of whether the processes involved are purely local, that is, limited to the wounded area itself and the tissues immediately about it, or whether the whole skeletal system is affected.

In a previous paper[6] we published some results of experiments in which the uptake of radiocalcium in bones of rats struck by an electric current was determined quantitatively. On this occasion a generalized skeletal response on local trauma was discovered and confirmed in roentgen-irradiated bones too.

In another experiment, reported here, a local burn in white male Wistar rats weighing 120 gm was caused by putting an iron bar heated at 400° C on the right thigh. Thus, a circumscribed third degree burn of 1–2 per cent skin area arose. This procedure was carried out in 35 rats, another 35 animals serving as controls. At different intervals after beginning the experiment (4, 11, 16, 29, 42, 66 and 83 days) groups of five experimental and five control animals were formed. Each animal then received an injection of 20 μc of calcium chloride labelled with calcium-45 in 1 ml saline intraperitoneally. The rats were then killed, ether anæsthesia being used, 48 hr after injection of the isotope. Both tibiæ and scapula were cleaned. In each tibia the proximal epiphysis and metaphysis were separated from the diaphysis, and the bone samples were treated in the same way as described in our previous paper[6].

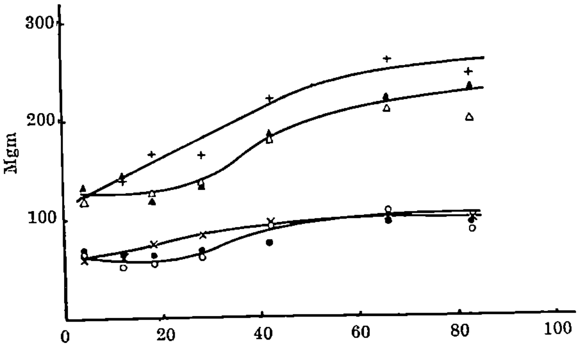

Fig 1. Weights of dry bone. Control group, ×, epiphysis, +, diaphysis, experimental group, burned leg, ○, epiphysis, △, diaphysis, unburned leg, ●, epiphysis, ▲, diaphysis

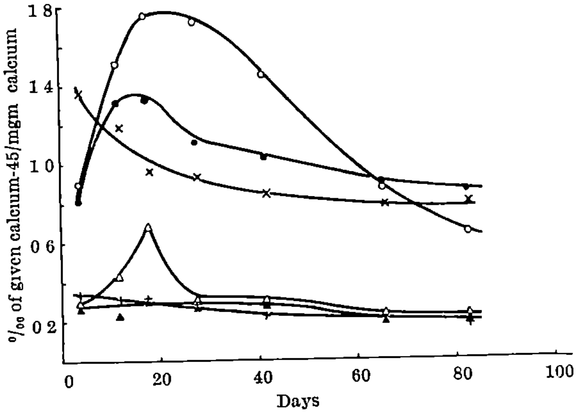

Fig 2. 48-hr accretion of calcium-45 in the tibia. Symbols same as in Fig 1

Eleven days after the injury, distinct differences appeared in the dry weight of epiphyses and diaphyses of the tibiæ on injured animals in comparison with the control group (Fig 1). The depression of weights of the epiphyses in burned animals persisted until the 42nd day and those of the diaphyses even longer, the differences being significant on the 16th and 29th day ($P < 0.05$). Analogous changes occurred also in the scapula, which is situated far from the injured place.

More distinct changes are evident when tracing the specific activity by measuring the values of two-day accretion of calcium-45 in the bones of injured and uninjured legs of the experimental animals and in the bones of the control animals Fig 2 shows that in the bones of injured animals an increased accretion of isotope at first took place, especially in the injured bones, but also in the tibia of uninjured side The calcium-45/mgm calcium in both is considerably higher than that of the tibia of intact animals ($P <$ 0 05–0 01) Again, the increase in activity was localized not only in the tibia, but was found also in the shoulder-blade, which is a bone of differently growing type Comparing both Figs 1 and 2 it is also seen that, while the weights of dry bones show a decline, the specific activity increases Even at the end of the experiment (83 days after injury) the conditions of metabolism had not become normalized, although the skin wounds were healed in most instances in about four weeks

The results of these experiments show a long-persisting and generalized metabolic skeletal response on local skin burn which was hitherto undescribed, because attempts to prove the imbalance of skeletal metabolism in burned organism, for example, by following-up the plasma calcium-levels, gave negative or uncertain results The generalized metabolic response was again proved to be a natural reaction of skeleton to local trauma The discovery of such a deep metabolic reaction to a circumscribed local burn may in future throw more light on the mechanisms involved in the forming of periarticular soft-tissue calcifications and ossifications, reported in 3 per cent of burned persons[5]

JAROMÍR KOLÁŘ
ARNOŠT BABICKÝ

Radiological Clinic,
Charles University,
Prague, and
Biological Institute of
the Czechoslovak Academy of Sciences,
Prague

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PHARMACOLOGY

Adrenergic Neurone Blocking Tropine Derivatives with Long-lasting Hypotensive Action

RECENTLY, long-lasting hypotensive agents have been described which act by blocking adrenergic neurones of these, guanethidine[1] and bretylium[2] have been widely used clinically

Among the many tropine derivatives produced in this Institute, *N* 361 (*N*-4-phenylbenzyl-3-benzoyl-σ-tropinium bromide) was found to have a strong ganglion-stimulating effect[3] When this is given in high, almost toxic, doses, however, the strong pressor effect is followed by a long-lasting fall in blood-pressure, caused by the blockage of postganglionic adrenergic neurones[4]

Because of its strong ganglion-stimulating effects *N* 361 cannot be considered for therapeutic use, but by varying the chemical structure we have attempted to obtain compounds which had the long-lasting hypotensive adrenergic neurone blocking action of *N* 361 without the ganglion stimulant and other side-effects Several compounds of this type have been examined and two of them found to be sufficiently selective in their action on the adrenergic neurones to be suitable for clinical trial They are *N* 830 (N_a-ethyl-N_b-(4-*cyclo*hexylbenzyl)-3-benzoyl-*nor*-α-tropinium bromide), and *N* 856 (*N*-(4-*cyclo*hexylbenzyl)-3-*p*-tolyl-α-tropinium bromide[5]

Both these are adrenergic neurone blocking agents which produce a long-lasting fall in blood pressure and few side-effects In cats they inhibit the contraction of the nictitating membrane in response to both pre- and post-ganglionic stimulation of the cervical sympathetic nerve They also block the contraction of the spleen in response to stimulation of the splenic nerve and in adrenalectomized animals they inhibit the pressor effects of tetramethylammonium bromide (TMA) They do not antagonize the effects of adrenaline but they produce a transient block at ganglia In this they are respectively 1 2 and 2 times as active as TMA, but the rise in pressure lasts less than 1/100 as long as the subsequent fall in pressure

In anæsthetized cats *N* 830, in a dose of 1–3 mg/kg intravenously, caused a slight and transient rise in blood pressure—not exceeding 25 mm/mercury, and lasting 20–25 sec *N* 856, in a dose of 1–1 5 mg/kg, had no immediate pressor effect on the blood-pressure but had an action similar to that of *N*-830 when the dose was increased to 2 mg/kg In this action they are significantly less active than guanethidine and bretylium

Both compounds, in doses of 1 mg/kg, intravenously, lower the blood-pressure of anæsthetized cats The effect begins within a few minutes, and from an initial level of 140–146 mm/mercury, the pressure is reduced to about 100 mm/mercury The effect increases with the size of the dose, after 2 mg/kg the pressure falls to less than 70 mm/mercury, and this fall lasts for more than 2 h In cats, both compounds block the reflex rise in blood-pressure in response to carotid occlusion for a long time and are effective in unanæsthetized as well as anæsthetized animals They are active in dogs and rats as well as in cats but higher doses must be given to produce a comparable fall in blood-pressure

In the anæsthetized cat, to produce the same reduction in the blood-pressure as 1 5–2 0 mg/kg given intravenously, it is necessary to give 3–6 mg/kg intraperitoneally 5–15 mg/kg subcutaneously, or 20–25 mg/kg into the stomach When administered by these routes, however, the drugs do not produce any apparent initial rise in blood-pressure

The compounds do not antagonize the actions of acetylcholine in the cat or, to any significant extent, in isolated rat intestine They do not affect the respiration and, in doses which lower the blood-pressure, have no action on the responses of the tibialis anterior muscle to stimulation of the sciatic nerve

A great advantage of *N* 830 and *N* 856 is that their pressor effects are so transient, lasting less than half a minute—as compared with guanethidine and bretylium, in which the pressor effects last much longer (30–120, and 2–20 min respectively) Further advantages are the rapid onset of the fall in blood-pressure and the relatively high oral absorption In cats the ratio of the oral dose to the intravenous dose producing equal effects is as low as 10 Quaternary compounds are often absorbed only very poorly from the gastro-intestinal tract and the unusually favourable absorption of *N* 830 and *N* 856 can be ascribed to their selective uptake in low concentrations by postganglionic adrenergic fibres (this has been shown in infusion experiments) and their slow rate of elimination

The compounds are relatively non-toxic, the LD_{50} in mice is greater than 20 0 mg/kg, which is larger than for guanethidine and bretylium As smaller doses are needed to produce a fall in blood pressure the therapeutic indices of *N* 830 and *N* 856 are better than those of guanethidine and bretylium

M Dóda
L Gyorgy
K Nádor

Department of Drug Research,
Institute for Experimental Medical Research,
Hungarian Academy of Sciences,
P O B 87, Budapest 9

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HÆMATOLOGY

Oxygen Equilibrium of Hæmoglobin A_2 and Its Variant Hæmoglobin $A_2{}^1$ (or B_2)

Evidence has been presented in a previous investigation[1] that the normally occurring minor hæmoglobin component A_2 has a considerably higher affinity for oxygen than normal Hb–A The Hb–A_2 preparations, which were isolated by carboxymethyl-cellulose (CMC) chromatography[2], however, contained relatively large quantities (up to 20 per cent) ferri–Hb or ferri–Hb derivatives In the work reported here we redetermined, therefore, the oxygen equilibria of normal Hb–A_0 and Hb–A_2, both being isolated as oxyhæmoglobin without notable oxidation to ferri-hæmoglobin derivatives The abnormal variant of Hb–A_2, known as Hb–$A_2{}^1$ or Hb–B_2 (ref 3), has also been included in this work

Blood samples from normal individuals, from thalassæmia trait carriers with increased quantities of Hb–A_2 and from heterozygous Hb–B_2 carriers containing approximately 1 per cent of the abnormal minor hæmoglobin fraction[3] were used Each blood sample was washed three or four times with isotonic saline and hæmolysed by the addition of 1 vol of deionized water and 0 5 vol of toluene per vol of packed cells The hæmolysates were dialysed at 4° C against distilled water for 12 h followed by dialysis against a 0 005 M phosphate buffer of *p*H 9 0 for 36 h The minor Hb–A_2 and Hb–B_2 were isolated by DEAE cellulose chromatography[4] 40–60 ml of the appropriate hæmolysate with a hæmoglobin concentration of about 10 gm per cent was applied to a DEAE-cellulose (Brown Co , 55 Main Street, Berlin, New Hampshire) column of 35 × 5 cm, the DEAE-cellulose being equilibrated with the same phosphate buffer as used for dialysis The elution was performed by application of a 0 005 M phosphate buffer *p*H 8 6 at a flow-rate of about 100 ml /h Hb–A_2 was eluted as a separate component in front of Hb–A, while the elution-rate of Hb–B_2 was even higher than that of Hb–A_2 The combined effluent fractions of each component and of Hb–A_0, which was eluted from the DEAE-cellulose column with the use of a 0 01 M phosphate buffer *p*H 6 0 (0 15 M sodium chloride added), were finally concentrated by CMC chromatography as described before[2] The whole isolation procedure, which lasted less than 7 h, was carried out in the cold When tested for purity by starch-gel electrophoresis[5] less than 5 per cent of Hb–A was found to be present in the Hb–A_2 fraction and less than 5 per cent of Hb–A_2 in the Hb–B_2 preparation The isolated hæmoglobin fractions were dialysed versus a large volume of distilled water at 4° C for 4 h followed by dialysis versus 0 1 M phosphate buffer of a *p*H varying from 6 8 to 7 4 at 4° for 15 h Spectral analyses of the oxygenated components were carried out with the use of the Beckman *DK*–2 spectrophotometer No notable amounts (less than 3 per cent) of ferri-hæmoglobin were detectable The hæmoglobin concentration of each fraction was finally adjusted to 1 0 gm per cent, determined by the cyanmethæmoglobin method[6]

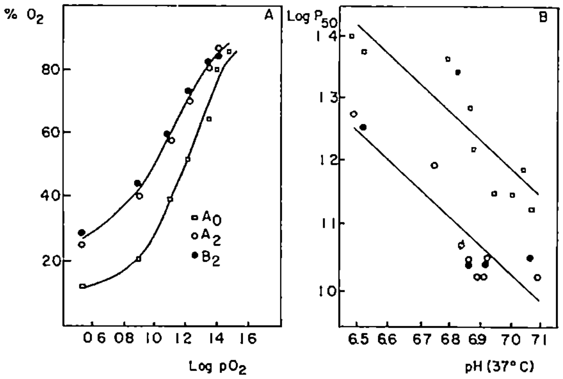

Fig 1

The procedure for the determination of the oxygen equilibria has been described previously[1] Equilibration of 1 ml samples with gas mixtures was accomplished in a tonometer of 100 ml capacity at 37° C The P_{CO_2} was maintained at a constant level of 40 mm mercury in all determinations The *p*H of an aliquot of each Hb-sample equilibrated at a P_{O_2} of 21 5 mm mercury and P_{CO_2} of 40 mm mercury was determined at 22° C using the Radiometer *PHM*4*C* model *p*H meter and finally corrected for the difference in temperature

Fig 1*A* represents examples of the oxygen dissociation curves of Hb–A_0, Hb–A_2 and Hb–B_2, the percentages of oxygen saturation were plotted against the log P_{O_2} values The relations between the changes in *p*H and the log values of the P_{O_2} producing 50 per cent oxygenation (log P_{50}) are shown in Fig 1*B* From these results the following can be concluded (*a*) Hb–A_2 and Hb–B_2 showed a substantially greater oxygen affinity than normal Hb–A_0 No difference was noted between the Hb–A_2 isolated from the blood of normal individuals and that of thalassæmia trait carriers The shapes of the oxygen equilibrium curves of Hb–A_0, Hb–A_2 and Hb–B_2 were found to be identical, the *n* values, defining the slope of the line obtained by plotting log $Y/1-Y$ (Y is the fractional oxygenation) versus log P_{O_2}, was between 1 4 and 2 0 under our experimental conditions (*b*) All three components showed identical Bohr effects (Fig 1*B*) (*c*) No notable difference in the oxygen affinities of the Hb -A_2 and Hb–B_2 fractions could be demonstrated

These investigations confirm the previously described observation of an increased oxygen affinity of Hb–A_2 as compared with normal Hb–A_0 The difference between these hæmoglobin types is located in the β-chains (Hb–A_0) and the δ-chains (Hb–A_2), these being different in more than one amino-acid residue[7,8] Elucidation of the amino-acid sequence of the δ-chain seems necessary for a better understanding of the observed difference in oxygen affinity The finding that the oxygen equilibrium of Hb–B_2, which is different from Hb–A_2 in the replacement of one unknown amino-acid residue for arginine in each δ-chain[3], is identical with that of Hb–A_2 suggests the possibility of using oxygen equilibrium experiments for demonstrating the presence of altered δ-chains in unknown minor Hb-components

This work was supported by a grant of the U S Public Health Service, No *H*–5168 and the Institutional research grant of the American Cancer Society, No *IN*–75

T H J Huisman
A M Dozy
C. Nechtman
R B Thompson
Departments of Biochemistry and Pathology,
Medical College of Georgia,
Augusta,
Georgia

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Glucose-6-Phosphate Dehydrogenase Activity in the Red Blood Cells of Monkeys

In human beings there exists an inborn character of the red blood cells which is usually harmless, but which, in certain individuals, can lead to severe hæmolysis if the red cells are challenged by the ingestion of certain drugs or foods The main abnormality in these individuals is a deficiency of the enzyme glucose-6-phosphate dehydrogenase in the red cells, which usually have a correspondingly low amount of reduced glutathione in them Further, when such sensitive red cells are incubated in certain chemicals, for example, acetylphenylhydrazine, there is a marked fall in the level of reduced glutathione, which does not occur with erythrocytes of normal persons This is the glutathione stability test of Beutler[1], and it has been pointed out by Beutler *et al*[2] that the glutathione instability of the drug-sensitive cells probably results from their deficiency in glucose-6-phosphate dehydrogenase A simple test for demonstrating this enzyme deficiency state has been developed by Motulsky[3]

To find out if a similar abnormal enzyme metabolism exists in animals, and in an attempt to obtain suitable experimental animals to examine these problems, I have examined 158 monkeys of both sexes—34 *Macca irus*, 75 *M mulatta*, 21 *M nemestrina*, 20 *Presbytis cristatus* and 8 *P obscurus* The results are given in Table 1

Table 1

Species of monkey	No examined	Motulsky test (min)	No examined	Reduced glutathione Incubation	
				before	after
M irus	34	40 – 85	5	98 4 – 70 8	96 5 – 70 0
M mulatta	75	40 – 90	5	91 5 – 80 8	89 6 – 80 4
M nemestrina	21	> 5 h	11	86 7 – 52 0	81 6 – 36 5
P cristatus	20	70 – 120	5	70 8 – 52 4	62 8 – 36 5
P obscurus	8	45 – 80	—	—	—

The blood for Motulsky test was obtained from the ear of the monkeys, and preserved in a small amount of acid–citrate–dextrose–inosine solution The test was usually carried out within 1 h, but always within 12 The time for the glucose-6-phosphate dehydrogenase activity test in normal non-sensitive men was found (in this laboratory) to be between 35 and 90 min while a time of more than 120 min was regarded as indicating a definite deficiency of the enzyme The results in all species of monkeys except one are more or less in agreement with those found in men, although in *P cristatus* the time for this test was slightly elevated compared with that in men The results for all specimens of *M nemestrina*, however, were definitely different, the time being far greater than that found in the other species and in human beings

It was expected that the level of reduced glutathione and the glutathione stability test would also be abnormal in this species, but these were found to be rather normal for all species A fall in glutathione content after incubation was noted in several monkeys, but was not so excessive as is usual in enzyme-deficient human beings

Two blood samples from *M nemestrina* were preserved in acid–citrate–dextrose–inosine solution and sent to Dr E Beutler for the glutathione stability test The values he obtained were within the range found for *M nemestrina* in this laboratory.

It would be of interest to know what the essential basis is for the discrepancies encountered in *M nemestrina* Most probably the different biochemical behaviour is a physiological characteristic peculiar to this special species, since all members of the group showed the same biochemical aberrations It is possible that the carbohydrate metabolism in this species follows different pathways The sex of the animal and the presence or absence of malarial parasites did not seem to influence the results

In a preliminary examination of other animals, the results for the glucose-6-phosphate dehydrogenase activity test were found to be within the normal range in 22 cats and 100 rabbits when compared with those found in men

I thank Dr D E Eyles of the Far East Research Unit, U S Public Health Service (Kuala Lumpur), and his staff for their co-operation , the Division of Malaria of this Institute for assistance , Mr G L Coombs and Mr C S Smith, who did most of the bleeding of the monkeys for glutathione study ; and Dr Ernest Beutler for confirming my glutathione stability test findings in two *M nemestrina*

This work was supported in part by U S Public Health Service grant No *E*4189

Lie-Injo Luan Eng
Institute for Medical Research,
Kuala Lumpur,
Federation of Malaya

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PATHOLOGY

N-Nitroso-*N*-methyl-urethane: a Potent Carcinogen

RECENTLY, we reported the induction of carcinomas of the fore stomach in rats by nitrosomethylurethane, given in the daily drinking water[1] By intravenous injection, this substance proved to be extremely toxic, the LD_{50} being only 5 mg /kg body-weight Repeated injections of 1 mg/kg every 9 days produced squamous epithelial- and alveolar cell-carcinomas of the lungs in all rats so treated[2] The medium induction time was 400 days, the total amount administered per rat only 8–9 mg The actual carcinogen is diazomethane[3], easily formed from the unstable nitrosomethylurethane by hydrolysis *N*-nitroso-*N*-methyl-*p*-toluene-sulphonamide, which is also used for the preparation of diazomethane[4] but relatively stable, proved to be practically non-toxic and non-carcinogenic when given in the daily diet of rats over their whole life-span[2]

Since nitrosomethylurethane is widely used in chemistry, we feel it necessary to point out that this dangerous substance should be handled with greatest care and be replaced in the preparation of diazomethane by the use of the non-toxic nitrosomethyl-*p*-toluenesulphonamide wherever possible

H DRUCKREY
R PREUSSMANN

Laboratory of the Surgical Clinic,
University of Freiburg,
Breisgau,
Germany

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Induction of Resistance to an Isologous Tumour by Ultra-violet Attenuated Tumour Cells

IN a previous publication from these laboratories it was demonstrated that the intravenous injection of a viable cell suspension of tumour, the *dbrB* adenocarcinoma, indigenous to the strain of origin, the *DBA*/1 mouse, elicited complete resistance to the growth of a subsequent subcutaneous implant of this same tumour, but that many of the animals died of pulmonary tumours resulting from the primary intravenous injection of viable tumour cells[1] Further, when this same strain of animal was pretreated intravenously with similar tumour cells attenuated or killed with alcohol, in an effort to eliminate the development of lung tumours, only partial resistance to challenge growth was effected[2]

In the investigations reported here the work on attenuation has been extended to the use of ultra-violet light as the attenuating agent and the *BW*5147 lymphatic leukæmia in *AKR* mice as the tumour–host combination The ultra-violet source in these studies was a high-pressure lamp having less than 2 per cent of its emission outside of the spectrum range of 2800 Å and 3650 Å All tumour suspensions were irradiated in thin layers in small Petri dishes at a distance of 8 in from the light source, the Petri dishes being immersed in an ice bath to maintain constant temperature The periods of irradiation were 0, 1, 3, 6, and 10 min All animals were treated with 0 1 ml of the irradiated cell suspensions containing 9×10^6 cells per ml and challenged 16 days later with 0 1 ml of a viable tumour cell suspension containing $6\ 75 \times 10^4$ cells per ml

Although resistance to challenge tumour growth was effected by this method of treatment, this resistance was not complete and was manifested principally by a decrease in the rate of challenge tumour growth No tumours developed in any of the animals as the result of treatment with the ultra-violet attenuated cells Best results were obtained with those suspensions irradiated for periods of 6 and 10 min As shown in Table 1, illustrative of 10 experiments so performed, the average tumour growth in these two groups of animals was respectively 73 and 39 per cent smaller than the control animals which received no treatment

Table 1 RESISTANCE ELICITED IN ANIMALS CONDITIONED WITH ULTRA-VIOLET IRRADIATED CELL SUSPENSIONS

Group No	No of animals	Ultra-violet irradiation of conditioning materials (min)	Percentage of animals with primary tumours	Average volume challenge tumours* (cm³)	Percentage of difference control tumour*
I	18	0	100	—	—
II	18	6	0	0 701	−73
III	19	10	0	1 625	−39
IV	10	None	Controls	2 635	—

* Average of volumes of tumours and percentage differences from the control tumour taken on the fourteenth day after challenge

Further investigations revealed that the density of the cell population at the time of exposure to ultra-violet irradiation was of apparently equal importance as time of irradiation in eliciting the inhibiting response The results presented in Table 1 were obtained when the cell population during irradiation was relatively sparse, $9\ 0 \times 10^6$ cells/ml When irradiation was applied to a more dense cell population, $3\ 15 \times 10^7$ cells/ml , and this was afterwards diluted to $9\ 0 \times 10^6$ cells/ml for animal treatment, it was found to be accompanied by enhanced growth of challenge tumour (63 7 per cent) implanted 16 days later (Table 2)

Table 2 EFFECT OF CELL POPULATION DENSITY IN THE ULTRA-VIOLET EXPOSURE CHAMBER ON THE HOST CONDITIONING TO CHALLENGE TUMOUR GROWTH

Group	No of animals	Cell population in irradiation chamber*	Average volume challenge tumours (cm³)†	Percentage of difference from contol tumour†
Exp *A*				
Pretreat	18	$9\ 0 \times 10^6$	0 701	−73 0
Control	10	none	2 635	—
Exp *B*				
Pretreat	10	$3\ 16 \times 10^7$	5 030	+63 7
Control	10	none	3 073	

* All groups (pre-treated) were inoculated with cell suspensions that were irradiated for 6 min

† Averages of volumes of tumours and percentage of differences from the control tumour were taken 14 days after challenge

Apparently the degree of inhibition or stimulation of challenge tumour growth is dependent on the duration of irradiation and the density of the cell suspension irradiated

In an earlier publication from our laboratories, Loofbourow[3] had reported that cells injured by ultra-violet rays secrete substances which absorb in the 2500–2600 Å region of the ultra-violet spectrum and that these substances stimulated growth It was also shown that a greater quantity of this 'growth promoter' was produced when the ultra-violet injury was prolonged[4] To ascertain whether the attenuation of the leukæmia cells in our experiments was accompanied by the production of similar 'growth-promoting' substances, spectroscopic studies of the cell-free media were undertaken These investigations revealed that those suspensions irradiated when sparsely populated produced very low quantities of substances absorbing between 2500 and 2600 Å, while the media from densely populated suspensions contained greatly increased quantities of these materials as compared with non-irradiated cells suspending media These results indicate a correlation with the growth promoting and inhibiting effects of the materials on tumour growth

This work was aided in part by grant *T*–127 from the American Cancer Society, Inc

M I COSBY
L G NUTINI
E S COOK
G S SPERTI

Institutum Divi Thomae,
1842 Madison Road,
Cincinnati 6,
Ohio

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IMMUNOLOGY

Unresponsiveness to Unrelated Antigens induced by Paralysing Doses of Bovine Serum Albumin

IT is generally admitted that immune paralysis is a phenomenon having a specificity comparable with that of immunity This conception is supported by the observations of Felton *et al* [1] and Dixon *et al* [2] However, it can be shown that, under certain conditions, adult guinea pigs, while in a state of immune paralysis to one protein, fail to be sensitized by another non-related protein antigen injected in quantities suitable for sensitization[3] The present report concerns itself with more detailed results on the question of the specificity of the immune paralysis

The experiments were performed on adult guinea pigs weighing about 400–500 g Immune paralysis was induced by daily administration of 800 mg of bovine serum albumin (BSA) injected by intravenous and intraperitoneal routes This treatment regularly resulted in immune paralysis of the injected adult guinea pigs, tests for sensitization by Schultz–Dale reaction and for serum antibodies by passive cutaneous anaphylaxis and by passive hæmagglutination (*bis* diazobenzidine method)[4] were negative for at least two months after the end of the paralysing treatment

Groups of immunologically paralysed guinea pigs, as well as control animals, were injected at various times after the beginning of the paralysing treatment with 10 mg of alum-precipitated egg albumin or rabbit γ-globulin Sensitizing injections were given by intraperitoneal, intravenous or subcutaneous routes The paralysing treatment was stopped 3–4 days after the sensitizing injection In one experiment, sensitization was attempted 8 days after the end of 15 days treatment Tests for sensitization of treated and control animals were made 15 days after the sensitizing injection by the Schultz-Dale reaction, by the passive cutaneous anaphylaxis and passive hæmagglutination methods The injections of alum-precipitated egg albumin or rabbit γ-globulin always produced a high degree of sensitization in control animals evidenced by maximal Schultz–Dale reactions, positive passive cutaneous anaphylaxis reactions of the sera (dilutions 1/128–1/512) and positive passive hæmagglutinations (dilutions 1/1024–1/4096) We considered those animals as non-sensitized which presented negative Schultz–Dale reactions, negative passive cutaneous anaphylaxis reactions with undiluted sera or a titre of passive hæmagglutination less than 1/32

The results of these experiments are reported in Table 1 It appears that if the injection of egg albumin or rabbit γ-globulin was made simultaneously with the first injection of bovine serum albumin, all treated guinea pigs were found to be sensitized

Table 1 GUINEA PIGS IMMUNOLOGICALLY PARALYSED WITH BOVINE SERUM ALBUMIN (800 MG/DAY/ANIMAL) AND SENSITIZED WITH AN UNRELATED ANTIGEN

Sensitizing antigen	Route of injection	Day of sensitization after the start of the immune paralysing treatment						
		0	3	6	8	10	14	18
Ovalbumin 10 mg alum pptd	I P	5/5*	1/2	3/5	0/2	0/5	—	—
	I V	—	2/3	1/3	—	0/4	—	—
	S C	—	—	—	—	3/3	2/4	0/3
Rabbit γ-globulin 10 mg alum pptd	I P	5/5	—	3/5	1/5	0/4	—	—
	I V	—	3/5	2/3	—	0/5	—	—
	S C	—	3/3	—	—	3/3	—	0/3

* Number of animals sensitized/total number of animals injected with the sensitizing antigen
I P Intraperitoneal, I V, intravenous, S C, subcutaneous

However, if the sensitizing antigens (egg albumin or rabbit γ-globulin) were injected after the beginning of the paralysing treatment, the proportion of sensitized animals became smaller as the interval between the start of the paralysing injections and attempted sensitization was increased When the injection of egg albumin or rabbit γ-globulin was given on the 10th day of the paralysing treatment, by the usual route (intraperitoneal or intravenous), no animals were found to be sensitized When sensitizing injections were made subcutaneously, the length of the paralysing pretreatment, necessary to inhibit sensitization, was even longer, only sensitizations attempted on the 15th–20th day of the treatment with bovine serum albumin were inhibited

The inhibition of sensitization to the non-related antigens observed when immunization is attempted under the above conditions is long lasting Guinea pigs tested two months after a sensitization attempted during the paralysing treatment had no antibodies to the sensitizing antigens as well as none to the paralysing antigen The capacity of paralysed animals to be sensitized against another non-related antigen is nevertheless rapidly restored guinea pigs treated with the same doses of bovine serum albumin during 15 days were readily sensitized if injection of alum-precipitated egg albumin was performed 8 days after cessation of the paralysing treatment This is in contrast to specific immune paralysis which may last 3–6 months, depending on the quantity of the paralysing antigen injected

These experiments support the conclusion that immune paralysis, induced in adult guinea pigs, is specific only in an early and in a late phase, that is, during the first few days of treatment and after the end of this treatment During the series of paralysis-inducing injections, as the treatment is prolonged, the immune unresponsiveness may spread to affect other protein antigens unrelated to the one used to induce immunological paralysis

P LIACOPOULOS
B N HALPERN
F. PERRAMANT

Centre de Recherches Allergiques et Immunologiques,
Hôpital Broussais,
96 rue Didot,
Paris, 14

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Binding of Ragweed Antigen labelled with Iodine-131 by Sera of Untreated Allergic Subjects

IT has recently been shown that purified horse serum albumin and two other mammalian serum proteins labelled with iodine-131 were bound by sera of allergic individuals with cutaneous reactivity to these proteins[1] The binding was demonstrated by immunological precipitation of human γ-globulin from a pre-incubated mixture of human serum and labelled antigen The quantity of antigen in the precipitate was then estimated by its radioactivity This procedure has been applied to the detection of iodine-131 ragweed antigen combined with the sera of untreated ragweed-sensitive individuals

A highly purified antigen and a high specific activity of labelled antigen are required for best results with this procedure The purified ragweed used was supplied by T P King of the Rockefeller Institute The specific activity of the labelled ragweed preparations varied from 1 to 10 mc of iodine-131 per milligram of antigen *N* Routinely, either 0 05 ml undiluted or 0 5 ml of ten-fold diluted human serum was incubated for 2 h at 37° C with varying amounts of labelled ragweed Sufficient rabbit anti-human γ-globulin was then added to precipitate all the human γ-globulin in the mixture and incubation at 37° was continued for another hour Radioactivity of the precipitate and of the supernatant fluid was measured A correction was made for the quantity of supernatant fluid entrained in the precipitate The percentage of the total activity found in the precipitate and the antigen nitrogen added was recorded A plot of such results was made for each serum with the former values as the ordinate and the logarithm of the latter values as the abscissa In all instances a flat region parallel to the abscissa, which was indicative of maximal binding, was observed in antibody excess followed by a region of inhibition as the quantity of antigen added was increased This type of curve is also typical of the binding of labelled horse serum albumin by sera of individuals sensitive to this protein[1], of the binding of labelled insulin by sera of some insulin-resistant diabetics[2] and of the binding of purified antigens by precipitating rabbit antisera[3]

A number of different sera were tested by this technique The maximum percentage of binding for several groups of sera, each tested in antibody excess, is shown in Table 1 The sera of all untreated individuals with immediate cutaneous reactivity to ragweed and all who had received hyposensitizing injections demonstrated significant binding of labelled antigen The reaction was inhibited in each instance in the presence of excess unlabelled whole ragweed extract The sera of non-atopic individuals who had not received injections of ragweed were negative

Table 1 BINDING OF RAGWEED ANTIGEN LABELLED WITH IODINE-131 BY HUMAN SERA

Subject status	No of individuals	Maximum percentage binding*
Atopic, untreated	17	30 ± 6
Atopic, treated	5	50 ± 3
Non-atopic, untreated	7	6 3 ± 2 5

*Expressed as mean ± average deviation

The maximal binding of 30–50 per cent suggests that 30–50 per cent of the labelled antigen used is immunologically competent with respect to homologous antibodies in human sera The difference in maximum binding of the immunized and non-immunized groups suggests that more than one antigen is present in the purified ragweed preparation It is evident that the ability to detect binding will decrease with decreasing purity of the labelled antigen When single pure antigens are available it will be possible to determine quantitatively the binding capacity of individual sera

J J PRUZANSKY
R PATTERSON
S M FEINBERG

Allergy Unit, Department of Medicine,
Northwestern University Medical School,
Chicago

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RADIOBIOLOGY

Strontium-90 Plasma-levels and Excretions in Young Adults

WITH the future of atmospheric testing of nuclear weapons uncertain, it remains of interest to determine in man the metabolism of strontium-90 derived from past tests Considerable information on strontium-90 retention has been obtained from analysis of human bones[1,2] and teeth[3], from work with animals, from the metabolism of stable strontium[4,5], and from the use in man of strontium-85[6,7] This communication reports some implications of a study of strontium-90 metabolism conducted on ten young adult volunteers who received a constant diet containing approximately 1 1 g of calcium per day on a metabolic ward for 3 periods of 6 days each The intake of strontium-90 during the study averaged 13 4 $\mu\mu$c /day, most of it ingested with milk Prior to the study, less milk was probably consumed, and less strontium-90 ingested

Fæcal excretion of strontium-90 averaged 86 per cent of intake Average net absorption, disregarding the amount absorbed and then re-excreted in the stool, was therefore 14 per cent, with a standard error of 3 1 per cent Absorption calculated from studies with strontium-85 on patients between 50 and 75 years of age averaged about 20 per cent[8], and 5 per cent was re-excreted in the stool, the net absorption of 15 per cent being in good agreement with the present results.

Table 1 lists values of strontium-90 found in urine, compared with urine values expected from intake. Calculations were made on the assumption that 20 per cent of the strontium-90 ingested was absorbed, and that the amount excreted in the urine depended on urinary calcium, the formula first used[8] being slightly modified[9]. The 12-day urinary excretion of strontium-90, in per cent of absorbed intake, is

$$80 - \frac{8000}{x + 100}$$ where x is urinary calcium in mg/day.

To allow for excretion of strontium-90 ingested before the study began, and for excretion beyond a 12-day period, this is multiplied by 1.2 (ref. 8). If intake prior to the study was lower than during the study period, as seemed probable, this factor may be slightly too great.

Table 1. STRONTIUM-90 IN URINE AND IN PLASMA COMPARED WITH VALUES CALCULATED FROM INTAKE

Volunteer	Urinary Ca (mg/day)	Urinary ^{90}Sr (μμc./day) predicted	Urinary ^{90}Sr (μμc./day) found	Plasma ^{90}Sr (μμc./l.) calculated from urine	Plasma ^{90}Sr (μμc./l.) calculated from food
1	45	0.91	0.69	0.315	0.416
2	148	1.75	1.2	0.222	0.323
3	91	1.20	0.66	0.171	0.311
4	360	1.97	1.78	0.204	0.226
5	75	1.05	0.78	0.234	0.315
6	61	0.94	0.66	0.234	0.333
7	110	1.29	1.0	0.226	0.291
8	159	1.51	2.0	0.353	0.226
9	96	1.19	1.3	0.324	0.297
10	202	1.63	1.7	0.260	0.249
Average	134.7	1.344	1.177	0.254	0.303

Renal clearances of strontium-90 were calculated from calcium clearances[8,9], and strontium-90 in plasma was determined by dividing urinary excretion by clearance.

Correlation between urinary calcium and urinary strontium-90 found was 0.727, significant at the 0.02-level, the equation of the regression line being $y = 4.50 + 0.034x$, where y is urinary strontium-90 as per cent of intake and x is urinary calcium in mg/day. Table 1 indicates that urinary strontium-90 could be predicted reasonably well from intake and urinary calcium, the correlation between predicted and found values being 0.785, significant at the 0.01-level. The average value found was 88 per cent of that predicted, possibly because retention was greater in our volunteers than in older persons.

The correlation between plasma values calculated from intake and from urine values was 0.171, not significant, while the average calculated from urine values was 84 per cent of that calculated from intake. On the assumption that a litre of plasma contains 100 mg calcium, the ratio of average strontium-90/calcium in plasma to that in food is 3.03/13.4, or 0.23, if plasma levels are calculated from food, and 2.54/13.4, or 0.19, if calculated from urine. Harrison[10], by direct analysis of 2 pools of plasma, found strontium-90 values of 1.1 and 1.4 μμc./g calcium on an assessed intake of 6 μμc./g, corresponding to an observed ratio (plasma/diet) of 0.18 and 0.23 respectively.

Table 2 lists strontium-90/calcium ratios in the stool, as well as ratios for stool/food. The coefficient of variation of the stool/food ratios was 19 per cent, while that of urine/food ratios, not listed individually, was 29 per cent. The use of the strontium-90/calcium ratio in urine has been proposed by Schulert[11] and by Comar and Georgi[12] for estimating the strontium-90/calcium ratio in food. The lesser variability of the stool/food ratio suggests that use of the strontium-90/calcium of stool would be superior to that of urine. Schulert's ratio is the reciprocal of the observed ratio, urine/diet (O.R.) of Comar and Georgi. Schulert's average ratio of 2.11 thus corresponds approximately to an average O.R. of 0.47. For his Peruvian and Vietnamese groups, the 95 per cent confidence limits of the O.R. may be calculated as 0.41–0.75 and 0.26–2.5 respectively. The data for Comar and Georgi's O.R. for rats (their Table 1) can be calculated to give a coefficient of variation of 54 per cent. The data in their Table 2, for human subjects give a coefficient of variation of 36 per cent. However, their data are drawn from a heterogeneous population, the values for children, first group of five, their Table 2, differing significantly from the values for adults, second group of 5. (Their third group includes some of our own volunteers, whose data are reported here.) Even for adults, however, in view of the difference between the values of Schulert and of Comar and Georgi, an average value of the ratio should be used with caution.

Table 2. STRONTIUM-90/CALCIUM RATIOS IN STOOL, AND IN STOOL COMPARED WITH FOOD

Volunteer	μμc. ^{90}Sr/100 mg Ca Stool	Stool/Food
1	1.49	1.11
2	1.60	1.19
3	1.96	1.72
4	1.86	1.65
5	1.45	1.31
6	1.66	1.43
7	2.19	1.99
8	1.96	1.73
9	2.01	1.70
10	2.09	1.82
Average	1.827	1.565
S.E.	0.082	0.091
Coeff. variation	14%	19%

As strontium-85 excretion rises less rapidly than urinary calcium[8,9], a negative correlation was expected between urinary calcium and the urinary strontium-90/calcium ratio. This correlation was found to be −0.680, significant at the 0.05-level. This suggests that the value of this ratio for estimating the corresponding ratio in food can be increased by taking urinary calcium into account. However, when urinary calcium is very low, the fraction of the ingested strontium-90 appearing in the urine is small and its determination highly uncertain. By contrast, the major portion of the ingested strontium-90 is found in the stool, the ratio of average stool strontium-90 to average urinary strontium-90 in the present study being more than 9/1. The stool/urine ratio is much greater for individuals with very low urinary calcium than for the average person in the present group, a collection of urine from a single individual of two weeks or more being needed to provide as much strontium-90 for analysis as a single day's collection of stool.

In addition, the absolute amounts of strontium-90 in food and urine bear no simple relation to each other, whereas the amount of strontium-90 in food may be calculated from that in stool by use of the factor 1.0/0.86 = 1.16. Stool analyses are therefore superior to urinary assays for strontium-90 on a number of counts, the most important of which are that they provide more accurate estimates of the strontium-90/calcium ratio in food and good estimates of total strontium-90 ingested.

This work was supported by the U.S. Atomic Energy Commission, and the analyses for strontium-90 were performed in the Atomic Energy Commission's Health and Safety Laboratory in New York.

JOSEPH SAMACHSON
HERTA SPENCER-LASZLO

Metabolic Research Unit,
Veterans Administration Hospital,
Hines, Illinois.

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Cytoplasmic Localization of Deoxyribonucleic Acid in *Allium cepa*

IT is believed generally that the normal complement of deoxyribonucleic acid (DNA) occurs exclusively on the chromosomes. However, that this is true of all plant cells has been contested. Thus ultra-violet absorption microscopy was used to show that it occurred predominantly in the cytoplasm of the root meristem cells of *Vicia faba* which had been treated with pectinase[1]. These results were supported by results obtained from fractionated homogenates of roots of *V. faba* and *Allium cepa*[2]. It was claimed that this localization was dependent on the lifelike preservation of the cytoplasmic particles, and that if these were damaged in any way the DNA would diffuse to the nuclei. However, these results were inadequate, partly because of the extreme variability of the commercial pectinase samples that were available[1], and partly because it was just as reasonable to postulate that homogenization caused the DNA to diffuse from the nuclei instead of to the nuclei[3].

The development of the controlled low-temperature sectioning technique[4] has made available a routine procedure by means of which the cytoplasm can be preserved in a life-like manner. The use of this technique, in conjunction with autoradiography, has now allowed the re-investigation of the localization of DNA in these cells.

Bulbs of *A. cepa* were grown in tap-water until the roots were about 2 in. long, when they were transferred to tap-water containing tritiated thymidine (sp. act. 0·360 mc./mM) at a concentration of 1 μc./ml. Root tips were removed from each plant after feeding for periods of 24 and 48 h, and were washed in tap-water. The majority were immersed in 5 per cent aqueous polyvinyl alcohol[4] for 2 h prior to freezing and sectioning. For comparison, other roots were treated by the more customary cytological procedures which, whether by artefact or not, ensure that all the DNA is nuclear. Thus after fixation for 3 h in Clarke's[5] acetic-alcohol, some were used for Feulgen squash preparations and others were embedded in paraffin wax and sectioned. Stripping-film autoradiographs[6] were set up with preparations from all three methods. Difficulty was encountered in the preservation of the frozen sections during the photographic processing of the autoradiographs. It was not possible to fix the sections prior to setting up the autoradiographs because many chemical fixatives destroy the cytoplasm, and it was postulated[1] that such damage would cause diffusion of the DNA to the nucleus. Other fixatives, such as the Newcomer–Zirkle technique[7], which might have produced good cytological effects, could not be used since they affected the photographic emulsion. The apposition method of autoradiography was considered inadvisable, both because of the poorer resolution of the method and because the recombination of the developed autoradiograph and the section depend on the worker.

It has been found that fixation of a frozen section with 10 per cent formalin can be achieved in the presence of a photographic emulsion without causing any damage to the emulsion. This form of fixation does not seem to cause an increase in the background count of the autoradiograph (Brain, E. B., private communication). Consequently, the exposed emulsion and frozen section were immersed for 15 min in 10 per cent formalin immediately prior to photographic development of the autoradiograph; at least this preserved the cell walls and the outline of the cell nuclei.

In sections of meristematic cells which had been fixed in acetic-alcohol and embedded in paraffin wax, as well as in those which had been subjected to fixation and macerated with hot acid, the radioactivity was localized only in the interphase and mitotic chromosomes. This radioactive, chromosomal material was insoluble in 1 per cent trichloroacetic acid at room temperature; however, extraction with 5 per cent trichloroacetic acid at 95° C for 15 min or by treatment with a suitably controlled deoxyribonuclease digestion, completely removed the labelled material. Thus, this Feulgen-positive, tritium-labelled material had the properties generally considered characteristic of DNA.

By contrast, in the frozen sections, a predominantly cytoplasmic localization was found in 82 per cent of the labelled cells of roots fed for 24 h, and in more than 90 per cent of labelled cells in those fed for 48 h. As in the paraffin sections, this labelled material in the frozen sections was insoluble in 1 per cent trichloroacetic acid at room temperature, but was extracted with a suitably controlled digestion with deoxyribonuclease or by 5 per cent trichloroacetic acid at 95° C for 15 min. That is to say, this tritium-labelled material in the frozen sections behaved like the DNA of the paraffin sections.

Consequently, this difference in the localization of the labelled material between the sections prepared by the two techniques is a true difference in the localization of the DNA, and would seem to depend on the mode of preparation.

It was postulated that the DNA would diffuse to the nuclei if the cytoplasmic granules were destroyed. Accordingly, frozen sections were treated with 1 per cent trichloroacetic acid prior to setting up the autoradiographs; the labelling was then found to be predominantly nuclear.

Grain counts made from both the paraffin and frozen sections showed that the mean number of grains per cell was of the same order for both types of section (Table 1). Furthermore, the ratio of the number of grains in the cytoplasm to that of the nucleus in the frozen sections was reversed after these sections had been treated with dilute acid (Table 2)

Table 1

Cell No	Paraffin section	Frozen section		
	Grains per nucleus	Grains per nucleus	Grains per cytoplasm	Total grains
1	39	9	23	32
2	40	12	22	34
3	58	10	51	61
4	51	15	51	66
5	73	—*	—*	93
6	100	9	19	28
7	68	25	45	70
8	42	17	54	71
9	72	14	65	79
10	57	8	28	36
Average	60	13 2	39 8	57

Grain counts, corrected for background, showing that both paraffin embedded and frozen sections have similar numbers of grains per cell Standard deviations are not given since these may correspond to a bimodal distribution which may be equivalent to 2 c and 4 c values

* Only total cell count given because of difficulty in distinguishing the nucleus sufficiently well

Table 2

Cell No	Untreated frozen section		Adjacent frozen section Treated 1 per cent TCA for 5 min	
	Percentage of grains present		Percentage of grains present	
	Nucleus	Cytoplasm	Nucleus	Cytoplasm
1	28 1	71 9	90 0	10 0
2	35 3	64 7	77 2	22 8
3	16 4	83 6	85 5	14 5
4	22 7	77 3	74 3	25 7
5	—*	—*	77 8	22 2
6	32 1	67 9	65 1	34 9
7	—*	—*	98 2	1 8
8	35 7	64 3	76 7	23 3
9	23 9	76 1	70 2	29 8
10	17 7	82 3	100 0	0
11	22 2	77 8	88 7	11 3
12	—*	—*	77 7	22 3
Average	22 6 ± 7 1	77 3 ± 7 1	81 8 ± 10 3	18 2 ± 10 4

Percentage of grains per cell over nucleus and cytoplasm demonstrating the reversal of the distribution of the grains on damaging the cell cytoplasm with dilute acid

* Only total cell counts given because of the difficulty of distinguishing the nuclei sufficiently clearly

From the preceding data it appears that not only is the labelled material of the frozen sections similar in behaviour to that of the paraffin sections, but also quantitatively similar Further, the behaviour of this material is such that it must be considered to be DNA It must be assumed that 'acid-soluble' nucleotides have been lost when the cryostat sections are immersed in water, during the autoradiographic procedure, this extraction of the so-called 'acid-soluble' fraction by the water will be facilitated by the thinness of the section, and by the fact that the protoplasm is not coagulated[4] The only difference, therefore, between the paraffin and frozen sections is one of the localization of the DNA Thus, confirmation has been obtained for the original data from which it was claimed that most of the normal complement of the DNA of the interphase root meristem cells of *A cepa* was in fact localized in the cytoplasm That treatment with acid caused most of the DNA to be found in the nuclei is further confirmation of the hypothesis that this cytoplasmic localization of the DNA is indeed dependent on the integrity of the cytoplasmic particles

We thank Prof G J Cunningham for his advice We wish to acknowledge partial financial support from the British Empire Cancer Campaign

P B Gahan
J Chayen
A A Silcox

Department of Pathology,
Royal College of Surgeons of England,
Lincoln's Inn Fields,
London, W C 2

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Role of Mercaptoethylamine in Repeated Monthly Exposures to Gamma-Radiation in Mice

The beneficial effect of administering mercaptoethylamine (MEA) to mice prior to a single acute exposure to X- or γ-radiation as reported by Bacq *et al* [1] has been demonstrated repeatedly in other laboratories Daily administration of MEA or other protective amino-alkylthiols to reduce the biological damage due to daily radiation exposure has not been successful[2] and may even be harmful[3] The role of such compounds in reducing damage to repeated radiation exposures once recovery is virtually complete has not been explored The experiment reported here was designed to determine how often mice could be challenged by a large exposure to radiation with adequate MEA administered before each such exposure A period of 30–32 days between exposures was selected as the time by which recovery from acute damage would be complete

Two hundred female Walter Reed Bagg Swiss mice, 6–7 weeks of age, 20–25 g in weight, were divided into two groups, a group of 80 was to be exposed monthly with 150 mg/kg of MEA (base) 1 5 per cent solution in distilled water, *p*H 7–7 5 freshly prepared, administered intraperitoneally 10–15 min prior to exposure The remaining group of 120 mice would serve as controls so that age would not be a factor The controls received comparable volumes of saline intraperitoneally prior to exposure Radiation was done in a specially designed cobalt-60 irradiator that has a radiation chamber of 2 ft × 2 ft × 4 in The irradiator contains approximately 1,100 c

Table 1 Effect of MEA on Repeated Exposures to 975 r at 30–32-Day Intervals in Mice

No of exposures	No of animals		Survival-time in days of decedents		30-day survival			
	Controls	Treated	Controls	Treated	Controls		Treated	
1st	10	40	11 5	15 4	0/10	—0 per cent	35/40	—89 per cent
	10	40	11 4	12 5	0/10		36/40	
2nd	20	30	11 3	16 4	0/20	—2 5 per cent	23/30	—78 3 per cent
	20	30	10 5	16 2	1/20		24/30	
3rd	24	22‡	12 0	20 3	0/24	—0 per cent	14/20	—75 per cent
	24	24	12 7	14 6	0/24		19/24	
4th	20*	32§	9 6	14 3	0/20	—0 per cent	15/29	—51 7 per cent
5th	35†	15¶	10 0	12 5	0/35	—0 per cent	0/11	—0 per cent

* 8 mice 6–7 weeks of age † All mice 6–7 weeks of age ‡ 2 deaths within 48 h of MEA injection, excluded from 30-day result § 3 deaths within 48 h of MEA injection, excluded from 30-day result ¶ 4 deaths within 48 h of MEA injection, excluded from 30-day result

cobalt-60, half above and half below the radiation-chamber. The rate varied from 116 r./min. to 110 r./min. during the period of this experiment.

Mice were confined in a circular plastic holder large enough to accommodate 50, and shallow enough to prevent huddling during the exposure. The holder was slowly rotated on a turntable within the radiation chamber, placed so that the animals were in the centre of the chamber. All exposures were 975 r. (approximately 9 min.) as measured in air at the mid-position of the animals. Post-exposure the mice were housed 10 to a wire-bottom cage, each cage containing treated and control mice. Mortality was recorded daily.

In the first exposure 80 mice were given MEA and exposed with 20 controls, in two groups of 50, 40 treated and 10 controls. The second exposure was 60 treated (survivors of first exposure) and 40 controls. By the fourth exposure the supply of age controls was exhausted so mice 6–7 weeks of age were used as controls.

The results are shown in Table 1. For the first three exposures survival in the treated mice is good. The results are consistent when the two internal and independent groups are compared for each of the first three exposures. Recovery from the third exposure was not complete 32 days later as the mice were thin, their coats were ruffled and there was gross evidence of diarrhœa. In spite of this they were exposed for a fourth time with the result as shown. Recovery from the fourth exposure was not complete 32 days later as the surviving mice appeared worse than after the third.

Better survival for the fourth and fifth exposures might have been obtained if a longer period of recovery had been allowed although a 30–32-day recovery period appeared ample after the first and second exposures. Mortality during the fourth and fifth periods was probably influenced by residual damage from the preceding radiation exposures although this cannot be quantitated from these data. It would have been of interest if treated mice could have been saved after each exposure to determine long-term survival after two, three, four and five such exposures. Also of interest would have been a third group of mice which without MEA received 490 r. each time treated mice received 975 r. With such data the actual protection offered could be described more accurately. There was increasing acute toxicity from later injections of MEA but this is probably due to the poorer condition of the mice as mentioned previously.

In spite of these faults, it is apparent that MEA does offer considerable protection to at least three repeated fully lethal exposures provided 30–32 days are allowed between exposures for recovery. After the third exposure the results may be obscured by residual damage due to a total exposure of several thousand r. There is no evidence of increased sensitivity to radiation exposure under these conditions.

MICHAEL P. DACQUISTO
SHIRLEY M. BENSON

Division of Nuclear Medicine,
Walter Reed Army Institute of Research,
Washington 12, D.C.

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BIOLOGY

Photoperiodism in Relation to the Origin of African *Trifolium* Species

THE genus *Trifolium* contains several hundred species of which the majority occur in the north temperate regions above 30° N. The genus is also represented in South Africa and South America, while a little-known group of *Trifolium* species occurs in the highlands of equatorial Africa. Some of the African species are promising pasture plants and are assuming importance in certain areas.

It is generally accepted that the genus *Trifolium* originated in the Mediterranean region. Gillett's[1] monograph on the African species contains some interesting observations on the origin and distribution of these clovers. One entire sub-section, the Ochreata, is at present confined to tropical Africa, and Gillett expresses the view that this group probably originated in equatorial Africa. The distribution of the other African species, as depicted in the remaining sections and sub-sections, is characterized by a great concentration of species in northern Ethiopia and Eritrea, and is consistent with the theory of the migration of the genus from the Mediterranean region. On the other hand, it has been suggested that the tropical African species, together with those inhabiting the mountains in South America and California, are relics of a truly ancient population[2].

Studies of photoperiodism have previously been applied as an aid in determining the origin and dispersal of plants[3–6]. So far as can be ascertained, the photoperiodic responses of at least 22 *Trifolium* species have been determined to date[7–9]. These include numerous species indigenous to the southern Mediterranean region (30–35° N.) and to Europe. All species have been classified as long-day plants of varying sensitivities. So far, no similar studies appear to have been published in respect of African clovers.

A summary of some of the recent results from an experiment on the reaction of five African clovers

Table 1. EFFECT OF DAY-LENGTH AND NIGHT TEMPERATURE ON THE FLOWERING RESPONSE OF AFRICAN *Trifolium* SPECIES
Values represent mean number of inflorescences formed per plant (means of 18 or 21 plants per treatment per species)

Treatment		East African				South African
Night temperature (° C.)	Day-length (h)	*T. usambarense*	*T. rueppellianum* var. *ruepellianum*	*T. semipilosum* var. *semipilosum*	*T. cheranganiense*	*T. africanum* var. *lydenbergense*
10	8	163.7	27.5	2.8	12.8	0.0*
	12	88.8	5.1	5.2	15.0	0.05
	16	0.7	4.0	7.0	17.3	19.8
15	8	139.6	40.2	1.7	6.1	0.0*
	12	55.9	10.0	2.9	10.8	0.0*
	16	0.2	4.3	7.0	13.8	23.9
21	8	99.3	8.0	0.7	1.5	0.0
	12	14.0	8.8	3.9	3.6	0.05
	16	0.0*	4.1	6.4	5.4	10.3

* The plants in these treatments were entirely vegetative as determined by microscopic examination of growing points.

to night temperature and length of day is presented in Table 1. The plants were grown in pots, kept in the open by day, and transferred in the afternoon to temperature-controlled growth rooms where the appropriate day-lengths were provided. The plants were harvested after 6.5 months. From Table 1 it is clear that the characteristic long-day flowering response of Mediterranean and European species did not obtain for all the African clovers. The reaction of *T. usambarense* Taub. was that of a sensitive short-day plant, with an optimum photoperiod of less than 12 h. *T. rueppellianum* Fres. also responded as a short-day plant, but appeared to be less sensitive than *T. usambarense*. The remaining three species all behaved as long-day plants, *T. africanum* Ser. from the eastern Transvaal (26° S.) being especially sensitive to photoperiod. (It should be noted that the terms 'quantitative' and 'qualitative' long- or short-day plants have been avoided in support of the recent appeal by Best[10], who showed by means of response curves that these terms are neither justified nor tenable; results of the present work (not shown) support his findings.)

The present results indicate that while genotypic differences in flowering behaviour may not be apparent between species when observed under the 12-h day-length régime of the equator, they may become clearly revealed when the material is transferred to different environments.

The physiological evidence in support of the existence of genes which determine the short-day flowering habit of *T. usambarense* in the sub-section Ochreata would, on the face of it, appear to support the hypothesis of Gillett[1], based on morphological and ecological evidence, that this presently endemic group evolved in tropical Africa. It is significant that *T. usambarense* is reported to show the greatest adaptation to tropical conditions, occurring at only 850 m near the equator[1].

The unusual existence of long-day plants on the equator does indeed suggest that *T. semipilosum* Fres., *T. cheranganiense* Gill. and related species may well have evolved from plants of the higher latitudes, probably of the north temperate region. It should be noted that this does not imply that all species and strains in the Ochreata can be classified as short-day plants or that the converse applies to the Euamoria.

On the basis of the available evidence there does not appear to be sufficient support for the view that the tropical African species are relics of an ancient population. It seems more likely that the Ochreata evolved from the widespread Euamoria some time after species of the latter group reached the African Highlands.

The fact that the predominant and aggressive Ochreata have, in recent times, failed to move out of a relatively narrow tropical band into apparently suitable areas in the higher latitudes to the north and south accords with the views of Allard[5], namely, that, among other factors of climate, day-length exerts a rigid control over the northward and southward distribution of many plants, and prevents their free colonization of lower or higher latitudes.

It is hoped that investigations in progress on the *Rhizobium* affinities and photoperiodic reactions of additional species and strains of African clovers will shed more light on the subject.

I thank Prof. N. Grobbelaar, head of the Margaretha Mes Institute of Plant Physiology and Biochemistry, for the use of controlled environment facilities, the Grassland Research Station, Kitale, Kenya, for supplying seed of the East African species, and the Director, Royal Botanic Gardens, Kew, Surrey, for identifying the material.

A. JOFFE*

Division of Crops and Pastures,
Department of Agricultural Technical Services,
Pretoria,
South Africa.

* Present address: Margaretha Mes Institute of Plant Physiology and Biochemistry, University of Pretoria.

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Dry-Matter Production by Evergreen Perennials

SOME years ago, Heath and Gregory[1] made the generalization that the mean net assimilation-rate was constant for species and environments. Subsequent work has demonstrated some large departures from their mean value of about 0.55 g/dm^2/week, although Watson[2] has more recently supported the validity of their general conclusion that leaf growth is the main yield determinant. Most of these studies have been made on annual crop plants and very few estimates are available for mean net assimilation-rates of perennials.

Recent work on the growth of aerial parts of the oil palm, *Elaeis guineensis*, Jacq., has indicated that a mature plantation, aged 17–22 years, has an annual crop growth-rate of 1.95 × 10^4 kg/hectare, a value very similar to that obtained for *Pinus*[3]. This is associated with a mean leaf area index of 4.9, and a leaf area duration of more than 250 weeks. The mean annual value for net assimilation rate is therefore 0.078 g/dm^2/week, a figure lower than any quoted in the literature. In terms of efficiency of solar energy conversion, the annual dry matter production (estimating 1 g dry matter as equivalent to 3,740 calories) represents 0.56 per cent of total radiation or 1.22 per cent of energy utilization in the spectral region 400–700 mμ (when the available energy in this spectral region is taken to be 0.46 of the total energy received). In terms of carbon fixation (assuming the total dry weight to contain an average carbon content of 40 per cent) the annual value is 2.1 g carbon/m^2/day.

Nursery oil palms have a higher crop growth-rate; in a spacing experiment the maximum value for the production of dry matter, including roots, was 0.58 g/dm^2/week over a 27-day period (equivalent to 3.03 × 10^4 kg/hectare/yr if this rate were maintained throughout the year). This figure corresponds to solar energy conversion of 0.81 per cent for total radiation, 1.76 per cent for radiation of wave-length 400–700 mμ, and 3.3 g/carbon/m^2/day.

The crop growth-rate values obtained for the adult oil palm are very much lower than the few other data obtained in the tropics. Values of 7.2 and 7.8 × 10^4 kg/hectare/yr are reported[4,5] for dry matter accumulation by sugar cane, with similar values for papyrus[6]. Preliminary data for the dry-matter production of the rain forest trees *Tectona*, *Altingia* and *Ochroma* in Java are 5 4, 6 8 and 6 9 × 10⁴ kg/hectare/yr, respectively[7], while the aerial parts of the grasses *Panicum maximum* and *Pennisetum purpureum* yield 2 3 and 3 4 × 10⁴ kg/hectare/yr respectively in a climate similar to that in which the oil palm data were obtained[8]

In annual crops yield differences are usually accounted for by differences of leaf area index or leaf area duration In the oil palm, as in other evergreen perennials, leaf area duration must be nearing the maximum value (apart from climatic and edaphic limitations), and the interception of solar radiation must be nearing maximum efficiency The very large difference between the crop growth-rate of the adult oil palm and other tropical plants must be due to differences in mean net assimilation-rate, rather than in leaf area index or leaf area duration The variation in net assimilation-rate must be of greater importance in many cases than differences in leaf area and leaf production, which, from work on annual plants, have been assumed to play the greater part in yield determination

Recent results published by Warne[9] for the crop growth-rate of kale indicate that net assimilation-rate in annuals may also assume greater importance than leaf area index or leaf area duration

If allowance is made for the absence of root weight data for the adult palms, there is still a very large difference between the adult palm and nursery palm production This is undoubtedly due to the effect of age in reducing the proportion of photosynthesizing to total living parts, which would reduce the mean net assimilation-rate to a low level despite a possibly high rate of photosynthesis Monsi[10] comes to the same conclusion in his consideration of the perennial-leaf tree system of dry-matter production

Yield increases in evergreen perennials must be looked for, not from increases in leaf area or leaf area duration, but from improvements in the net assimilation-rates

A R REES

West African Institute for Oil Palm Research,
Near Benin City,
Nigeria

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Determination of the Quantity of Carbon and Nitrogen in the Rhizosphere of Young Plants

HILTNER[1] introduced the concept of the rhizosphere to express the zone in which the living root exerts an influence on microbes in the soil Much information has been gleaned since then on the microflora of the rhizosphere and on the substances given off by the roots, but there is much less information on the total quantities of organic substances which the roots lose The facts so far known are in relation to plants grown in sterile nutrient solutions or in sterile quartz sand without any adsorption capacity[2-4] The quantities of nitrogen given off by leguminous plants can be estimated from the total nitrogen present in non-leguminous plants cultivated together with the former in a nitrogen-free soil[5,6]

Scarcely anything is known about the total quantity of the substances given off by the roots, especially concerning their carbon and nitrogen amount and their C/N ratio in a soil with a natural absorption capacity in which the rhizosphere is somewhat limited in space In general, the extent of the rhizosphere is insufficiently known In a recent publication Papavizas and Davey[7] discussed this subject, and made estimations from a set of micro-borings in the rhizosphere of young lupins In these small soil samples they estimated the total amounts of bacteria, fungi and actinomycetes using the dilution plate technique The *R/S* values (total numbers of micro-organisms in the rhizosphere/total numbers of micro-organisms in non-rhizosphere soil) were thus obtained, and indicated that the rhizosphere is rather extensive and can have a radius up to 18 mm in unamended soils In amended soils the rhizosphere may be negligible

We have attempted to obtain exact information concerning the quantities of organic matter (that is, carbon and nitrogen) given off by the roots and the distance which these substances spread from the root surface The roots are forced to grow in a small chamber made from a thin nickel plate (0 1 mm thick) densely perforated with punctures 60μ in diameter This chamber is placed in a big container, and both are filled with the same substrate The space within the perforated chamber becomes filled with a mat of roots, and consequently may be considered to belong entirely to the rhizosphere, while the space outside the chamber is still in connexion with the roots and serves as a stock of moisture and nutrients

The substrate used consisted of a mixture of 70 per cent pure sand, 25 per cent finely ground potassium-feldspar and 5 per cent kaolinite with some finely granulated pumice stone to improve the structure and aeration of this artificial soil In some experiments the amount of feldspar and kaolinite was higher This substrate is very poor in organic substances, containing only 0 022 per cent carbon on dry-weight basis

200 ml of a solution of Crone's mixture, containing 1 5 g/l, is added to the content of every container holding about 600 g of dry soil During the experiment water is added if necessary

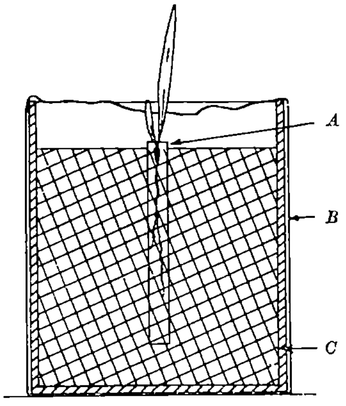

Fig 1 Diagrammatic scheme of the apparatus *A*, Root chamber, *B* black plastic sheet to prevent growth of algæ, *C*, glass pot with soil

Table 1 ROOT EXCRETIONS OF CARBON AND NITROGEN (MG)

Plants	Carbon Per 5 g dry soil			Carbon Total	Nitrogen per 5 g dry soil			C/N	Carbon/100 mg dry roots	Season
	a	*b*	*c*		*a*	*b*	*c*			
Wheat	1 7	—	—							winter
	*8 7	1 4	0 5		0 56	0 10	—	15–16		spring
	2 7	0 1	—		0 19	—	—	14–15		spring
Spinach	2 1	0 1	—		0 30	0 02	—	7		spring
	*2 6	—	—		0 25	—	—	10–11		spring
Vetch	1 6	—	—	11 8					2 9	late summer
	0 7	0 1	0 2	7 0					1 6	late summer
	†0 6	—	—	3 6					2 2	late summer
	†0 7	—	0 1	5 4					2 6	late summer

* Soil with an enhanced clay minerals content

† Experiments under conditions as for sterile cultures

—, No differences with control values

Wheat and spinach were grown for 2 months, vetch for 6 weeks In each pot 4 plants were grown

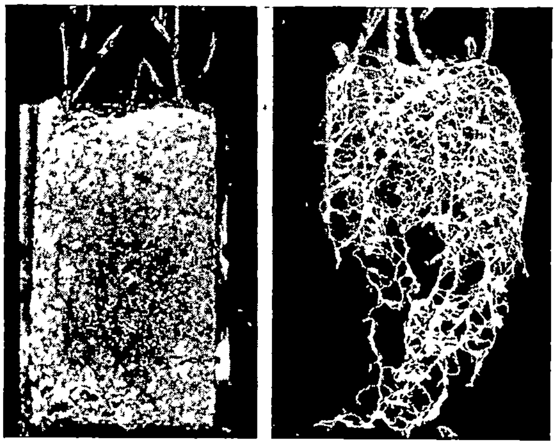
A B

Fig 2 *A*, root chamber opened, the roots grown in it are visible, *B*, the same roots, washed free from soil

The nickel plate proved not to be harmful to the roots and had scarcely any oligodynamic effect on the micro-organisms in the soil It is also inert against the substances produced by the roots and is impervious to the roots

In the upper part of the root chamber germinated sterilized seeds are planted and allowed to grow for a required period A diagram of the apparatus is shown in Fig 1

To determine the amounts of carbon and nitrogen in the rhizosphere the content of the opened root chamber (Fig 2) is washed free from soil The washing is dried at 105° C and analysed (Table 1, *a*) Further analyses are performed on the 1-cm thick layer of soil surrounding the root chamber (*b*) and on the rest of the soil in the container (*c*)

The nitrogen is determined by the micro-Kjeldahl method, the carbon by the dry-combustion method

The preliminary results so far obtained are given in Table 1 The carbon figures are corrected for the blank (0 022 per cent carbon), the nitrogen figures could not be corrected in this experiment since ammonium nitrate was added in the Crone mixture, they are therefore less reliable

A distinct concentration gradient from the root chamber outward is apparent in these experiments The technique consequently proved workable, but the distance of diffusion of the substances given off is smaller than was expected This may be brought about by the constant flow of water and solutes towards the roots as a result of the transpiration of the plants

Enhancement of the sorption complex of the soil seems to lead to larger amounts of carbon preserved in the rhizosphere

When considering the figures in Table 1 it must be realized that these experiments were not performed under aseptic conditions Part of the organic substances given off by the roots certainly had already been decomposed by micro-organisms The results therefore represent the minimum of excretion, correct figures presumably being higher

Following and improving this technique further, experiments are being started to obtain more information about the total quantities of carbon and nitrogen produced in the rhizosphere of different plants of various ages, grown under sterile, as well as non-sterile circumstances, in soils of different composition It might be expected that thereby a better insight will be gained in the part played by the rhizosphere in the cycle of carbon and nitrogen in the soil, providing us also with new information about formation and disintegration of humus

G W HARMSEN
G JAGER

Institute for Soil Fertility
Groningen,
The Netherlands

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Longevity of *Schizophyllum commune* II

IN 1945 the late G R Bisby[1] reported that sporophores of the agaric *Schizophyllum commune* Fr collected by A H R Buller[2] at Winnipeg in 1909 and 1911 and freeze-dried in 1910 and 1912 had retained their viability up to 35½ years Pure cultures were obtained and in June 1945, two months after submitting his communication for publication, they developed sporophores

On December 17, 1949, Bisby opened another tube containing two pieces of sporophore freeze dried in 1912 and afterwards kept for three weeks in liquid air at −190° C (Buller[2]) One piece (collected in 1909) was dead, the other (collected in 1911) was alive, shed spores, and was air-dried on December 20

A pure culture was again obtained, and sporophores had developed by February 21, 1950, when the culture was dried This extended the longevity to 38⅙ years

On April 25, 1962, I opened two more tubes One tube contained a single piece of sporophore collected in October 1909 and freeze-dried in December 1910 Within 24 h of being moistened this sporophore was shedding spores On April 27, a spore print was obtained for preservation on a glass slide and the specimen cultured by allowing spores to fall on to plates of potato dextrose agar The specimen was then air dried and on May 1, through the kindness of Mr H Proom, of the Wellcome Research Laboratories, re-dried (in two pieces) *in vacuo* over phosphorus pentoxide at room temperature The other tube, similar to that opened by Bisby in 1949 contained two pieces of sporophore, one collected in 1909, the other in 1911 The 1909 piece was dead The 1911 piece was viable, shed spores, and cultures were obtained This specimen, too, was air-dried on April 27 and freeze-dried (in two pieces) on May 1 By May 14 cultures from both the living specimens had developed sporophores

These tests extend the longevity of the 1911 collection to 50½ years and that of the 1909 collection to 52½ years, which is probably a new world record

A particularly interesting feature is that it is the sporophore tissue which remains viable On being moistened the gills uncurl and hymenial activity revives to give a succession of normal spores which readily germinate Unfortunately, whether spores which had been freeze-dried for fifty years were viable was not tested

The Buller specimens tested by Bisby in 1945 and 1949 and then found to be viable were all dead when re-tested May 4–6, 1962, as was the 1935 specimen from the Herbarium of the Royal Botanic Gardens, Kew, found viable by Bisby in 1945

Eight of Buller's original series of tubes still remain to be opened

G C Ainsworth

Commonwealth Mycological Institute,
Kew, Surrey

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Psilotum triquetrum, Swartz in Basutoland

Psilotum triquetrum, Swartz (*Psilotum nudum* (L) Beauv) has been listed recently around Port St John's, Cape Province (Jacot-Guillarmod, A personal communication, 1961) and in Natal, where it occurs locally on the coast, in bush swamp and, rarely, on mud flats[1] Engler and others noted this plant in the Rain Forest, Victoria Falls, and I have seen it in Ghana, in the Aburi Botanic Garden, growing on a tree stump (1958) In a general discussion with the Rev J Gomes, O P, mention was made that a plant like *Psilotum* had been seen in the Roma Valley, Basutoland Dr F Zeylmaker, previously of this Department, had made an entry in the Herbarium (1959) of a specimen of *Psilotum triquetrum* without making a clear record of the locality Consequently, during an endeavour to 'rediscover' this plant, the Cave Sandstone on the eastern escarpment of the Roma Valley was explored and a single colony of eighteen fronds of *Psilotum triquetrum* noted The plant was found growing in a cleft in the rock-face in running water The water was richly mineralized leaving a coralline encrustation on the surface of the more mature fronds of the colony The plant showed all the usual features of angularity of the stems, distinct flattening in the initial dichotomy of the frond and erect growth form Most accounts of this genus state that it is distinctly tropical and almost certainly epiphytic Manton[2] comments on its establishment in certain botanic gardens and greenhouses in non-tropical areas The climate of Basutoland is certainly not tropical, hence the observation of *Psilotum* growing here, particularly in a well-established non-epiphytic habitat, is worthy of record

Specimens of the Basutoland plant have been deposited in the British Museum (Natural History)

D Morgan

Department of Botany,
University College,
Roma, Basutoland

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A Vegetative Method for producing Virus-free Nucellar Citrus Budwood

To reduce the hazard of virus diseases in citrus-developing areas it is commonly recommended that only budwood of nucellar origin be used for multiplication purposes

Nucellar budwood is normally obtained from certified seedling trees In countries where these are not yet available their establishment is tedious, expensive and slow due to the long delay before budwood is produced for extensive budding It is thought that a quicker method could be adopted by using grafted nucellar plants instead of seedling plants, the former coming to maturity earlier and thus permitting an early varietal identification This is necessary, as in rare cases polyembryony may not be of nucellar nature but may originate by scission of the sexual embryo and the resulting seedlings are likely to differ from the parent

A method is described here for producing vegetatively propagated virus-free parent material in a relatively short time

Raising scion and rootstock seedlings Scion and rootstock seedling plants are raised under glass-house conditions Seed is sown in a sterilized compost mechanically consolidated into blocks, the compost consisting of three parts of soil to one of sand Standard mechanical analysis of a soil suitable to be composted and consolidated is as follows (per cent)

Clay and silt	15
Fine sand	70
Coarse sand	15

Two seeds are sown per soil block, but only one seedling is allowed to grow after germination The soil is watered as necessary and preventive pesticides should be applied

Approach grafting scion and rootstock seedlings Two months after germination both scion and rootstock seedlings should be approximately 15 cm high and approach grafting can be carried out The two stems are defoliated up to 10 cm from ground-level, and a 1 5–2 cm long cut is made in them at a height of 5 cm This cut should expose bark, phloem and xylem for

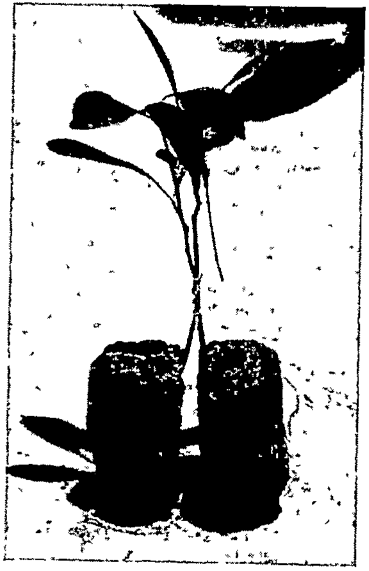

Fig. 1. Approach grafting scion and rootstock seedlings

half the stem thickness. Each soil block has one side removed and the two cut surfaces are placed in contact so that the two stems are close together. The two exposed surfaces in the stems are firmly tied together with sterile material (Fig. 1).

Scion and rootstock seedlings so treated are potted, and three weeks after grafting the rootstock seedling is topped 1 cm. above the graft union. The scion seedling is cut 1 cm. below the graft union four weeks after grafting and its roots are removed from the pot. Tying materials and the stub above and below the union should also be removed four weeks after grafting.

Although the method described proved successful under normal daylight conditions, the union of the two plants and the subsequent growth of the graft were faster under an artificial light supply of 18 h. a day.

A. BRUNO

Regional Research Station,
Ministry of Agriculture,
Samaru, Zaria,
N. Nigeria.

Long-Distance Migration of Salmon

In 1956 a salmon tagged in Scotland was recaptured in Eqaluq Fjord, south of Sukkertoppen, on the west coast of Greenland[1]. Information about the recapture of a second British salmon has recently been received from the Ministeriet for Grønland. The more recent recapture was made on October 19, 1961, near Kangamiut (65° 50′ N., 53° 21′ W.), a small settlement north of Sukkertoppen and just outside the Arctic Circle. The fish was a male, 73 cm. overall and weighed 2.4 kgm. when gutted. It was tagged as a smolt approximately 20 cm. in length in the estuary of the River Usk, Monmouthshire, on April 27, 1960. The distance between tagging and recapture positions is about 2,000 miles.

This recapture is of especial interest because the smolt was one of a number which entered the cooling-water system of the Uskmouth Generating Station, near Newport, Monmouthshire, and were netted out of the fine screen chambers, tagged and released lower down the estuary in the course of the rescue operations organized by the Central Electricity Generating Board.

In 1960 a salmon tagged as a smolt in the estuary of the Miramichi River, New Brunswick, Canada, was recaptured on the south-west coast of Greenland[2], and more recently the recapture of another Canadian salmon in that area has been reported (Nielsen, J., private communication).

Large numbers of salmon, both smolts and adults, have been tagged during recent years, and it is possible that these very-long-distance migrations may be more common than was earlier supposed. Whether fish that have travelled so far would normally return to their native rivers to spawn is, of course, still unknown. However, these recent reports show that salmon from both sides of the Atlantic can appear together in the coastal waters of south-west Greenland at some stage during their marine life.

We thank Dr. Jørgen Nielsen, of the Ministeriet for Grønland, Charlottenlund, Denmark, for supplying details of this recapture.

A. SWAIN
W. G. HARTLEY

Freshwater Fisheries Laboratory,
Ministry of Agriculture,
Fisheries and Food,
Whitehall Place, London, S.W.1.

R. B. DAVIES

Central Electricity Generating Board,
South Wales Division, Cardiff.

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ENTOMOLOGY

Influence of Illumination on Moulting and Regeneration in the Cockroach *Blattella germanica* (L.)

In *Blattella germanica*, at temperatures between 18° and 30° C., regeneration of a metathoracic leg, if begun before the critical period, adjusts the moulting cycle so as to provide the exact amount of extra time needed for completion of the regeneration process before ecdysis[1-4]. (If the level of regeneration is such as to require no muscular reorganization, this effect is absent[5-7].) In animals regenerating a whole leg, but not in controls, the corpora allata and 'head lobes' (ventral glands) show marked volume oscillations suggesting a circadian rhythm[5,11]. Photoperiod effects are important in re-setting timing of developmental processes in the diapause of many insects[8], and the onset of the dark phase in particular influences circadian activity rhythms, neurosecretion, and abnormal morphogenesis leading to tumour formation in the cockroach *Periplaneta*[9]. Far-red illumination has an adverse effect on growth in *B. germanica*[10].

Time-regulation of the temperature-dependent moulting and regeneration processes may involve some temperature-independent factors such as illumination. Preliminary to a study of possible photoperiod effects, an investigation of the influence of single changes in illumination on moulting and regeneration has been made.

Mass-cultures of *B. germanica* were reared for at least two generations in a 'light box' continuously illuminated by a fluorescent daylight lamp (40 W , correction factor 0 91) giving a light intensity in the cultures of 70–75 foot-candles (*L*-series), or in a similarly constructed 'dark box' in continuous total darkness (*D*-series) In both boxes, constant conditions of 29° ± 0 5° C , 60–70 per cent relative humidity were maintained Ootheca-bearing females were isolated, and the progeny of each divided into 4 groups of 6–10 individuals In groups I and II, the left metathoracic leg was removed at the same known age, while groups III and IV were controls Groups I and III were kept in the conditions in which they had been reared, while groups II and IV were transferred, at the time of removal of the leg, to the alternative conditions (that is, *L*-series to dark box, *D*-series to light box) A total of 1,850 *D*-series animals was used, treated at 0, 1, 2 and 3 days old and on days 1 and 3 of instar II Because of much higher mortality, the *L*-series yielded only 420 animals in the same period, these were treated at 0, 1 and 2 days old

24-hourly inspections yielded a comparison between the mean ages at ecdysis of regenerating and control animals kept in darkness and their sibs transferred to constant light, and vice versa All were necessarily exposed during inspection, between 3 and 6 p m each day, to diffuse room lighting for about 30 min and to intense light under the microscope for about 1 min This regular brief interruption of constant darkness régimes is unlikely to have been significant (the light phase is unimportant in entraining photoperiod-controlled circadian rhythms in *Periplaneta*[9])

The results (Tables 1 and 2) show a similar relation between regeneration and the moulting cycle to that already reported Transfer from light to darkness accelerates ecdysis and vice versa, but neither transfer affects regeneration as such Effects are marked only if transfer is made during a photosensitive period shortly after hatching or ecdysis, in instar II, but not instar I, there is some evidence of a recurrence of photosensitivity just after the critical period

Table 1 RETARDATION OF ECDYSIS IN *L*-SERIES MATERIAL TRANSFERRED TO DARK BOX AT AGES SHOWN IN FIRST COLUMN

Age in days at time of transfer	Difference (± S E), in days, between mean age at ecdysis of animals transferred to dark box and that of their sibs kept in constant light		
	Unoperated controls	Operated animals producing a Papilla	Operated animals producing a Regenerated leg
0–1	+0 5 (±0 095)	none recorded	+0 8 (±0 123)
1–2	−0 04 (±0 1)	none recorded	+0 2 (±0 095)
2–3	+0 05 (±0 18)	+0 12 (±0 26)	+0 33 (±0 31)

Table 2 ACCELERATION OF ECDYSIS IN *D*-SERIES MATERIAL TRANSFERRED TO LIGHT BOX AT AGES SHOWN IN FIRST COLUMN

Age in days at time of transfer	Difference (± S E), in days, between mean age at ecdysis of animals transferred to light box and that of their sibs kept in constant darkness		
	Unoperated controls	Operated animals producing a Papilla	Operated animals producing a Regenerated leg
0–1	−0 6 (±0 085)	none recorded	−0 8 (±0 085)
1–2	−0 2 (±0 085)	none recorded	−0 1 (±0 07)
2–3	−0 2 (±0 082)	+0 1 (±0 19)	−0 15 (±0 085)
3–4	+0 02 (±0 04)	−0 15 (±0 094)	none recorded
First day of instar II	−0 6 (±0 125)	none recorded	−0 5 (±0 11)
Third day of instar II	−0 2 (±0 09)	−0 5 (±0 16)	−0 2 (+−0 13)

In 4 *D*-series and 1 *L*-series batches which were followed through to metamorphosis, with the operated animals undergoing repeated regeneration, the 2nd and 3rd ecdyses were almost synchronous in both boxes, and the later ones somewhat delayed (not accelerated) in the light box, to which supermoulting by regenerating animals was confined The accelerating effect of light and the retarding effect of darkness are therefore not cumulative, the effective stimulus seems to be the change from dark to light or vice versa

Evidence[3] that regeneration virtually sets up a new moulting cycle needs re-interpreting in the light of the present results, which show no indication that the photosensitive phase of the normal cycle is repeated in regenerating animals Even those operated on at 0–1 day old merely show an 'excess' delay in moulting comparable with that originally reported[1,4] This may be due to shortage of neurosecretion to trigger the endocrine system, it does not appear in the corpora cardiaca until 24–48 h after hatching[11]

In *Periplaneta*, the circadian activity rhythm is undetectable just after moulting, when neurosecretion in the allato-subœsophageal nerve is minimal[8] In *B germanica*, the period of sensitivity of the moulting cycle to change of illumination coincides with a low level of (visible) neurosecretory activity Transfer to light or darkness may affect the moulting cycle by influencing the production or transport of neurosecretion and perhaps also modify the endocrine rhythm Further experiments, using photoperiodic as well as single illumination changes, and measuring the circadian activity rhythms, may elucidate these problems

This work was supported by a grant-in-aid from the New South Wales State Cancer Council

A F O'FARRELL
A STOCK

Department of Zoology,
University of New England,
Armidale,
New South Wales

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Houseflies Resistant to Organic Phosphate in Australia

DDT resistance in the housefly (*Musca domestica* L) has been known in Australia since 1950[1] In addition, 'Dieldrin' resistance was recorded as early as 1956 (Shanahan and Wright, personal communication) During the past year, resistance to organic phosphate has occurred on both the east and west coasts of Australia

In November 1960, a report from Perth, Western Australia, suggested that larvæ feeding in manure heaps was resistant to a 'shotgun mixture' of DDT, 'Dieldrin' and 'Diazinon' following 3 years successful control Shortly after, a report was received that 'Diazinon' was losing efficiency in controlling houseflies in an abattoirs-effluent disposal area at Casino in northern New South Wales However, control could still be achieved by applying 'Diazinon' more frequently

Samples of larvæ were collected from both areas, and laboratory strains established and afterwards tested for resistance A reference strain was also established from adult flies caught in the Royal Botanic Gardens, Sydney Contrary to expectations it was completely resistant to type I and II chemicals[2] These flies may have originated from areas with a history of DDT and BHC treatments The reference strain was not resistant to organic phosphates Practically all flies in the Perth and Casino strains were found to be homozygously resistant to the ethyl-*O*-phosphate group within the organic phosphate insecticides, but were not resistant to the methyl-*O*-phosphate group[3]

The insecticide was applied in risella oil or kerosene to the thorax of 4–5 day-old female flies anæsthetized with carbon dioxide After treatment, the results of which appear in Table 1, the flies were kept for 24 h at 25 5° C At the higher concentrations of DDT 10 per cent acetone was added to the kerosene to assist in dissolving the insecticide

Table 1 TOXICITY OF SOME INSECTICIDES APPLIED TO THREE STRAINS OF *M* *domestica*

Insecticide	LD_{50} (μg/female) Sydney	Perth	Casino
Diazinon	0 056	0 50	0 48
Parathion	0 014	—	0 148
Bayer 21/199	0 08	—	0 43
Malathion	0 40	—	0 50
DDT	> 40	> 40	> 40
γ-BHC	0 94	1 0	0 95

Susceptible flies were unavailable for comparison but the LD_{50} levels of DDT and γ-BHC in Table 1 are such that on comparison with previously reported tolerances of susceptible strains there is no doubt that each strain is significantly resistant to these chemicals

Although it is usually accepted that selection with organic phosphate insecticides alone can confer cross-resistance to chlorinated hydrocarbons There was probably adequate use of DDT and BHC at Perth and Casino for the development of DDT and BHC resistance Both phosphate-resistant strains gave every indication, by the discriminating dose technique of Milani[4,5], that they were homozygously resistant to DDT and γ-BHC as well as 'Diazinon'

Hybridization experiments between the Sydney and Perth strains to test the inheritance of resistance in the Perth strain were undertaken Openoorth[6], and Nguy and Busvine[7] have already shown that 'Diazinon'-resistance in the housefly is due to a single fully dominant gene A dose known to discriminate against susceptibles, applied to F_2 flies (interbred F_1 from a resistant by susceptible cross), gave a 3 1 (*R*/*S*) ratio When the F_1 flies were back-crossed to the susceptibles, a 1 1 ratio resulted tending to confirm the results of the earlier workers In addition, resistance to 'Diazinon' has been held through six back crosses to the Sydney strain, which in itself is strongly indicative of a simple mode of inheritance[8]

Acknowledgment is made to Geigy (Australasia) Pty , Ltd , for permission to publish this work

R J HART

School of Wool Technology,
University of New South Wales,
Kensington,
New South Wales

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Duration of Life, Egg Number and Parthenogenesis in the Stick Insect *Orxines macklotti* De Haan

IN the Phasmoptera bisexual and parthenogenetic reproduction both occur In several species males have never been found, or appear to be extremely scarce Some observations under natural conditions and many in captivity have established that the reproduction of these species is parthenogenetic and thelytokous[1–6] Well-known examples of this group are *Carausius morosus* Br from India, and the south European populations of *Bacillus rossii* F and *Clonopsis gallica* Charp

On the other hand, in species where males are common and where, at least in captivity, copulations have frequently been observed, parthenogenetic reproduction was found to lead to abnormalities in developmental processes It has been stated[4] that in a species of the genus *Leptynialla*, which has about 65 per cent males in natural conditions, only 3 per cent of parthenogenetically produced eggs hatch , the same author reports a 'weakening' of the third parthenogenetic generation in *Eurycnema* The development of unfertilized eggs of *Clitumnus extradentatus* Br takes about 2–3 weeks longer than that of normal ones[5] The following is another example of the biological significance of bisexual reproduction in a species of stick insect in which males are frequent

The species concerned, *Orxines macklotti* De Haan, was bred in large cages in the laboratory at an average temperature of about 24° Celsius and a relative humidity of about 90 per cent The insects were fed with *Rhododendron* leaves Further information on breeding and a description of some aspects of the behaviour of *Orxines* in captivity have already been given[7,8] When breeding *Orxines* in normal populations the sex ratio remains roughly 1 1 and copulations are very often seen

Table 1 HISTORY OF TEN NORMALLY AND TEN PARTHENOGENETICALLY REPRODUCING FEMALES OF THE WALKING STICK *Orxines macklotti* DE HAAN, AND OF THEIR OFFSPRING

Parents			Offspring				
Beginning of oviposition	Copulations	Females died	No of eggs	1st larva	% hatched	Sex ratio M F	Further breeds
About mid-November, 1957	Yes	1st individ on 3rd Dec 1957 , rest between 12th Febr and 1st May, 1958	1,673	March, 21st, 1958	81	1 1	Normal
About mid-November, 1957	No (No males in cage)	1st individ on 6th Febr 1958 , rest between 2nd May and 24th August, 1958	1,845	May 2nd, 1958	4 4	1 80*	20 of these 80 female larvæ reached adulthood, and laid 300 eggs, none of which hatched

* Absolute numbers

In late summer 1957 about 50 medium-sized, distinctly female larvæ were taken from the mixed populations and brought together in one cage. In the beginning of October two groups of ten larvæ from this purely female stock were put into two separate cages. Care was taken that all larvæ were of the same size (age). To the first group an ample number of males was added, as soon as the first larvæ had reached adulthood; copulations were often seen here. The second group remained without males. None of the eggs produced here can have been fertilized. Both groups began egg laying about mid-November. Table 1 shows how much the further history of the two groups differs.

P. KUYTEN

Zoological Laboratory,
Groningen,
Netherlands

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Biosynthesis of Cholesterol in an Insect, Silverfish (*Ctenolepisma* sp.)

MUCH nutritional[1-4] and biochemical[5,6] evidence suggests that the insects, as a phylum, are incapable of synthesizing sterols. This biosynthetic deficiency results, in general, in a dietary requirement of insects for some sterol without which they cannot grow to maturity. The apparent capacity of two species to thrive on a sterol-free diet has been shown to reflect the sterol-synthesizing activity of their intestinal micro-organisms which supply sterols (as well as vitamins of the B group) in quantities sufficient to satisfy the insects' growth requirements[7]. Similarly, in the cockroach, *Blattella germanica*, incorporation of dietary [1-^{14}C] acetate into sterol, which occurs under non-aseptic conditions, has been shown to be almost entirely abolished in insects reared aseptically[6]. It has been shown that the sterol synthesis observed in this insect under non-aseptic conditions is due to initial synthesis of ergosterol by the intestinal flora, followed by the conversion of this compound to 22-dehydrocholesterol in the tissues of the insect. The rate of formation of 22-dehydrocholesterol by this process is, however, not sufficient to render the cockroach independent of a supply of sterol in its food[8].

In the course of these experiments with *Blattella*, it was noted that even in insects reared aseptically, so that the biosynthesis of ergosterol and 22-dehydrocholesterol was abolished, there was a small, though significant synthesis of cholesterol itself. Evidence for this was the incorporation of very small amounts of dietary [1-^{14}C] acetate into cholesterol in the total absence of its incorporation into 22-dehydrocholesterol. The possibility had been suggested previously[5] that the most primitive insects may retain the capacity to synthesize sterols, in contrast to the higher forms in which this capacity has been lost in the course of evolution. According to this view, the trace of cholesterol synthesis observed in the sterile cockroach might represent a residuum of cholesterol-synthesizing capacity which might be quantitatively more important, and, therefore, more amenable to study, in species of insects lower in the evolutionary scale.

In order to examine this question further, we have undertaken a study of the sterol metabolism of the silverfish, *Ctenolepisma* sp., an insect more primitive than the cockroach. Some preliminary findings are reported here.

The silverfish used in the experiments to be described were non-sterile insects at various stages of growth which had been trapped in the laboratory. A group of 24 such insects were maintained for 7 months under ordinary laboratory conditions of temperature and humidity with a food supply which consisted of 1 gm. dog food containing 1 mgm. sodium [1-^{14}C] acetate (25×10^6 c.p.m.) and drinking water supplied in a soaked cotton plug. At the end of this period, the insects were extracted in turn with hot ethanol, acetone, and ethyl acetate. The total lipid extract so obtained contained 60,000 c.p.m. This low level of incorporation of acetate was due to the very small overall food consumption of the insects which were prone to cannibalism. The dried, extracted residue weighed 100 mgm. Hydrolysis of the lipid extract with 10 per cent potassium hydroxide in methanol/water, 4:1, yielded a non-saponifiable fraction containing 10,000 c.p.m. This material was combined with 5 mgm. pure cholesterol and chromatographed on deactivated alumina[6] with recovery of the sterol fraction which contained 8,000 c.p.m.

The sterol was acetylated and the major portion (6,500 c.p.m.) was subjected to careful chromatography on 7 gm. Woelm alumina deactivated with 3 per cent of its own weight of 10 per cent aqueous acetic acid. Elution with pure hexane in 9 ml. fractions yielded a single peak of material extending from fraction 35 to 74, which contained 6,000 c.p.m. and a total of 4.3 mgm. material. The peak of radioactivity coincided with the peak of weight in this chromatogram and no significant radioactivity was recovered from the column in a further 95 fractions of hexane.

These conditions of chromatography have previously been shown to give complete separation of cholesteryl acetate from 22-dehydrocholesteryl acetate and ergosteryl acetate[6,9]. Coincidence of weight and radioactivity curves in the present experiments is therefore good evidence for the identity of the labelled material with cholesteryl acetate and for the absence of 22-dehydrocholesterol from the tissues of this insect. To confirm that the labelled sterol was indeed cholesteryl acetate, 2.9 mgm. of this material (4,000 c.p.m.) was mixed with 37.1 mgm. non-radioactive, pure cholesteryl acetate. The resulting material, with a specific activity of 98 c.p.m./mgm., was precipitated as the dibromide and the free sterol acetate regenerated by a modification of the method of Fieser[10]. The recovered recrystallized acetate (10 mgm.) had a specific activity of 96 c.p.m./mgm.

It is concluded that ingestion of sodium [1-^{14}C] acetate by the silverfish leads to the appearance of labelled cholesterol in the tissues of the insect. No 22-dehydrocholesterol was detected. If the appearance of labelled cholesterol is due to a primary synthesis of ergosterol by intestinal symbionts, then it follows that the silverfish, unlike the cockroach, is able to reduce the Δ^{22}-bond in the sterol side-chain and thus complete the conversion of ergosterol to cholesterol. The alternative possibility that the tissues of the insect itself can synthesize cholesterol is at least as reasonable in view of the fact that so large a percentage (about 10 per cent) of the total incorporated label of acetate is in the sterol fraction This is to be compared with a level of approximately 1 per cent of the total incorporation of carbon-14 which appears in 22-dehydrocholesterol in the non-aseptic cockroach and about 0 004 per cent in the aseptic cockroach A decision between these two possible explanations must await the outcome of further experiments which are in progress and will be reported in due course

This work is supported by grants from the American Heart Association and the National Institutes of Health One of us (R B C) is an Established Investigator of the American Heart Association

R B CLAYTON
ANNE M EDWARDS
KONRAD BLOCH

Conant Chemical Laboratory
Harvard University
Cambridge, Mass

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MICROBIOLOGY

Reversal of Mitomycin c-induced Growth and Division Inhibition in a Species of *Erwinia*

MITOMYCIN *c*, an anti-tumour agent from culture filtrates of *Streptomyces caespitosus*, is effective against both Gram-positive and negative bacteria, and is bacteriocidal to growing and non-growing bacteria[1] It is an extremely potent inhibitor of growth and cell division of an *Erwinia* species, using a synthetic medium[2] normally supporting excellent growth

Growth inhibition can be partially overcome by increasing the concentration of one of certain divalent cations (calcium = manganese > magnesium > ferrous iron), or by increasing *p*H of the medium prior to inoculation Cobalt and zinc are inactive in overcoming growth inhibition, although zinc may reverse division inhibition to some extent Calcium, iron, manganese and zinc are in the basal medium in trace amounts , magnesium is at the level of 0 026 mgm / ml , in the form of magnesium sulphate

Increased levels of calcium or manganese partially reverse division inhibition caused by mitomycin *c* (0 2 μgm /ml), added iron or magnesium do not Neither added sodium chloride nor potassium chloride has any effect on growth or cell length in the presence of 0 2 μgm /ml of the antibiotic Representative data on the effects of various cations are given in Table 1

Increasing initial *p*H of the mitomycin *c* containing medium from 7 0 to 7 6 increases growth by a factor of 4–5, however division inhibition is not reversed Ammonium chloride and L-α-alanine, effective reversers of D-amino-acid induced division inhibition[2], do not reverse division inhibition caused by mitomycin *c*, although ammonium chloride overcomes growth inhibition to some extent

Table 1 EFFECT OF VARIOUS INORGANIC SALTS ON GROWTH AND CELL LENGTH OF *Erwinia* SP IN THE PRESENCE OF MITOMYCIN *c*

Additive and *M* concentration	Mitomycin *c* (μgm /ml)	Optical density	Cell-length (percentage in each range) 3–20μ	20–50μ	> 50μ
None	None	1 00	(70% 3–5, 30% > 5)		
None	0 2	0 22	30	50	20
None	0 4	0 02	60	40	
$CaCl_2$ 8 9 × 10^{-4}	0 2	0 60	80	20	
$MnCl_2$ 2 5 × 10^{-4}	0 2	0 73	60	30	10
$MgSO_4$ 3 0 × 10^{-4}	0 2	0 58	30	50	20
$FeSO_4$ 3 6 × 10^{-4}	0 2	0 43	45	40	15
$ZnSO_4$ 1 08 × 10^{-3}	0 2	0 18	90	10	
$CoCl_2$ 3 6 × 10^{-8}	0 2	0 15	50	30	20
$NaCl_2$ 1 5 × 10^{-2}	0 2	0 25	30	55	15
KCl 1 5 × 10^{-}	0 2	0 22	30	50	20
NH_4Cl 7 4 × 10^{-2}	0 2	0 36	20	40	40
$CaCl_2$ 8 9 × 10^{-4}	0 4	0 32	95	5	
$MnCl_2$ 2 5 × 10^{-4}	0 4	0 21	70	20	10
$MgSO_4$ 1 0 × 10^{-3}	0 4	0 23	60	38	2
$FeSO_4$ 2 7 × 10^{-4}	0 4	0 07	30	50	20

Optical density readings determined on a *B* and *L* Spectronic 20 spectrophotometer at 540 mμ, after incubation for 16 h at 25° C on a rotary shaker (ref 2)

Pantoic acid, pantoyl lactone, and sodium pantothenate (able to reverse growth inhibition and/or division inhibition caused by D-serine or penicillin[3]) do not reverse growth inhibition caused by mitomycin *c* However, pantoyl lactone effectively overcomes division inhibition, it is the only substance tested which produces a really significant degree of reversal In general, the spectrum of reversal of mitomycin *c* cell division inhibition, with this species of *Erwinia*, resembles that of penicillin[3] in many respects, particularly in that pantoyl lactone is the only really effective division inhibition reversing agent Results of the effect of organic compounds in the presence of mitomycin *c* are given in Table 2

In the presence of supplements of calcium, manganese, or magnesium (0 2 μgm mitomycin *c*/ml), pantoyl lactone or pantoic acid completely nullify the ability of the cations to stimulate growth Calcium may even enhance the slight toxicity of pantoyl lactone However, calcium enhances reversal of division inhibition by pantoyl lactone All three cations stimulate a certain degree of reversal of division inhibition by pantoic acid, a compound completely unable to reverse division inhibition in the absence of metal ion supplements

Pantoyl lactone, pantoic acid, and calcium chloride, which have the ability to trigger division of 12-h D-serine induced filaments, in the presence of D-serine[4], were tested in a similar manner with mitomycin *c*-induced filaments Since mitomycin *c*-induced filaments are, in all probability, deficient in DNA[5], it may be that, with such a deficiency, essential prior steps

Table 2 EFFECT OF CERTAIN ORGANIC COMPOUNDS ON GROWTH AND CELL LENGTH OF *Erwinia* SP IN THE PRESENCE OF MITOMYCIN *c*

Additive and *M* concentration	Mitomycin *c* (μgm /ml)	Optical density	Cell length (percentage in each range) 3–20μ	20–50μ	> 50μ
None	None	1 00	(70% 3–5, 30% 5–10)		
None	0 2	0 22	30	50	20
L-α-alanine 3 36 × 10^{-2}	0 2	0 14	80	20	
Pantoyl lactone 4 0 + 10^{-2}	0 2	0 14	(80% 2–5 20% 5–10)		
Pantoic acid 4 0 × 10^{-2}	0 2	0 14	60	30	10
α-ketoisovaleric acid 1 4 × 10^{-2}	0 2	0 23	55	40	5
Sodium-D-pantothenate 1 4 × 10^{-3}	0 2	0 17	40	40	20

Conditions for growth and measurement as given in Table 1 Pantoic acid was prepared by hydrolysing pantoyl lactone with an equimolar amount of sodium hydroxide for 10 min at 100° C for terminal division cannot occur We observed that mitomycin *c*-induced filaments did not divide nearly as readily as D-serine-induced filaments, although with pantoyl lactone or calcium chloride some division did occur Pantoic acid was inactive

Mitomycin *c* apparently causes damage of cell wall or membrane Supernatants from cultures grown in the presence of the antibiotic have a high 260/280 mμ absorbance ratio as compared with uninoculated medium indicating leakage resulting from damage to the osmotic barrier Lysis of cells occurs when mitomycin *c* is added to exponentially growing cultures

Mitomycin *c* depolymerizes DNA *in vivo* when tested with *Escherichia coli*[5] Much evidence exists that the mechanism of DNA breakdown *in vivo* is by means of the activation of a DNase, although *in vitro* activity of DNase from *E coli* is not stimulated by mitomycin *c*[6] Reich *et al* [5] state that magnesium is needed for an optimal depolymerization of DNA, in view of this it would be expected that increased magnesium concentrations would enhance, rather than diminish, mitomycin *c* growth inhibition It is possible that with this *Erwinia* species the optimum concentration for magnesium activation of DNase is exceeded at levels of magnesium which reverse growth inhibition If this were true, the mechanism of growth inhibition by mitomycin *c* would involve some factor other than activation of DNase On the other hand, a certain concentration of magnesium (or other divalent cation) may serve to stabilize the DNA molecule, as it has been shown to do with RNA from yeast[7], or tobacco mosaic virus a calcium–magnesium co-ordination complex[8]

The differential effects on growth and cell division by the various agents reported here indicate that the action of mitomycin *c* on *Erwinia* cannot be explained completely in terms of scission of preformed DNA The relationship of the known action of mitomycin *c* on DNA to its effects with *Erwinia* obviously requires further scrutiny

This investigation was supported by grant *G*9848 from the U S National Science Foundation We wish to thank Dr R D Coghill National Institutes of Health, for a gift of mitomycin *c*

MARY M GRULA
E A GRULA

Department of Bacteriology,
Oklahoma State University,
Stillwater

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Taxonomy of the Fish Furunculosis Organism

RECENTLY, Vickers and McClean[1] described cases of fish furunculosis in Lough Neagh pollan, *Coregonus pollan* Thompson They have named the organism isolated *Bacillus salmonicida* (Emmerich and Weibel) This is erroneous, as revealed by the description of characteristics given Since the organism is Gram-negative, non-sporulating and able to ferment sugar to acid, it cannot belong to the genus *Bacillus*, which is defined as consisting of Gram-positive sporulating bacteria, obligate aerobe, with highly oxidative metabolism The furunculosis organism was discovered by Emmerich and Weibel[2] in 1894 and described as *Bacterium salmonicida* This name has been in general use[3–6] until in recent years when the organism was incorporated in the new genus *Aeromonas* Kluyver and Van Niel[7,8]

The description of biochemical properties of the organism isolated by Vickers and McClean[1] conforms with the official descriptions, except its lack of indole production The regular strains should produce indole in broth cultures, but not hydrogen sulphide However, although this is an important difference from the *forma typica*, it is not enough to separate the organism from the species *Aeromonas salmonicida*, belonging to the family Pseudomonadaceae of the Eubacteriales

C VAN DUIJN, JUN

Prinses Margrietlaan 241,
Zeist, Netherlands

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VIROLOGY

Echo Virus Type 23 (Williamson) in Monkey Kidney Tissue Culture

THE cytopathic effect produced by *Echo* virus type 23 in rhesus monkey kidney tissue culture has been unsatisfactory, where propagation in this system has been accomplished[1,2], certain disadvantages made it necessary that an easier and less complicated method be devised for the regular detection of growth of this viral agent

This report describes some improvements in the technique of the propagation of *Echo* 23 in rhesus monkey kidney tissue culture and its application in the optimal dilution (checker board) neutralization test

Rhesus monkey (*Macaca mulatta*) kidney tissue culture tubes and bottles were prepared as previously described[3] in Eagle's growth medium with 5 per cent calf serum (*p*H 7 0) After eight days growth and just before virus was inoculated growth medium was removed, the cells were washed twice with phosphate-buffered saline and 0 1 c c and 1 0 c c of 10^{-1} dilution of *Echo* 23 (Williamson) was inoculated into tubes and milk dilution bottles respectively After 15–20 min 1 0 c c and 10 0 c c of Sabin's maintenance medium consisting of 5 per cent lactalbumin hydrolysate in Earle's solution with 0 22 per cent sodium bicarbonate, *p*H 7 5 was added either to tubes (1 0 c c) or bottles (10 0 c c) Control tissue culture tubes and bottles were treated in the same way as infected cultures, except that phosphate-buffered saline in the same volumes as those inoculated with tubes and bottles was added instead of virus

In order to check the specificity of virus obtained by serial passage, an optimal dilution neutralization test was carried out on the 7th and 8th passaged stock with anti-*Echo* 23 monkey serum[3] The test consisted essentially of mixing equal volumes of every dilution of virus in phosphate-buffered saline (10^{-1}–10^{-6}) with every dilution of uninactivated serum (1 · 1,024–1 256,000) in phosphate-buffered saline The mixtures were incubated in a 37° C water bath for 1 h 0 1 ml of each mixture was then inoculated into each set of six tubes devoid of growth medium and washed with phosphate-buffered saline This was followed 15–20 min later with 1 5 c c of Sabin's maintenance medium Incubation of the test was at 37° C and tubes were examined microscopically for cytopathogenicity daily for seven days

The cytopathic lesions of *Echo* 23 are typified in the early phase by rounded or ovoid cells in grape-like clusters and with clear cytoplasm and refractile edges usually at the periphery of the cell sheet As more virus is produced the whole periphery of the cell sheet shows this typical degeneration, and occasionally similar clusters of rounded cells are found in the centre of the cell sheet The cytopathic effect usually begins 24–36 h after infection Cellular destruction is usually not complete

Table 1 FINAL DAY (7) READING OF AN OPTIMAL DILUTION NEUTRALIZATION TEST WITH *Echo* 23 IN MONKEY KIDNEY TISSUE CULTURE

Virus dilutions	Antiserum dilutions* 1,024	4,096	16,384	65,536	262,144	Controls *PBS*	*NMS* 1,024
10^{-1}	0/6	0/6	0/6	6/6	6/6	3/3	4/4
10^{-2}	0/6	0/6	0/6	6/6	6/6	4/4	4/4
10^{-3}	0/6	0/6	0/6	2/6	6/6	4/4	4/4
10^{-4}	0/6	0/6	0/6	0/6	6/6	2/4	4/4
10^{-5}	0/6	0/6	0/6	0/6	0/6	0/4	2/4
10^{-6}	0/6	0/6	0/6	0/6	0/6	0/4	0/4
PBS	0/4	0/4	—	—	—	—	0/4

Inoculum 0 1 c c of virus–serum mixture

* Reciprocals of dilutions

Numerator, No showing cytopathic effect, denominator, total tubes per dilution mixture

NMS, Normal monkey serum, *PBS*, phosphate-buffered saline

The results of a typical optimal dilution neutralization test with this virus are shown in Table 1 By this method it has been possible to determine the TCD_{50} of the virus and the neutralizing titre of the serum without first resorting to a separate titration test for the virus, and then determining the neutralizing titre of serum in another test Conventional neutralization test on *Echo* 23 using the same serum as above and 100 TCD_{50} virus gave an *SDE* of 1 65,536 So far we have been able to maintain serial passages (11 passages) of *Echo* 23 in monkey kidney tissue culture without loss in infectivity

The *Echo* virus (Williamson) was kindly supplied by Dr Albert B Sabin

This work was supported in part by a U S Public Health Service research grant *C*–6006, from the National Institutes of Health

AKINYELE FABIYI
LELIA M OLSEN
JEAN WESTON
HERBERT A WENNER

Section for Virus Research,
Department of Pediatrics,
University of Kansas
School of Medicine,
Kansas City

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Monkey Pox Disease in Irradiated Cynomologous Monkeys

A POX-LIKE infection was observed in a female cynomologous monkey (*B*-39) approximately 45 days following exposure to whole-body irradiation of 350 r Monkey *B*-39 had been maintained in the same area for approximately ten months and was one of the eight survivors of a group of monkeys used in a series of irradiation studies All were apparently free from any noticeable infection until the appearance of the pox lesions Similar pox lesions were seen in one other irradiated monkey (*B*-44) in an adjacent cage a few days later

Clinical lesions similar to those described by von Magnus *et al* [1] and Prier and Sauer[2] were observed These lesions included pox-like skin eruptions, severe facial and cervical œdema, hæmorrhagic ulcerations, dyspnœa, and bloody diarrhœa Both infected monkeys died twelve days following the outbreak of the disease Only one non-irradiated monkey (*C*-302) became infected with the agent This monkey had cervical œdema and ulcerated areas on the arms and forehead similar to the lesions seen in the other two animals (*B*-39 and *B*-44), however, this animal survived the infection No signs of infection were seen in the remaining survivors of the irradiation study, nor in the 19 other non-irradiated monkeys that were housed in the same room

Virus isolation was attempted from scabs obtained from the thigh area of monkey *B*-39 An agent which produced a cytopathogenic effect in monolayers of monkey kidney epithelial cells was isolated Transmissibility was established by serial passage of the isolate in monkey kidney tissue culture and in rabbit kidney tissue culture The appearance of a typical cytopathogenic effect (Figs 1 and 2) was used as the criterion for virus multiplication First, monkey

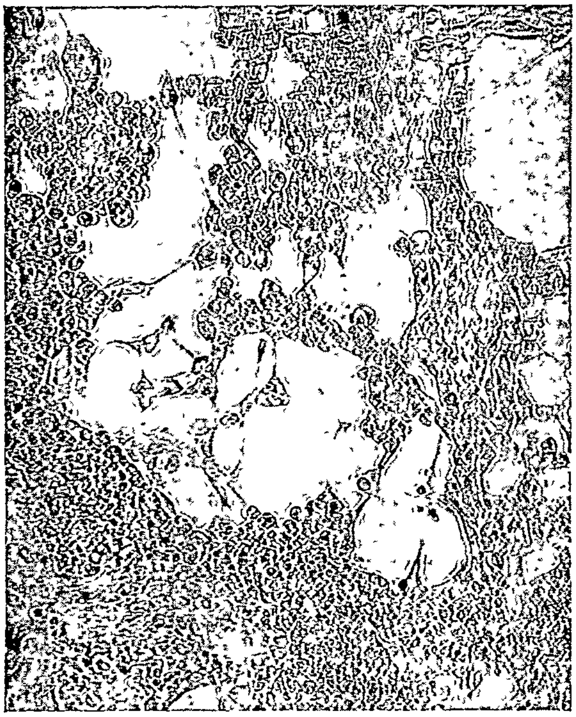

Fig 1 Cell destruction in monkey kidney epithelial cells infected with 'monkey pox agent' four days post infection (× 104)

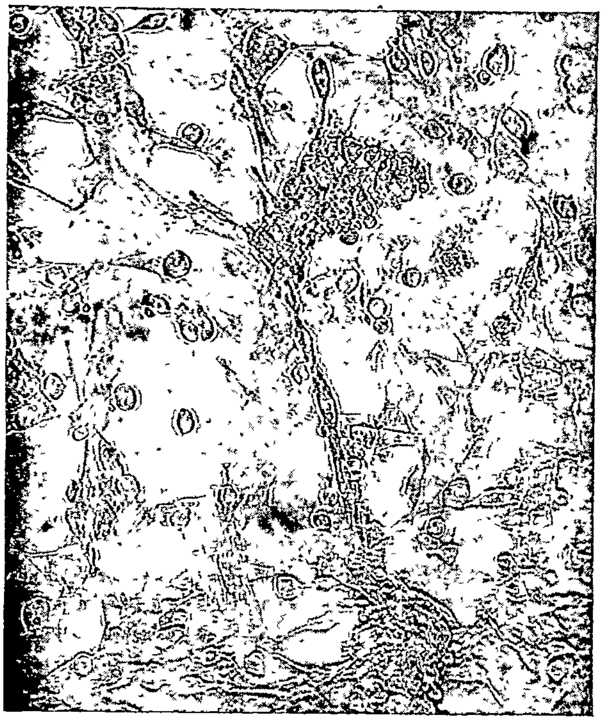

Fig 2 Cell destruction in rabbit kidney epithelial cells infected with 'monkey pox agent', five days post infection (× 104)

kidney tissue fluid containing the isolate when inoculated on the chorioallantoic membrane of 13-day-old embryonated eggs produced lesions with considerable necrosis and hæmorrhage A 20 per cent suspension of the infected membranes in physiological saline solution hæmagglutinated susceptible chicken red blood cells with a titre of 1 128 Two hæmagglutinating units of antigen were inhibited by 1 640 dilution of hyperimmune vaccinia rabbit serum which had been adsorbed with chick red blood cells The agent was then classed as a member of the variola-vaccinia group This agent was not neutralized in tissue culture by herpes *B* (Herpes simae) hyperimmune serum

Animal inoculation techniques differentiated the isolate from variola Death resulted in 3-week-old mice when inoculated intracranially, but not when inoculated intraperitoneally as reported previously by Prier *et al*[3] The agent was successfully passed serially in the epidermis of adult rabbits, thus differentiating it from variola[4]

A serological survey of our monkey colony was conducted at this time to determine the number of monkeys with detectable hæmagglutination-inhibition antibody to the isolated agent The results are given in Table 1

Table 1 SEROLOGICAL SURVEY OF MONKEY COLONY FOR MONKEY POX VIRUS

Species of monkey	No exposed to irradiation	No with clinical monkey pox disease	Hæmagglutination inhibition titres			
			0 titre	1 10– 1 40	1 80– 1 640	1 1,280 and higher
Cynomologous	Irradiated 8	2	2 *N D*	1	5	0
	Not irradiated 22	1	1	1	18	2
Cynomologous*	Not irradiated 45	0	30	10	4	1
Rhesus	Not irradiated 67	0	9	6	46	6
African green	Not irradiated 14	0	4	4	6	0

N D, Not done

* Monkeys not in area of occurrence of monkey pox disease

The room in which the monkey pox disease occurred showed 89 3 per cent of the 28 cynomologous monkeys to have a significant hæmagglutination inhibition titre of 1 80 and higher The highest hæmagglutination inhibition titres, 1 2,560 and 1 1,280, were found respectively in the serum of the non-irradiated cage mate (*B*-38) and in the convalescent serum of the non-irradiated infected monkey (*C*-302) In contrast only 11 per cent of the 45 cynomologous monkeys caged in other separate rooms showed significant hæmagglutination inhibition titres One cynomologous monkey out of this total had a high (1 1,280) hæmagglutination inhibition titre, while 30 were without titres and 10 had titres of 1 40 It is interesting to note that 77 5 per cent of the 67 rhesus monkeys had hæmagglutination inhibition titres of 1 80 and higher

From the results obtained, it would appear that the monkey pox virus may be carried by some species of monkeys as a latent virus Irradiation, by decreasing host resistance, may lead to the appearance of clinical disease Other monkeys can then acquire the disease clinically or, more likely, sub-clinically

STEWART J MCCONNELL
YAYE F HERMAN
DONALD E MATTSON
LEROY ERICKSON

Departments of Veterinary Microbiology
and Laboratory Animals,
Walter Reed Army Institute of Research,
Washington 12, D C

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Airborne Transmission of Influenza Virus Infection in Mice

THE mode of transmission of influenza has been the subject of great controversy[1] Human volunteers can be experimentally infected by dropping infected fluids into the nasopharynx[2], by inhalation of finely atomized liquid suspension[3], or by inhalation of room air into which virus has been introduced by aerosol spray[4] However, there is no direct evidence to prove whether epidemic or pandemic influenza is spread by direct contact by large droplets, or by finely dispersed airborne droplet nuclei

Indirect evidence of airborne transmission was found by McClean[5], in an examination of a hospital unit equipped with ultra-violet irradiation The incidence of influenza was lower among patients housed in the irradiated unit than among patients housed in a non-irradiated unit

Direct experimental evidence showing that ferrets could transmit influenza virus infection by the airborne route has been given by Andrewes and Glover[6]

A model has been developed in this laboratory for the study of transmission of influenza virus infection in mice Mice are infected with influenza virus by exposure to an aerosol spray of *A*2 (Asian) virus We have found that infected mice transmit influenza virus to non-infected animals almost exclusively during the period 24–48 hr. after the initiation of their infection[7].

Twenty-four hours after initiation of their infection, mice were placed in a wire mesh cage inside a modified autoclave chamber. Non-infected mice were placed in the same cage, but were separated in two of the four experiments from the infected animals by a double mesh wire screen that assured at least a ¾-in. separation. A 24-hr. exposure period was allowed under conditions of measured air-flow through the chamber. At the end of this period the previously uninfected mice were quarantined for a 48-hr. period in separate individual cages. The lungs of these previously uninfected animals were then tested individually for the presence of virus by inoculation of a 10^{-2} dilution of ground lung suspensions into 10–13-day-old chick embryos.

The fundamental factors involved in the transmission of respiratory infections have been defined by Wells[8] as follows:

$$C = r \times I \times S$$

where C = the number of new cases, I = the number of infectors, S = the number of susceptibles, and r = the effective contact-rate.

Table 1. INCIDENCE OF TRANSMITTED INFECTION AT VARYING RATES OF AIR-FLOW

C	r	I	S	V
16	0·0727	11*	20	2·3 l./min.
12	0·0600	10*	20	2·3 l./min.
7	0·0233	6	50	6 l./min.
2	0·0067	6	50	30 l./min.

C, No. of previously uninfected animals with demonstrable pulmonary virus; r, effective contact-rate; I, No. of infectors; S, No. of susceptibles; V, air-flow through the chamber (l./min.).

* Infectors and susceptibles separated by double mesh wire screen.

It was possible in four separate experiments to determine values for each of these factors at varying rates of air-flow (V) through the chamber. As illustrated in Table 1, the effective contact-rate (r) was inversely proportional to the rate of air-flow through the chamber, the greater the air-flow, the lower the effective contact-rate. When the values for r are plotted logarithmically against those of V a nearly straight line relationship is obtained.

We believe that the profound effect of varying air-flow-rates on the rate of transmission can best be explained by the conclusion that mice transmit influenza virus infection almost exclusively by airborne droplet nuclei. Droplet nuclei, because they are dispersed in the air, must obviously be reduced in concentration as the environmental air-flow is increased. The chance of acquiring airborne infection is thus inversely related to the ventilation[8,9]. It is notable that in experiments with ferrets[6], Andrewes and Glover found that a fan providing rapid air-flow diminished the transmission of influenza virus infection. We have also found that the rate of transmission when free intermingling of infected and contact mice is permitted is not appreciably different than when the two groups are separated by a double wire screen, an observation that supports the hypothesis of airborne transmission.

Measurements of the relative humidity inside the chamber were made during the exposure period, with values for different experiments ranging from 47 per cent to 70 per cent. The effect of air-flow (V) on the effective contact-rate (r) can be corrected by multiplying the two values. When the resulting product (rV) for each experiment is plotted against the relative humidity, the curve given in Fig. 1 is obtained. There is a significant decline in the product rV (and hence in r) as the relative humidity rises from 47 to 70 per cent. Hemmes *et al.*[10] and Harper[11] have shown that the decay-rate of airborne droplet nuclei of influenza virus increased rapidly as the relative humidity was raised from 40 to 70 per cent.

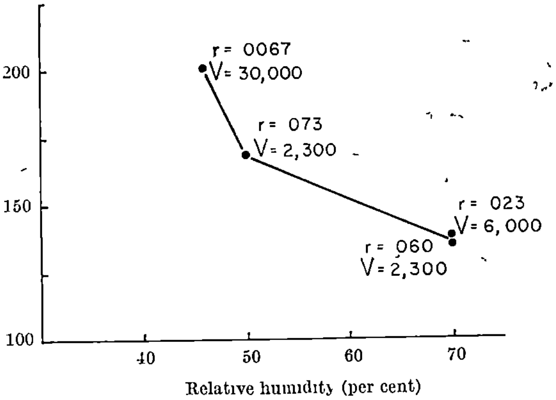

Fig. 1. Airborne transmission of influenza virus infection in mice. r, Effective transmission-rate; V, air-flow (c.c./min.)

The combined effects of increased ventilation and high relative humidity in decreasing the effective rate of transmission of influenza virus infection may, in part, explain the seasonal factors in influenza. The present investigations have demonstrated that the transmission of influenza virus infection in mice is enhanced by conditions of low relative humidity and diminished ventilation, conditions which correspond to the usual winter, indoor environment of man. However, recent preliminary studies have also suggested that seasonal factors may act directly on the mice affecting their ability to transmit influenza virus infection.

This work was carried out under the auspices of the Commission on Influenza of the Armed Forces Epidemiological Board and was supported in part by the Office of the Surgeon General and also aided by a grant from the National Tuberculosis Association—American Thoracic Society.

JEROME L. SCHULMAN
EDWIN D. KILBOURNE

Division of Virus Research,
Department of Public Health,
Cornell University Medical College,
1300 York Avenue,
New York, 21.

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FORTHCOMING EVENTS

(*Meeting marked with an asterisk * is open to the public*)

Sunday, September 16—Saturday, September 22

International Union of Pure and Applied Physics (at Queen Mary College (University of London), Mile End Road, London, E 1)—Eighth International Conference on Low Temperature Physics

Monday, September 17

Institute of Metal Finishing (at Northampton College of Technology, St John Street, London, E C 1), at 6 15 p m —Mr M Clarke 'Porosity in Electrodeposited Tin"

Monday, September 17—Wednesday, September 19

Faraday Society (in the University Laboratory of Physiology, The University, South Parks Road, Oxford)—General Discussion on "High Resolution Nuclear Magnetic Resonance"

Monday, September 17—Friday, September 21

Conchological Society of Great Britain and Ireland, and the Malacological Society of London (at the British Museum (Natural History), Cromwell Road, London, S W 7, and Queen Elizabeth College (University of London), Campden Hill Road, London, W 8)—First European Malacological Congress

Tuesday, September 18

Institute of Fuel (at the Institution of Civil Engineers, Great George Street, London, S W 1), at 5 30 p m —His Excellency E N van Kleffens "The European Communities (E E C , E C S C , and EURATOM)", Mr S Nora "Energy in the Context of the European Communities" *

Tuesday, September 18—Friday, September 21

Battersea College of Technology, Department of Metallurgy (at Battersea Park Road, London, S W 11)—Materials Summer School—"Materials for Vessels and Containers for Low Temperatures"

First International Congress of Food Science and Technology (at the Imperial College of Science and Technology, South Kensington, London, S W 7)

Thursday, September 20

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 p m —General Meeting

Friday, September 21

Biochemical Society (in the Department of Biochemistry and Chemistry, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London, E C 1), at 2 p m —Colloquium on The Teaching of Biochemistry to Medical Students"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Research Assistants (2) (with a degree in an engineering or science subject, or equivalent qualification and knowledge of two modern foreign languages, preferably with library experience) in the Science Museum Library, for duties including classifying and cataloguing scientific and technical literature and preparing bibliographies—Civil Service Commission, 17 North Audley Street, London, W 1, quoting S/5540/62 (September 20)

Head (with suitable qualifications and experience to supervise teaching and ancillary staff and 150 students following a full-time pharmacy course) of the New Zealand School of Pharmacy—The High Commissioner for New Zealand, 415 Strand, London, W C 2, and the Principal, Central Institute of Technology, P O Box 177, Patone, New Zealand (September 21)

Assistant Lecturer or Research Demonstrator in the Physics Department—The Secretary, Queen Elizabeth College, Campden Hill Road, London, W 8 (September 22)

Lecturer (with a degree in biochemistry, organic chemistry, physiology or pharmacology, postgraduate research experience of Ph D or equivalent standard essential, preferably with an interest in the study of natural products or biosynthesis) in Biochemistry in the Department of Physiology and Biochemistry—The Secretary and Registrar, The University, Southampton (September 22)

Research Fellow (with research experience in solid-state physics or physical electronics, familiarity with ultra-high vacuum techniques desirable) in a surface physics group investigating mechanism of photoelectric emission—The Secretary and Registrar, The University, Southampton (September 22)

Lecturer or Assistant Lecturer in Bacteriology—The Registrar, The University, Leeds 2 (September 24)

Physicist, Basic grade (with a first- or second-class honours degree in physics) in the Physics Department, to assist with the routine, development and research work of the Department in the application of physics to medical problems—The House Governor, The London Hospital, Whitechapel, London, E 1 (September 24)

Assistant in Applied Economics in the Department of Social and Economic Research—The Secretary of University Court, The University, Glasgow (September 25)

Director of the Institute of Education—The Secretary, The University, Exeter (September 29)

Lecturer (registered medical practitioner with previous experience of the subject) in Chemical Pathology, for duties including teaching, research and hospital diagnostic routine—The Secretary, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W C 1 (September 29)

Lecturer (preferably with a postgraduate science degree in pharmacology) in Pharmacology in the Department of Pharmacology and Therapeutics, Queen's College, Dundee—The Secretary of theUniversity of St Andrews, c/o Queen's College, Dundee (September 29)

Assistant Lecturer (with research experience in physical chemistry) in Physical Chemistry—The Secretary, Royal College of Science and Technology, Glasgow (September 30)

Lecturer (with a degree or equivalent qualification, and with appropriate teaching and industrial experience) in Electronics in the Department of Electrical Engineering, to teach electronics and allied subjects to post-H N C standard—The Principal, Reading Technical College, King's Road, Reading, Berks (September 30)

Lecturer or Senior Lecturer (non-medically qualified, preferably with interest and/or experience in immunological methods of hormone assay or hæmatological serology) in the Department of Obstetrics and Gynæcology—The Registrar, The University, Leeds 2 (September 30)

Research Assistant (university graduate, either a biologist with biophysical training or a physicist with biological interests) in the Wellcome Laboratories of Pharmacology, for investigations into the effects of hormones on the permeability of membranes—The Secretary of the University, College Gate, St Andrews (September 30)

Principal Scientific Officer or Senior Scientific Officer (honours graduate in animal husbandry or other appropriate science, with considerable research experience) in the Animal Studies Department, to work as the senior member of a team undertaking research into the nutrition of hill sheep and associated problems—The Secretary, Hill Farming Research Organization, 48 Palmerston Place, Edinburgh 12 (October 6)

Senior Assistant Librarian (graduate, with experience in librarianship, preferably in a university library)—The Registrar, The University of East Anglia, 4 Cathedral Close, Norwich, NOR16P (October 8)

Lecturer in the Department of Psychology—The Secretary, The University, Aberdeen (October 10)

Senior Laboratory Technician (M I L T or equivalent qualification), for the Ruakura Animal Research Station, New Zealand, for duties including assistance in biochemical studies of silage fermentation—The High Commissioner for New Zealand, 415 Strand, London, W C 2, quoting B 13/2/8 (October 11)

Lecturer in Philosophy at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S W 1 (October 15)

Lecturer in Physical Chemistry at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Reader (with a higher degree in electrical engineering, with experience in some aspect of electronics or communications and able to show evidence of experience in lecturing on theoretical topics to honours students and of ability to supervise and participate in research projects) in Electrical Engineering at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Research Assistant (with appropriate research experience, preferably to Ph D level) in Chemistry, for work on the oxidation of cysteine peptides, the project programme involving both kinetic and isotopic tracer studies—The Secretary, The University, Exeter (October 15)

Senior Lecturer or Lecturer (with an interest in electrical communications) in Electrical Engineering at the University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (October 15)

Senior Lecturer in Pharmacology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Senior Lecturer or Lecturer (with research and teaching experience in pharmaceutical chemistry, and preferably to have worked in the field of chemical mechanisms of drug action) in Pharmaceutical Chemistry at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Senior Lecturer in Social Anthropology at the University of Sydney, Australia, to assist in the organization of teaching and research in the Department of Anthropology and to supervise certain field projects—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Chair of Pathology at the Medical College, University College of Rhodesia and Nyasaland, Salisbury, Southern Rhodesia—The Registrar, The University of Birmingham, Edgbaston, Birmingham 15 (October 20)

Senior Lecturer or Lecturer in Biochemistry at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall London, S W 1 (October 27)

Lecturer (preferably with an interest in systematics and anatomy) in Botany in the School of General Studies, Australian National University—The Secretary Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 29)

Lecturer (with an honours degree or equivalent qualification with an interest in teaching and research in economic geography, particularly the geography of manufacturing and transport, an interest also in cartography and photo-interpretation or in the regional geography of either North America, Africa or South-East Asia would be an advantage) IN GEOGRAPHY at the Newcastle University College, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N S W, Australia (October 30)

LECTURER (honours graduate in engineering, physics or mathematics, and preferably with interest and/or experience in factory lay-out, production planning, metrology or metal forming) IN THE DEPARTMENT OF PRODUCTION ENGINEERING—The Registrar, The University, Nottingham (October 31)

ASSISTANT LECTURERS IN ORGANIC, INORGANIC, PHYSICAL or ANALYTICAL CHEMISTRY—The Registrar, The Manchester College of Science and Technology, Manchester 1

LABORATORY TECHNICIAN, to assist in research in the BIOCHEMISTRY DIVISION—The Assistant Secretary, Royal Veterinary College, Royal College Street, London, N W 1

LECTURER IN OPERATIONAL RESEARCH IN THE PRODUCTION ENGINEERING AND MANAGEMENT STUDIES SECTION, Imperial College of Science and Technology—The Dean, City and Guilds College, Exhibition Road, London, S W 7

LECTURER IN RADIATION CHEMISTRY IN THE NUCLEAR TECHNOLOGY LABORATORY, Department of Chemical Engineering and Chemical Technology—Mr G R Hall, Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S W 7

LECTURERS (with at least a second-class honours degree in either physics or mathematics) IN PHYSICS AND MATHEMATICS at the Royal Air Force Technical College, Henlow—Air Ministry, (C E 2m), London, W C 1

PEDOLOGIST (with an M Sc or Ph D degree in pedology, with at least five years experience in soil survey and classification and land classification, and able to make use of aerial photographic maps), for a technical assistance arrangement appointment to the Hashemite Kingdom of Jordan, to conduct and supervise soil survey and classification and land classification—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S W 1, quoting Ref RC 213/179/02

PHYSICAL CHEMIST, to join a research team on sodium transport—Dr Anderson, Medical Unit, King's College Hospital Medical School, London, S E 5

PHYSICIST, Basic grade (with a good degree), for routine and development work in hospital physics—The Secretary, Lambeth Hospital, Brook Drive, London, S E 11

RESEARCH ASSISTANT (graduate in agricultural chemistry, biochemistry, botany or chemistry) IN AGRICULTURAL CHEMISTRY, to assist with an investigation on aspects of the biochemistry of potato tubers affecting tuber quality—The Registrar, The University, Nottingham

RESEARCH ASSISTANT (graduate, preferably with some knowledge of statistics or mathematics), to work in the field of economics, pure and applied—The Clerk to the Faculty of Commerce and Social Science, The University, Birmingham 15

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (honours graduate, preferably with a Ph D in biochemistry, with interests in the isolation and chemistry of proteins, and in studies of enzymes) IN THE PHYSIOLOGY DEPARTMENT—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, Berks, quoting Ref 62/8

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Department of Scientific and Industrial Research Road Research 1961 The Report of the Road Research Board with the Report of the Director of Road Research Pp vi+134+8 plates (London H M Stationery Office, 1962) 8*s* 6*d* net [267

Department of Scientific and Industrial Research National Physical Laboratory Notes of Applied Science, No 27 Inspection of Gauging Dimensions Involving Linear and Angular Measurements By Dr J C Evans and C O Taylerson Pp v+42 (London H M Stationery Office, 1962) [267

Report of the Hydrographer of the Navy for the year 1961 Pp 31+1 chart (H D 523) (London Admiralty, 1962) [267

Ministry of Agriculture, Fisheries and Food Domestic Food Consumption and Expenditure 1960—Annual Report of the National Food Survey Committee Pp vii+167 (London H M Stationery Office, 1962) 12*s* 6*d* net [267

Commonwealth Mycological Institute Miscellaneous Publication No 16 Blue Mould of Tobacco—a Review of Current Information By R W Rayner and J C F Hopkins Pp 16 (Kew Commonwealth Mycological Institute, 1962) 5*s* [267

The British Non-Ferrous Metals Research Association B N F Analytical Conference Proceedings of a Conference on Analytical Methods held at Malvern, 24th–25th October, 1961 Pp 120 (London The British Non-Ferrous Metals Research Association, 1962) 50*s* [267

Horticultural Marketing Council Second Annual Report and Statement of Accounts for the year ended 31st March, 1962 Pp 19+4 plates (London Horticultural Marketing Council, 1962) [267

Department of Scientific and Industrial Research Report of the National Chemical Laboratory, 1961 Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1961 Pp iii+75+4 plates (London H M Stationery Office, 1962) 5*s* 6*d* net [307

The National Council for Technological Awards Report for the period April 1961 to March 1962 Pp 51 (London The National Council for Technological Awards, 1962) [307

College of Advanced Technology, Birmingham Principal's Report for the period September 1960—March 1962 Pp 40 (Birmingham College of Advanced Technology, 1962) [307

Ministry of Health Memorandum on Measures for the Control of Mosquito Nuisances in Great Britain Pp 38 (2 plates) (Memo 238/Med) (London H M Stationery Office, 1962) 2*s* 6*d* net [307

Other Countries

Federation of Malaya Report on Forest Administration for the year 1960 By Mohd Alwy bin Haji Suleiman Pp iv+110 (Kuala Lumpur Forest Department, 1962) 2 50 Malayan dollars [267

Memorie dell Istituto Italiano di Idrobiologica "Dott Marco de Marchi", Vol 14 Pp 595 (Verbania Pallanza Istituto Italiano di Idrobiologia, 1962) [267

FAO—WHO—OIE Animal Health Yearbook, 1961 Pp viii+315 (Rome Food and Agriculture Organization of the United Nations, London H M Stationery Office, 1962) 15*s*, 3 dollars [267

Food and Agriculture Organization of the United Nations FAO Atomic Energy Series, No 3 Dietary Levels of Strontium-90 and Cesium-137—a Summary of World Information Pp viii+78 (Rome Food and Agriculture Organization of the United Nations, London H M Stationery Office, 1962) 5*s*, 1 dollar [307

Journal of Verbal Learning and Verbal Behavior, Vol 1, No 1, (July 1962) Edited by Leo Postman Pp 78 Subscription Vol 1 (6 issues), 1962, 15 dollars (New York and London Academic Press, Inc , 1962) [307

Metropolitan Life Insurance Company Statistical Bulletin, Vol 43, June 1962 Recent Trends in Heart Disease Population Profile of Puerto Rico Frequency of Fatal Public Accidents Surgical Treatment of Retired Men Pp 12 (New York Metropolitan Life Insurance Company, 1962) [307

Corrosion Abstracts, Vol 31, No 1 Pp 67 (Stockholm Corrosion Committee of the Royal Swedish Academy of Engineering Sciences, 1962 Distributed by Almqvist and Wiksell, Stockholm) [307

Canada Department of Mines and Technical Surveys Geological Survey of Canada Bulletin No 71 Palaeomagnetism and Correlation of Keweenawan Rocks By P M Du Bois Pp xvi+76+3 plates 1 dollar Paper 61–22 Cross Lake Map-Area, Manitoba By C K Bell Pp ii+22 50 cents Paper 62–4 Illustrations of Canadian Fossils, Devonian of Western Canada Invertebrates By D J McLaren and A W Norris Plants By D C McGregor Pp i+35 (16 plates) 50 cents (Ottawa Queen's Printer, 1962) [307

World Health Organization Public Health Papers No 13 Aspects of Water Pollution Control—a Selection of Papers prepared for the Conference on Water Pollution Problems in Europe, Geneva, 1961 Pp 115 4 Swiss francs, 6*s* 8*d*, 1 25 dollars No 14 Deprivation of Maternal Care—a Reassessment of Its Effects Pp 165 7 Swiss Francs, 12*s*, 2 25 dollars Technical Report Series No 236 Planning, Organization and Administration of a National Health Laboratory Service—Third Report of the Expert Committee on Health Laboratory Services Pp 46 2 Swiss francs, 3*s* 6*d*, 0 60 dollars No 237 Requirements for Biological Substances 7 Requirements for Poliomyelitis Vaccine (Oral)—Report of a Study Group Pp 29 1 Swiss franc, 1*s* 9*d*, 0 30 dollars (Geneva World Health Organization, London H M Stationery Office, 1962) [307

Ceylon Administration Report of the Director of National Museums for 1960 (Part IV—Education, Science and Art (E)) Pp 38+2 plates Rs 1/10 A General Guide to the Colombo National Museum Edited by P E P Deraniyagala Pp 45 (10 plates) 50 cents Colombo Government Publications Bureau, 1961) [307

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Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO , LTD
ST MARTIN'S STREET, LONDON, W C 2
Telephone Number Whitehall 8831 Telegrams Phusis Lesquare London

Advertisements only should be addressed to
T G Scott & Son, Ltd , 1 Clement's Inn, London, W C 2
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ORGANIZING GOVERNMENT SERVICES FOR COMMONWEALTH COUNTRIES

In its full report for the session 1959–60 the Select Committee on Estimates, after carefully considering the arguments for and against the merging of the Colonial Office and the Commonwealth Relations Office, accepted the arguments in favour as desirable from the point of view of administrative efficiency in Whitehall and offering the best way of preserving, for the benefit of independent and dependent territories alike, knowledge and experience that might otherwise have been lost It also considered that the continued existence of two separate Departments of State to deal with the affairs of a rapidly developing and changing Commonwealth leads to a dichotomy of thought and approach that militates against the unity of the Commonwealth It suggested that the new Department, which should be formed on tripartite lines outlined by the Permanent Under-Secretary of State for the Colonies at the time, Sir Hilton Poynton, should be known as the Commonwealth Office and be administered by a single Secretary of State assisted by three or more Departmental Ministers

Although in the Government changes announced in July the Colonial Office and the Commonwealth Relations Office were placed under a single Cabinet Minister, no other effect has been given to these recommendations, although their force has been increased rather than otherwise in the subsequent two years They were criticized forcefully by the Earl of Listowel in opening a debate in the House of Lords on July 17 dealing with aid to Commonwealth countries Lord Listowel, who urged that in no field of policy was a new direction more urgently needed than in that of economic aid and support for the development of the less-developed areas, countries and dependencies in the Commonwealth, thought that the Government had taken the first opportunity of making the change, but he was very dubious as to how far the merger had gone He thought that the Colonial Office was in fact retaining its identity and that to stop short of complete integration was to show a deplorable misunderstanding of the situation, particularly in retaining both titles

Britain has no reason to be ashamed of her colonial record and the Colonial Office has a long and proud history Nevertheless, Lord Listowel is manifestly right in urging that the word should have been dropped in the new title and the office called Secretary of State for the Commonwealth, as indeed the Select Committee's recommendation implied As a consequence of lack of imagination a useful administrative change may well hinder effective co-operation in the economic field between the British Government and those of the new Commonwealth countries Lord Hastings, who concluded the debate for the Government, did indeed promise that these criticisms would be considered and also the further criticism that the Department of Technical Co-operation was too limited by Government policy Even that much response was not forthcoming when Mr P Wall raised the same question in the House of Commons debate on Commonwealth Co-operation on July 30 and suggested the Department of Technical Co-operation should become a Ministry of Overseas Aid, although in the meantime it has been announced that the Colonial Development Corporation is to extend its functions and become the Commonwealth Development Corporation

Much of the debate centred around the first year's activities of the new Department, in which it has spent some £27 million and recruited a further 855 British experts in addition to the 21,000 already serving abroad under its auspices The civil estimates for 1962–63 provide a further sum of about £18 million for the Department, and it expects to spend rather more than last year in aid altogether Lord Listowel thought that the Department should be authorized to handle financial aid also, such as long-term loans as well as grants, and that it should be headed by a Cabinet Minister The situation was urgent because in Africa British technical experts were leaving faster than they could be replaced, and he thought that the only solution was to incorporate such experts in the home Civil Service and then second them for service overseas

The extent of this wastage was rather questioned by Lord Hastings, but although he and other speakers quoted freely from the recent report from the Department of Technical Co-operation and from the annual report of the Colonial Development Corporation, little that was new emerged Lord Twining referred to the importance of the contribution which the Department of Technical Co-operation could make to strengthening the public service overseas Lord Casey again urged the value of international commodity price agreements and thought that the way was now open for practical discussion Technical aid, he suggested, brought in quicker and better results than grant-aid or loans, and his instinct would be to increase technical aid even at the expense of some grant-aid or loans He referred to the relevance of the Freedom from Hunger Campaign now being conducted in the United Kingdom which might raise up to £5 million, much of which would be used to finance and maintain agricultural institutes in the under-developed countries, while Lord Listowel had put Britain's contribution to the support of the poorer countries as some £180 million, apart from private investment

The extent of British aid to the under-developed countries was the other main aspect of the debate in which Government policy came under criticism

Lord Casey did not object to the Government placing a ceiling on the total of economic and technical aid, and agreed that we could not allow increased aid to these countries to endanger our balance of payments Nevertheless, the situation is urgent, at least as much in Asian as in African countries, and, as already noted, if it were necessary to limit the financial assistance, it is the more important that Britain put the utmost effort into technical assistance, and that obviously means giving the utmost support to the Department of Technical Co-operation

Lord Walston was more critical of the Government precisely because he doubted the sincerity of its support for that Department and whether the recent rearrangement meant any real change in policy He did not think that Britain's record of financial assistance compared favourably with that of some other countries, such as the United States or France, and he urged that it was entirely in Britain's own interest to do much more to help the new nations, especially those which show that they are ready to make sacrifices to help themselves It was, next to disarmament, the most vital problem at present confronting Britain

On the magnitude of Britain's contribution, Lord Hastings put Government investments by grant and loan in the Commonwealth and Colonial territories in 1961–62 at £141 million and the overall investment overseas, including private finance, at more than £300 million, or 1 3 per cent of the national income This, however, is not aid in the sense of the recommendation of the report of the Secretary-General of the United Nations which called for 1 per cent annually of the national income of the wealthier countries during the development decade 1960–70 With the vote for the Department of Technical Co-operation under a tenth of this figure, Britain has obviously some way to go before she can expect to escape criticism

What was most disappointing about the debate was that it drew from the Government no indication of any sense of urgency or of opportunity or any sign that imaginative thinking and constructive vigorous policy lie behind recent changes There is, for example, no evidence that the frustration at present confronting the Colonial Development Corporation is viewed with any concern or that the necessary action to remove the hindrances to its operation in the independent territories is being taken with vigour and foresight Here a change of title might be pedantic, but that cannot reasonably be argued of the merger of the Colonial Office and the Commonwealth Relations Office To retain the term 'Colonial' at the present time is impercipient, to say the least, and, on the other hand, the use of the word 'Relations' becomes inappropriate and inaccurate in the new setting The Government has only itself to thank if, on present evidence, it is charged with missing a great opportunity and with failure to measure up to the imaginative measures which the new situation demands, particularly when the way has been carefully sign-posted by the Estimates Committee.

The charge is the more credible in the light of the treatment which the Department of Technical Co-operation has so far received and of the absence of any indication of the future of overseas research The vital importance of an adequate research organization in the emergent nations has been repeatedly stressed during recent months, quite apart from Britain's own particular responsibilities and the uncertainty surrounding the future of the Overseas Research Council One such paper was that on the organization and orientation of applied research in under-developed areas which Dr W H R Lumsden, director of the East African Trypanosomiasis Research Organization, presented last autumn at the South Pacific Science Congress of the Pacific Science Association at the University of Hawaii (see p 1139 of this issue)

Dr Lumsden bases his discussion on his own experience in East Africa since 1947 and seeks to bring out the factors of chief importance and to define the main principles which should determine the organization of applied research in these under-developed areas He points out that while it will be comparatively easy to find support for research specifically and narrowly limited to practical problems, it may be necessary to make special arrangements for research, in which, while directed to the solution of practical problems, the approaches likely to be successful are yet undefined and the use of resources or time is not accurately predictable Much of the first type of research will be essential in the development of a backward country, but limitation of research to that type alone would be unfortunate and the two should be regarded as complementary, providing invaluable mutual stimulants

Dr Lumsden regards the arrangement of good communications as the most vital necessity in planning research in under-developed areas, especially in the scientific field, not only to maintain the impetus of research by continual reappraisal of beliefs and plans, but also in the administrative field so as to facilitate the recruitment of research and technical staff, their administrative support and the supply of materials and essential services Further, he suggests that units should be fairly large and include a variety of disciplines, and he notes that when internal subdivision of supervision is necessary the means of communication should be adequate to take care of problems of mutual understanding The administrative and financial arrangements should be as simple as possible, and since ultimately in these territories applied research will necessarily be carried out by local scientists, early attention should be given to the training and recruitment of suitable local graduates

Once again, the value of linking research institutions with universities is emphasized, both in this context of assisting local recruitment and as contributing to the stimulation of research and the attraction of research workers generally Nor does Dr Lumsden ignore the problem of retaining and recruiting sufficient expatriate workers until the higher educational institutions in the emergent countries are able to ensure a sufficient and continuous inflow of local graduates This is indeed, at present, the main problem, and it is political rather than economic in view of the political instability which often accompanies development

Whether the proposals outlined by the Department of Technical Co-operation in its White Paper on future policy in recruitment for service overseas will prove sufficient, or whether it is necessary to take such a further step as Lord Listowel suggested and incorporate such experts in the Home Civil Service, Dr Lumsden has indicated a field in which the Overseas Research Council should be able to do much useful work Moreover, a number of his suggestions are entirely in line with the thinking of the Zuckerman Report on the management and control of research and development, to the implementation of which the Government is already committed What is vital is that adequate and effective action should be taken early and, although the prime responsibility rests with the Government, the help of professional organizations will also be essential

It is remarkable that while Dr Lumsden nowhere mentions the Department of Technical Co-operation or the Overseas Research Council, his paper points most emphatically to the opportunities which confront the Overseas Research Council if the Department of Technical Co-operation is allowed to use it wisely and effectively The Council is clearly the appropriate instrument to handle some of the problems of communication and of the location of research institutions which were stressed by Dr Lumsden, and it might also contribute to easing the difficulties of recruitment The Council must be given the necessary status, and this seems to be recognized already by the Department of Technical Co-operation though there is no other evidence that the Council is a recognized instrument of the Government or that it possesses its own resources

This in turn means that the Department of Technical Co-operation must itself receive full financial support and that changes in the Government offices should be such as to strengthen, and not to weaken, its authority and status This applies not merely to the Colonial Office and to the Commonwealth Relations Office, though it is essential that Mr Dennis Vosper should be able to count on the understanding and co-operation of Mr Duncan Sandys It is no less important that he should be able to rely on the support of the Minister of Education and the Minister for Science All these, except Mr Vosper himself, are full Cabinet Ministers, and if the Government is really in earnest over technical aid, no step would do more to convince the Nation of its sincerity than to give the Minister in charge of technical co-operation cabinet status with full authority to vitalize the Overseas Research Council and use it effectively, and at the same time consolidate effectively the merger of the Colonial Office and the Commonwealth Relations Office with a title appropriate to the times

COMBINING COMPARATIVE ANATOMY WITH EMBRYOLOGY

Morphogenesis of the Vertebrates

Integrates into a single unit the usually separate considerations of comparative vertebrate anatomy and vertebrate embryology By Dr Theodore W Torrey Pp viii + 600 (London and New York John Wiley and Sons, 1962) 75*s*

THE student of zoology or medicine nowadays needs to consult far more numerous, larger and more expensive books than his predecessors fifty years ago He also has to cover in a smaller and more preliminary part of his course the basic knowledge of human or comparative anatomy and embryology which used to be the entire concern of students in their first two or three years at College It is these two changes of circumstance that Prof Torrey hopes to provide for in a conveniently compact book bringing together, in easily digestible form, the essentials of both vertebrate anatomy and embryology As a stimulating introductory course, this is an undoubted success the subject-matter is well chosen and vigorously presented, with a useful selection of references at the end of each chapter, including many recent reviews All the same, it is doubtful if the text is really digestible, either to the initiated or the uninitiated reader A somewhat colloquial style is adopted, derived no doubt from the spoken words of a lecture course But the slight exaggerations or vaguenesses that are allowable as a manner of speaking, because they can always be qualified or explained afterwards to questioners, are not acceptable in print in a scientific text-book, particularly in one designed for the student who may not have time for the further reading that would correct inaccurate first impressions Torrey's own students, reading this book as a supplement to what one cannot doubt would be a most stimulating and explanatory course of lectures, would certainly emerge well equipped The rest of the world cannot romp through his text with quite the same careless rapture, however, but must take cautious stock of his remarks

It is not easy for one author in one book to do justice both to the dynamic, experimental point of view of present-day embryologists and to the onlooker-antiquarian outlook of the devoted comparative anatomist Experts from either camp will find their point of view inadequately represented Embryologists will feel that Prof Torrey deals too summarily with much experimental work, particularly that on the early embryo, though some of his small-print supplements in chapters on organogeny are excellent A great loss is the lack of mention of experimenters' names though students cannot be expected to memorize these, they should be made to appreciate that any scientific subject is still developing and that well-known individuals are still actively finding out the information that later appears in books This is a great incentive to criticism, in place of the school-child's ready acceptance of facts

Reading the book one has moments of delight at the clarity and beauty of the illustrations, as well as miscellaneous small moments of discomfort over the text The histological illustrations are very good, but electron microscope work is largely ignored an unfortunate omission in the chapters on germ cells, fertilization and muscle The early chapters are excellently begun, with basic principles clearly expressed, but small inaccuracies shake one's confidence later on, such as the not exclusively Chordate character, metamerism, being mentioned as if it did not also occur in invertebrates, and pharyngeal 'clefts' also being listed although they scarcely occur in mammals. The "Preview of Embryogeny", a most helpful preliminary, has only one oddity in the otherwise pleasing sterograms: the foliate shape given to notochord cells, such as is never in fact seen, in life or death. The chapter on cleavage and gastrulation, after some splendidly clear first points, includes one or two dangerous colloquialisms such as that the yolk "makes its influence felt" on cleavage (how often do we have to point out to our own students the fallacy of this favourite text-book dogma?) On the whole, though, this and more particularly the following chapter on early avian development are good and clear, successfully embodying important recent findings, and ending with a well-balanced selection of references.

To describe, next, human development in preference to that of any other mammal is a refreshing innovation, well guarded by Prof. Torrey's emphasis that the human being is not representative of mammals generally. He also, fortunately, digresses to devote much of this chapter to an outline comparison of extra-embryonic membranes in various vertebrates. No other recent book has condensed the same information so lucidly.

In Part 3, "Morphogenesis of Organ Systems", major emphasis is laid on finished structure, and disappointingly little space is devoted to details of morphogenesis. Sometimes, one or two additional sentences, or a series of small diagrams instead of one large one, would remedy this defect. For example, in the chapter on skin two or three diagrams of developing sweat glands could have been added: this might also have precluded the misleading statement that they closely resemble developing mammary glands. In the same chapter too little justice seems done to work on the development of feathers. Reference to Rawles's review (*Biology of Birds*, 1960) could remedy this. Prof. Torrey is, of course, up against the difficulty that students reading his book presumably will not know any comparative anatomy from other sources. It is even doubtful if they could gain enough clear information from some parts of his later chapters, particularly on the development of more complex organ systems. Torrey has decided, allowably, to describe in detail only the mammalian condition, but then it is necessary either to omit the too sketchy references to other forms, or to fit the mammal clearly into a comparative vertebrate series first. One is lost when he leaves out the mammal from each of his series of comparative diagrams: for example, of cœlom divisions, urinogenital system, arteries, and veins. He goes on to illustrate the mammalian condition in detail, but one does need first a diagram in the same style as previous ones in order to see the mammal in its proper sequence. Admittedly, this part of the book is aimed more at the medical student than the comparative zoologist: for example, in its detailed description of human musculature. But medical teachers may be worried, too, by remarks like the optimistic suggestion that we understand the functions of cardiac jelly, and the simplified description of interventricular septum development, omitting all reference to the septum membranaceum, which is an important site of developmental defects of the heart.

The best balance between comparative description and references to present-day research is achieved in the chapter on the urinogenital system, as one might expect since this is the author's own research field. He reveals also his special interest in sense organs, in introducing them from the Protozoa upwards (yet why not choose Ciliates, with their delightful 'organelles', as examples in preference to *Amœba*?). A slight incongruity is the description of eye structure before its development: also the discussion of neural induction might well have come earlier in the book. Incidentally, it is surprising that he does not refer to Yamada's and Toivonen's recent work in this field (well reviewed by Yamada, 1961, in *Advances in Morphogenesis*).

The glossary, complete with derivations of words, is a valuable addition to the book and will increase the student's understanding of the subject. This is indeed a book well worth study, as it shows to the teacher as well as the student how hard it is to embody these two approaches in one course, and how essential it is to try to do so.

E. M. DEUCHAR

PLANNING THE WAY FOR UNIVERSITY EXPANSION

The Expanding University

A Report. By W. R. Niblett. Incorporating papers by Sir Charles Morris, Lord James of Rusholme, J. S. Fulton, D. G. Christopherson and R. M. Ogilvie. Pp. 132. (London: Faber and Faber, Ltd., 1962.) 18*s*. net.

THIS is a stimulating book; it is the outcome of a conference held by the University Teachers' Group in Oxford in October 1961, and it is built around a series of papers, which are given in full, by those leading university figures—Sir Charles Morris, Lord James of Rusholme, Mr. J. S. Fulton and Dr. D. G. Christopherson—who are playing an outstanding part in the shaping of Britain's existing and new universities. It includes also a most welcome and important paper by one of the new generation of academics, R. M. Ogilvie, on "The Role of the Teacher". It is sometimes difficult for those who were deeply involved in the universities before the Second World War to appreciate the changes in outlook of the post-war generations and, if only on this account, Mr. Ogilvie's paper is especially valuable. These individual papers are held together by summaries of the discussions which followed them and the whole has been admirably moulded by Prof. Niblett, dean of the University of London Institute of Education. It must have been a lively conference, and this is clearly reflected in the book.

There is a danger to-day that all thought on what universities should be and do ought to be left to the Robbins Committee; the appointment of a Royal Commission or its more modern counterpart is so often taken to be a sign for academic hypo-thermia. Nothing could be more unfortunate at this time. The Robbins Committee has been asked to deal with the pattern of higher education, and while it may well be that the universities will find themselves with stronger, and even new, partners in this field, it is most improbable that the Robbins Committee will recommend for the universities a smaller or less-responsible role than they fulfil to-day. Moreover, the pattern for the next ten years is already fairly well set by the Government's recent decisions on universities, colleges of advanced technology and the teacher training colleges This is not, therefore, the time to sit back waiting for Robbins, if the next generations of students are to get a fair deal

This book is also therefore timely While making a wide survey of the universities' academic and social problems of to-day, emphasis lies on what the universities could do better than they do now for the undergraduates The increasing impact of research and postgraduate education is not overlooked but discussion centres on the welfare of the undergraduates, including aspects of their life other than mere class-room and laboratory education This is perhaps brought out most clearly in Mr Ogilvie's paper He claims that the new student lacks interest and drive, but whose fault is this? He then singles out three qualities with which students should be equipped when they leave the university—individualism, interest and integrity He himself admits that these are not new ideas, but it is certain that they need restating to-day

One of the real dilemmas facing the universities is the dual claims of teaching and research The demands for more research, particularly in the pure and applied sciences, are increasing every day Unless the universities are put in a position to recruit and retain academic staff, the claims of research can be met only at the expense of the undergraduate It must have been fear of this disequilibrium that led the University Grants Committee to set up the Hale Committee to study undergraduate teaching methods and practices current in the universities

This book makes it clear that the universities are well aware of the academic problems arising from expansion and, given adequate resources, that they have the will to solve them It could not have arrived at a more opportune time, discussion now is the means of avoiding many of the real dangers of expansion

VITAMIN B_{12}

Vitamin B_{12} und Intrinsic Factor

Zweites Europaisches Symposium, Hamburg, 2–5 August 1961 Herausgegeben von Dr H C Heinrich. Pp xx+798 (Stuttgart Ferdinand Enke Verlag, 1962) 142 DM

SINCE the isolation of vitamin B_{12} in 1948 by Lester Smith in England and by Folkers and his co-workers in the Merck Laboratories in the United States two symposia have been held, both in Hamburg, to discuss the many aspects of interest in the vitamin The first symposium was held in 1956 at a time when the structure of the vitamin had just been established, in the main by the brilliant X-ray work of Mrs Hodgkin (Dr Dorothy Crowfoot) and her associates in Oxford The highlight of the second symposium (recorded in little more than four pages in the volume under review) is the disclosure of the structure of the vitamin B_{12}-containing coenzyme It is a cobalt-organo-metallic compound containing a cobalt–carbon bond derived in a formal sense from vitamin B_{12} by the displacement of the cyano-group by the 5′-methylene-group of 5′-deoxy-adenosine (the valence state of the cobalt in the coenzyme is in some doubt) This dramatic and startling discovery is again to be attributed to Mrs Hodgkin and her collaborators The other important contribution, that of H A Barker, from the Department of Biochemistry, Berkeley, California, concerns the observations (made mainly in his laboratory) of the remarkable biochemical reaction of vitamin B_{12} in converting L-glutamic acid to L-β-methyl aspartic acid Barker's paper is also a gem However, it is a pity that, in the volume as printed, speculations in Barker's paper concerning the constitution of the coenzyme are allowed to remain when, in the succeeding paper, Mrs Hodgkin details its structure It is left to the reader to refer back to the preceding paper by Barker and to make his own rationalizations

The volume under review contains eighty-one papers (from the seventy-two contributors), sixty-one of which are in English and twenty are in German Dr Heinrich, of the Physiological-Chemical Institute in Hamburg, has attempted the herculean task of editing these contributions, and he is to be congratulated on his effort

The contributions range from Prof A W Johnson's succinct discussion of attempts to synthesize the vitamin (or, at least, the fundamental heterocyclic system, corrin, of which vitamin B_{12}, cyanocobalamin, is a derivative) to aspects of the pathology of and the therapy of vitamin-B_{12} deficiency

It is clear that no editor can command a critical knowledge of so diverse fields, and the contributions quite naturally vary in quality Moreover, with contributors from Bethesda and Budapest, Stockholm and Sofia, it is not surprising to find an article on the analytical chemistry of the corrinoids written in English by a Polish biochemist and edited by a German physiologist Needless to say, one admires the attempt, but can only deprecate the results, with the attendant errors of mis-spelling and mis-interpretation On page 291, for example, there are at least seven spelling mistakes, none of them serious if one knows the field However, in Friedrich's article on the partial synthesis of B_{12}-type vitamins (pp 8–28) it is quite obvious that the author would never have embarked on the particular course he chose had he been aware of the now classical work of Winstein, in California, extending over the past twenty years, on neighbouring group participation

The volume is made up of eleven sections, ranging from the chemical through the biochemical and physiological to problems of nomenclature The quality of the production is excellent although the opening pages of photographs of selected participants seem ill-placed and irrelevant

One feels that the symposium was eminently worth while What must be asked, however, is whether the proceedings should have been published, particularly in their present form They are not of general interest Moreover, the book is inordinately expensive It contains no record of discussion, and it is plagued with errors, not only in spelling, but also in the formulation of ideas

It is hoped that the success of the second symposium (as stated in the preface to the present volume) will encourage a third such symposium, but I can only suggest that the proceedings are not published—at least in the present form The participants should be encouraged to publish in the accepted media of scientific intercourse and exchange

The title of the book mentions "intrinsic factor", but only one of the eleven sections is devoted to this topic and it ends with a contribution entitled "Does Intrinsic Factor Exist?"

The book takes as its leitmotive the quotation from Fanny Rosenblum that "B_{12} is not a vitamin, it is a fraternity" Considering the contributions of Mrs Hodgkin and her co-workers, one is tempted to ask if B_{12} is not better described as a sorority

V M Clark

The Genus Monadenium, with Descriptions of 21 New Species

By Peter R O Bally Pp 111+34 photographic figures+32 plates (Berne Benteli-Verlag, 1961) n p

THE genus *Monadenium* is a close relative of *Euphorbia*, the spurge genus, differing in the structure of the inflorescence It is altogether confined to tropical Africa, and almost restricted to East Africa Although, with some exceptions, the species are not distinguished for their ornamental qualities, their life-history is full of interest, particularly in the way that their structure has become adapted for the evasion or endurance of a rigorous climate Their habit ranges from herbs an inch or so high with underground storage-organs to succulent-stemmed trees of 18 ft

So happy a union of botanical skill and artistic excellence as that shown by Mr Bally is but rarely found The result is an authoritative taxonomic revision of the forty-seven species of *Monadenium* (twenty of which were first described by Mr Bally himself), coupled with 32 splendid plates, the majority coloured, from the author's talented brush The usual herbarium specimens of this genus are so deplorable that plates of this sort, made from living plants, become not only ornamental but also a necessity

Indeed, this revision indicates very strongly the value of a knowledge of the living plants in their native home in producing good systematic accounts of difficult genera which respond poorly to the normal methods used for preparing specimens for the herbarium

In spite of the sub-title, which has an odd contradiction between the dust-cover and title-page (18 new species on the former, 21 on the latter), only three of Mr Bally's species are described here actually for the first time. J P M BRENAN

The Pattern of Progressive Mineralisation in Human Dental Enamel

By Dr H S M Crabb and Dr A I Darling (International Series of Monographs on Oral Biology, Vol 2) Pp xi+99 (London and New York Pergamon Press, 1962) 35*s* , 5 50 dollars

THE nature and mode of development of dental enamel have engaged the attention of histologists and other workers for many years, but owing to the relative difficulty of working with this tissue it has not received so much attention as have most other tissues of the body Special techniques have also been more important in the microscopy of enamel than in the case of other tissues, and thus much of the evidence on which our present theories of the development and mineralization of enamel are based depend on microradiography and polarized light microscopy as well as on information derived from studies of decalcified material by ordinary transmitted light

Interpretation of the findings yielded by the various techniques have in the past shown considerable variation, and as a result opposing and contradictory theories of the pattern of enamel mineralization have been current Workers in this field will, therefore, welcome this authoritative monograph, which gives a clear and succinct account of recent research on enamel mineralization The authors discuss and criticize the older theories and put forward their own conclusions, which agree very closely with those recently obtained by other groups of workers It seems clear that the older views of enamel maturation cannot sustain critical re-examination, and that ideas much closer to those now put forward will take their place R B LUCAS

The Preservation of Timber

By Dr W P K Findlay Pp xiii+162+14 plates (London Adam and Charles Black, 1962) 25*s* net

MUCH has been written about the preservation of timber, but Dr Findlay has succeeded in putting into one book, and in easily understandable form, the information on the subject required by architects, builders, householders, farmers and a host of others The reader is given the necessary basic essentials of the subject in chapters on "The Nature and Durability of Wood", "Agencies of Destruction", "Preservative Methods" and "Wood Preservatives" Non-pressure, pressure and diffusion treatments are described, and the preservatives are also classified into the three main groups of tar-oil derivatives, water-borne chemicals and chemicals in organic solvents The advantages and disadvantages of each kind and the methods of application are discussed. Most of the succeeding chapters deal with wood preservation for specific requirements such as building timber, woodwork in garden and farm, timber in constant contact with water and the large category of poles, sleepers and mining timber The preservation of wooden antiques is also considered Fire-retarding methods are described and the author ends with well-chosen examples demonstrating some economic aspects of wood preservation

Dr Findlay has dealt with his subject in a concise and very masterly manner His book provides the information to enable the most suitable preservative to be chosen for a particular purpose and to be used to the best advantage In an appendix is a list of the preservatives generally available in Great Britain

All users of timber and timber products will welcome this informative and very well-illustrated book C J TAYLOR

Reports on Astronomy

Edited by D H Sadler (Transactions of the International Astronomical Union, Vol 11A—Reports) Pp viii+579 (London Academic Press, Inc (London), Ltd , New York Academic Press, Inc , 1962) 80*s* net , 11 50 dollars

THIS volume of *Transactions*, which has appeared in less than a year after the 1961 meeting of the International Astronomical Union, contains the draft reports of the Commissions as presented to the General Assembly and later amended As usual these reports review the work done in each field covered by the Commissions since the preceding meeting, together with an extensive bibliography The reports of the Commissions on Notation, Bibliography, Telegrams and Exchange of Astronomers have not been published in this volume These four reports will be published in Transactions 11 *B* in view of their administrative character

Volume 11 *B*, to be published later, will contain the proceedings at the 1961 General Assembly, the resolutions adopted, the invited discourses and other business of the Union This new policy of publishing separately the reports of the Commissions and the proceedings will be greatly welcomed since the rapid publication of the reports enhances their value as a reference work D MCNALLY

ORGANIZATION AND ORIENTATION OF APPLIED RESEARCH IN UNDER-DEVELOPED AREAS*

By Dr W. H. R. LUMSDEN

Director, East African Trypanosomiasis Research Organization, Tororo, Uganda

THE discussion regarding the organization and orientation of applied research in under-developed areas which is here presented is based on my own experience in this sphere of research in East Africa since 1947, and, particularly, as director of the East African Trypanosomiasis Research Organization since 1957 The factors important in relation to the organization and orientation of applied research are many and miscellaneous and so are difficult to summarize It seems most suitable to describe the subject, as it appears in my East African experience, under three headings (1) the scope of applied research, (2) the development of trypanosomiasis research, (3) general research development, 1947–61 —so as to exemplify and discuss points of importance, and then to attempt to define main principles

Scope of Applied Research in East Africa

If 'basic' research may be defined as research unrelated to any immediately obvious practical application, then practically all the biological research which has been done in East Africa, except for recent research in the University of East Africa, may be described as 'applied' in that its financial support has been derived from the existence of obvious practical medical or agricultural problems Examples are

(a) Trypanosomiasis research, related to sleeping sickness, still mainly limited in its impact on man only by leaving unoccupied the areas known to be infected, and to nagana, which still denies huge areas of Africa to cattle

(b) Virus research, related mainly to arthropod-borne virus diseases, even though yellow fever is restricted in importance by the application of vaccination, other virus diseases, such as chikungunya, are still liable to appear, causing extensive epidemics and temporarily incapacitating large populations

(c) Agricultural research dealing with a multitude of problems—the effects of soil characteristics and climate on crop growth, crop selection and improvement, methods of animal husbandry, the prevention of disease of livestock, and so on

Although all this research may be designated as 'applied' there are wide differences in scope between different investigations and between the programmes of different laboratories It is impossible rigidly to classify research, but as regards East Africa it will be convenient to recognize two main types of approach differing in scope

Type A Research specifically and narrowly limited to the practical problem in question Such research will usually involve the application of known techniques, and will usually be fairly accurately predictable as regards the time and resources needed to fulfil it An example might be the choice of a strain of crop suited to the climate of a given locality.

Type B Research, still directed towards the solution of practical problems, but in which the approaches likely to be successful are yet undefined Such research will not be accurately predictable either as regards the use of resources or time, and freedom must be accorded to the workers concerned in it so that they may explore any avenues which appear promising to them An example might be the development of laboratory methods for separating *Trypanosoma rhodesiense* from the morphologically similar, but non-man-infecting, *T. brucei* and for diagnosing infections with these organisms in wild or domestic animals; methods for these purposes can only be developed by trial and error of indefinite duration

Clearly, a great deal of type *A* research will be essential in the development of an emergent country but limitation of research to that type alone, which is a point of view often canvassed, has its pitfalls The results of investigations with this limitation, needed for immediate practical purposes, will usually be embodied in reports which may have only a restricted circulation, and so will not necessarily be subjected to the independent critical assessment which is one of the main functions of formal scientific publication Most type *B* research will be carried out with the view, ultimately, of publication, and so from type *B* workers may be expected to come the periodical challenge of beliefs which is essential if practices are to be thoroughly soundly based

So, for developing countries, both types of research scope are necessary, they should be regarded as complementary to each other, as the mutual stimulations between them are invaluable There exists, however, the possibility of frictions arising between the workers engaged in the two types Such difficulties will sometimes be the manifestation of competition for limited funds for research Money for a research project will, in countries with small financial resources, be the more easily forthcoming the earlier any economic or public health advantage appears likely to ensue Thus, type *A* research will be comparatively the easier to find support for and special arrangements may be necessary for the other type. Desirably, the support and supervision of type *B* research should be invested in some council of good scientific standing which can assess and balance without bias the importance of various competing points of view and which can protect type *B* research from the too importunate demands for immediate action on special aspects which may dislocate planned programmes Such an authority should be politically independent, difficulties in collaboration and co-operation may arise if bodies politically different, and therefore perhaps competing for funds, support different types of research within the same general field

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* Substance of a paper presented to the Tenth Pacific Science Congress of the Pacific Science Association, held in the University of Hawaii during August 21–September 6, 1961, and sponsored by the U S National Academy of Sciences, Bernice Pauahi Bishop Museum and the University of Hawaii (See also p 1133 of this issue)

In under-developed areas also, distance and poor communications may make contact between workers infrequent and so, sometimes, lead to misunderstandings and unproductive rivalries between workers engaged in the two general types of applied research Probably the most fruitful liaisons between research workers, and with the executive technical workers engaged in the practical applications of research, is by informal reciprocal visits But emergent Governments, anxious to ensure that they derive value for the money they expend on research, may demand more formal arrangements such as committees and seminars at which all types of worker may meet

Development of Trypanosomiasis Research in East Africa

The devastating sleeping sickness epidemics in the Lake Victoria basin early in the century stimulated work which established the basic facts of the ætiology and transmission of the trypanosomiases, and a great deal of well-balanced work, taking into account many aspects of the diseases, continued up until the 1920's A change in emphasis occurred in the 1920's and research became largely concentrated on one single aspect of trypanosomiasis—the ecology of tsetse flies (*Glossina*) The reasons for the decision to concentrate on this aspect, which was mainly due to C F M Swynnerton, appear to have been that, lacking efficient drugs for treatment or prophylaxis, attack on the insect vector appeared to offer the only hope for the eradication of the trypanosome diseases, that with the techniques then available, investigations of the interrelations of host and parasite—on immunity and 'tolerance'—gave only disappointing results, that much of the general interest in the field was by German scientists who withdrew when Germany lost her East African territory

Concentration of the main research effort on the ecology of *Glossina* continued practically unbroken until comparatively recently Only in the past few years has a return taken place to a more comprehensive approach in which the contributions of several different scientific disciplines are integrated The main considerations determining this recent readjustment were

In the event, the long-drawn-out concentration of research on the ecology of *Glossina* gave only disappointing results as regards its main aim—the development of economical and reliable methods for eradicating these flies as a single 'key' measure to prevent the diseases they transmit The great zoological interest of the work of Swynnerton and his successors must be recognized, for example, the studies of Jackson on methods of estimation of wild populations are of fundamental importance It is equally necessary, however, to recognize that the great body of work on tsetse biology has influenced practical control measures comparatively little Bush clearing and game destruction are still in use as main control measures with little fundamental change in the way that they are applied The most considerable change in methods of tsetse control or eradication in recent years has been the introduction of insecticides, that is, a change not primarily derived from ecological research on *Glossina* but from commercial chemical research, and even this development may leave the purely vector control approach with intractable problems of preventing re-infestation, after insecticidal application, either by re-invasion or by the expansion of relict populations

Secondly, the range and efficiency of drugs for the cure or prophylaxis of trypanosomiasis both in man and animals have enormously improved in the last forty years In particular, the introduction in the 1940's of drugs promising prolonged prophylaxis in cattle raised hopes of productive pastoral industries, at least in areas of low tsetse density, without the prior eradication of tsetse flies, even though these drugs were not without their attendant complications in use, such as the appearance of trypanosome strains resistant to them

When the trypanosomiasis problem was considered in the light of these two main arguments it was evident that there was need for better understanding of host–parasite relations and of the ecology of the diseases in Nature—of the mechanisms of mammalian immunity, or adjustment, to trypanosome infections, of interactions between these mechanisms and the effects of drugs, and of the ways in which particular trypanosome species survive and are transmitted in Nature Thus, present research is mainly concerned with problems which cover a wide front and require the concurrent contribution of several different disciplines for progress to be made Examples are the yet unexplained discontinuity of the distribution of *Trypanosoma rhodesiense* sleeping sickness within the distribution of those species of *Glossina* which are believed to be its main vectors, and the need to measure, in relation to the planning of régimes of drug prophylaxis, the degree to which cattle are exposed to infection in Nature—the trypanosome 'risk' Such problems demand the collection of information regarding the habits of the men or animals becoming infected, the re-orientation of entomological studies to provide information on the periodicity and spatial distribution of vector attack on man and animals, and the development of laboratory methods for the recognition of trypanosome species and infected hosts by immunological methods and for the measurement of certain of the important biological characteristics of trypanosomes, such as infectivity and drug resistance

The foregoing résumé of the development of trypanosomiasis research in East Africa illustrates the importance of a wide comprehension of the mechanisms of transmission of a disease if its prevention is to be logically and efficiently developed, undue preoccupation with a single 'key' measure may prove disappointing. It illustrates, also, the importance of the frequent questioning of research trends so that the momentum of research may be maintained by continually adapting to the changing situation Such reappraisal may not be automatic in research fields which tend to isolation from others, as did African trypanosomiasis because of the limited geographical distribution of its vectors Unless such reappraisal can be accomplished, research direction may be dominated by groups of workers of restricted experience who may not appreciate the possibilities of other approaches—or of combined approaches A first need, then, is to avoid the isolation of individual workers, or of groups of workers of a single discipline —situations which are peculiarly likely to arise in under-developed countries For them to continue actively original and self-appraising, research units should desirably not be too small (at least six workers) and should include a variety of disciplines Also, consideration may be given to arranging interchange of workers with other, related, fields from time to time and for reviews of the field by independent scientists of high standing, the reviews made by Buxton[1], Davey[2] and Hornby[3] have been invaluable in planning the recent re-organization of trypanosomiasis research

General Research Developments in East Africa (1947–61)

In the late 1940's the present research organizations of East Africa, in both medical and agricultural fields, were in the process of establishment In those days workers were fairly easily available, but there were scarcities of buildings, equipment and money Communications were little developed, and little difference in suitability as sites for research institutes appeared to exist between one place and another Thus, in the siting of research institutes, although isolation was sometimes recognized as to be avoided, availability of buildings and easy access to the field were more often determining factors Sometimes political factors were dominant

The perspective in 1961 was very different, development of communications had improved enormously the suitability of some sites while others remained practically unaffected In cities such as Nairobi and Kampala the facilities scarcely differ from those of a provincial university town in a temperate country, laboratories sited in such cities, or near them on main lines of communication, now contrast noticeably with those more isolated

From good siting much else with regard to organization and orientation appears to flow A research institute sited in good communication with a major centre of population will be much easier to run because of the availability of skills for the maintenance of general services and scientific equipment The better scientific contact also available in or near such centres is important for avoiding the intellectual stagnation which may overtake more isolated institutes Continual scientific contact is more generally important than continual access to the field so long as communications are good enough that access to the field is reasonably easy when it is required, as they are now, widely, in East Africa Siting is also important with regard to the orientation of research, orientation depends largely on securing workers interested and skilled in the particular kinds of investigation required For reasons of general amenity, as well as of good scientific contact, there will usually be a larger 'field' of applicants, from which suitable workers may be selected, for posts in institutes in good communication

Conclusions

The main thesis of this article is that the most vital necessity in planning research in under-developed areas is to arrange good communication, most importantly in the scientific field, to maintain the impetus of research by continual re-appraisal of beliefs and plans but also in the administrative field to facilitate the 'logistics' of research—the recruitment of research and technical staff, their administrative support, and the supply of materials and essential services Examples of the importance of good communication are cited with relation to (*a*) The maintenance of productive and stimulating relations between the workers engaged in different scopes of applied research and between them and executive technical workers (*b*) The maintenance of the impetus of research by frequent re-appraisal of beliefs and research plans by good communication both among the workers engaged in any particular field and between them and those engaged in different, but related, fields (*c*) For the attraction and selection of suitable workers (*d*) For general administrative reasons

There are a few other points regarding communication which require mention The desirability of units being fairly large and including a variety of disciplines has been mentioned It is difficult, however, for a single senior scientist to keep close scientific contact with more than a limited number, probably four or five, more junior workers Thus in units larger than this size some internal subdivision of supervision will be desirable So that reviews of progress and decisions as to the amendment, re-inforcement or abandonment of plans may be made rationally at suitable intervals, designation of certain areas of the work as named 'projects' may be necessary With regard to communication with another, non-scientific, cadre concerned in applied research—government administration—problems of reciprocal lack of understanding of points of view between scientists and administrators, not absent in more advanced countries, may arise Basically, administrative and financial arrangements should be as simple as possible

This article has been written on a basis of the research workers in an under-developed country being expatriates from more advanced lands, and this will typically be the initial situation in emergent countries Ultimately, however, applied research in these lands will necessarily be carried out by local scientists, and early attention should be given to the training and attraction into research of suitable local graduates A main problem will be retention and recruitment of sufficient expatriate workers to tide over until local scientists become available Salaries will require to be adequate in comparison with those offered in the countries from which the workers are derived, but, as development of a country is often accompanied by political instability, some special arrangements may be necessary to retain and attract staff Desirably, expatriate workers should be associated with some parent institution in their home countries so that they may not necessarily regard themselves as irrevocably devoted to expatriate careers and so that they may be reasonably confident of returning to suitable employment in their own countries when they so wish or when their services are no longer needed Probably the most productive arrangement for the future, so far as East Africa is concerned, would be the attachment of research organizations to East African and European universities so that, as well as the advantages mentioned above, research on the problems of under-developed countries could be kept in the view of senior undergraduates as a full and satisfying career

I thank Mr J S McClay, my administrative secretary, discussion with him has been invaluable to me in formulating the ideas here presented

[1] Buxton, P A, *Trypanosomiasis in Eastern Africa* (H M S O, London, 1948)

[2] Davey, T H, *Trypanosomiasis in British West Africa* (H M S O, London, 1948)

[3] Hornby, H E, *Animal Trypanosomiasis in Eastern Africa* (H M S O, London, 1952)

CERES: AN AUSTRALIAN PHYTOTRON

CERES, the Controlled Environment Research Laboratory of the Commonwealth Scientific and Industrial Research Organization, recently completed at Canberra, was officially opened by the Prime Minister of Australia, the Right Hon. R. G. Menzies, on August 29. Although operated by the C.S.I.R.O. Division of Plant Industry, a large group of biologists covering many disciplines, *Ceres* is to be a national facility available for use by other C.S.I.R.O. divisions, universities and Departments of Agriculture, as well as by visiting overseas scientists.

A phytotron is a building in which a large number of controlled environments is provided for the study of the responses of plants to climatic conditions. The first phytotron was built at Pasadena in 1949, and others on a scale comparable with *Ceres* have been built at Moscow and Paris, while there are also several smaller ones such as that recently built by the Colonial Sugar Refining Co., at Brisbane, for work on sugar cane[1]. All these consist of a relatively small number of rooms maintained at set conditions, and by moving plants from room to room they can be exposed to a large number of climatic conditions. Rather more than one-third of the plant-growing area in *Ceres* is organized in this way.

An alternative, of advantage when greater accuracy of environment control is required, is to have a large number of small cabinets, each under individual control, and each capable of providing a wide range of conditions. Five cabinet types, differing in size and in the climatic components controlled, have been developed to provide the remainder of the plant-growing area in *Ceres*. The majority of these, some 180 units, with a total area of 1,300 sq. ft., are naturally illuminated, but provide close control of temperature and day-length. Artificially illuminated growing space, about 800 sq. ft. in area, is provided in forty-five cabinets, some of which also provide control of humidity over a very wide range.

The high proportion of naturally illuminated space was determined by favourable light intensity and day-length conditions in Canberra, even in winter, and by the particular interest of the Canberra group in relating responses under controlled climatic conditions to those in the field. On the other hand, it is expected that with increasing sophistication and experience in the use of these controlled environment facilities will come increased pressure for greater accuracy and complexity of control, and ample space has been left in the building for an increase in the complement of artificially illuminated cabinets. It was in this space that the opening ceremony was held.

The naturally illuminated cabinets are placed in a series of 15 glasshouses along the north side of the building. Each glasshouse has independent temperature control, being conditioned by a number of reverse-cycle units specially developed for this purpose. These act as benches on which plants can be grown, and can be readily moved from one glasshouse

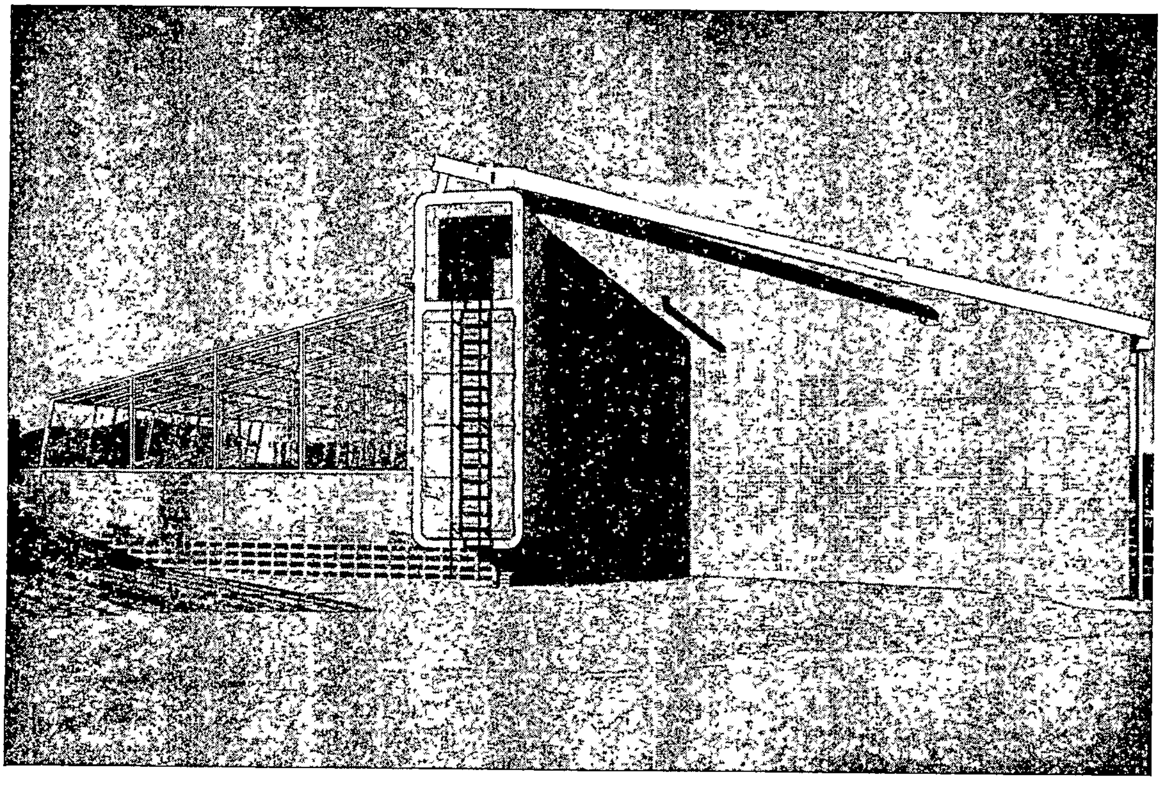

Fig. 1. *Ceres* from the west. The glasshouses are on the north side, at the left, with the grille of the thermal storage pond below them. On the south side of the building are the workshop, preparation area and artificially illuminated cabinets and dark rooms. The main fumigation chamber can be seen at the centre of the photograph.

to another—as can the cabinets—giving great flexibility in operation Heat removed from the glasshouses during the day, and from the sealed light canopies of the artificially illuminated cabinets, is stored in a large pond under the building, whence it may be withdrawn during reverse-cycle operation at night and in the hotter glasshouses by day

As in the Pasadena phytotron, *Ceres* will be kept free of pests and diseases by passing all air through electrostatic precipitators, by fumigating all equipment and plant materials, and by change of clothing for personnel, on entry to the building Additional details of the design have been given elsewhere[2]

The Canberra phytotron was initiated several years ago by Dr O H Frankel, then chief of the Division of Plant Industry. It was made possible by the development of a range of both naturally and artificially illuminated cabinets of a high level of performance and reliability by Mr R N Morse and his colleagues of the C S.I R O. Engineering Section They also developed the system of glasshouse conditioning, and suitable electronic controllers for both glasshouses and cabinets The building was designed by Grounds, Romberg and Boyd, with W E Bassett and Partners as consulting engineers It was built and equipped with funds made available by the Commonwealth Government in 1958, at a total cost of about £A600,000

L T EVANS

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[1] Glasziou, K T , *Nature*, 187, 745 (1960)

[2] Morse, R N , and Evans, L T , *J Agric Eng Res* , 7, 128 (1962)

EVIDENCE FOR A FURTHER IONOSPHERIC LEDGE ABOVE THE F_2 REGION

By PROF J. SAYERS, P. ROTHWELL and J H WAGER

Department of Electron Physics, University of Birmingham

SATELLITE *Ariel*[1], which was launched from Cape Canaveral on April 26, 1962, carries instrumentation to measure the local ionization density along the path of the satellite These data are stored point by point along each orbit by a tape recorder on board the satellite, and relayed by fast play-back over the telemetry link on command from a ground station *Ariel* is, therefore, the first satellite with instrumentation and a data-recovery system capable of providing a rapid world-wide survey of the distribution of ionization over the range of altitude and latitude covered by its orbit This, in effect, means a scan of ionization between latitudes 54° N and 54° S , and in the geocentric altitude range of 400–1,200 km

The electron or ionization density is measured by a new radio-frequency plasma probe which has been developed for ionospheric investigation The sensors are in the form of two disk-shaped grids of 4-in diameter and 3½ in apart These grids are mounted on insulating supports at the end of a 4-ft boom projecting radially from the equator of the satellite In operation a radio-frequency voltage of approximately 3 0 V root mean square and 10 Mc/sec frequency is applied between the grids

The radio frequency current which flows between the grids in response to this voltage is measured in magnitude and phase and the complex impedance of the plasma between the grids is thus determined at 10 Mc/sec frequency This enables the electron density to be calculated directly, and since radio

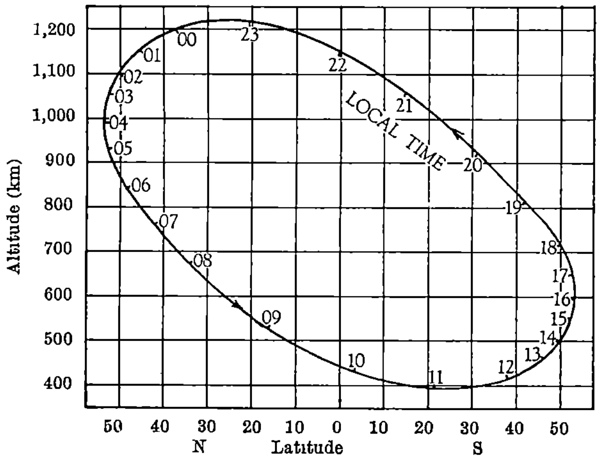

Fig 1 *Ariel* orbit of May 13, 1962

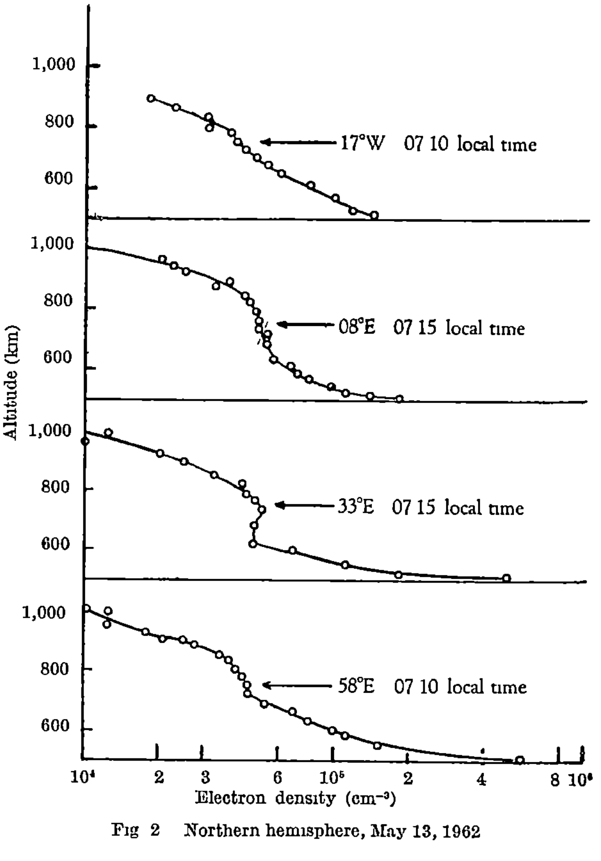

Fig 2 Northern hemisphere, May 13, 1962

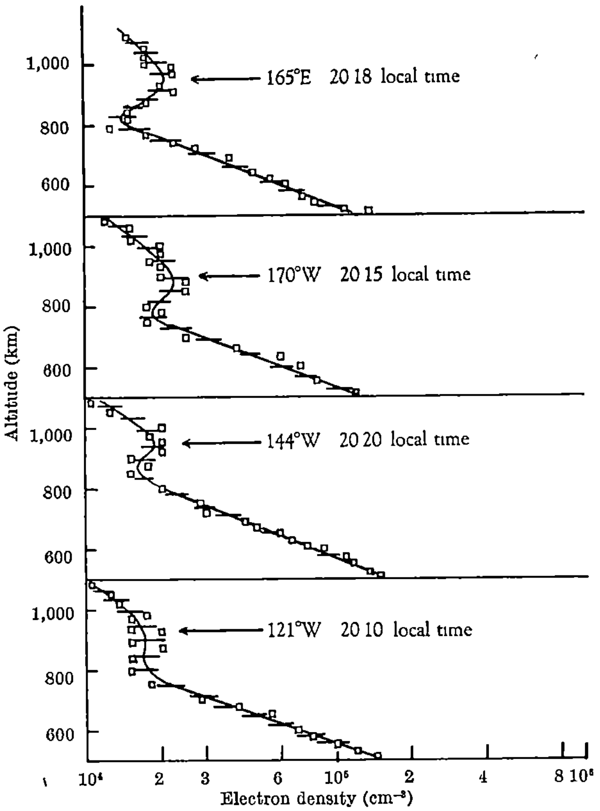

Fig 3 Southern hemisphere, May 13, 1962

frequency currents only are measured the d.c. currents due to photo-emission or secondary emission of electrons at the grids do not disturb the measurement. The effect of positive ion sheaths in excluding electrons from part of the region between the grids is eliminated by a d.c. sweep voltage applied to both grids and their support structure. This enables observations to be made with the grids at the plasma space potential.

The entire world-wide scan of ionization distribution requires several months of satellite orbit precession, and at the time of writing about half this scan is complete, and significant new results have resulted from the analysis of a representative selection of the data. The following conclusions have been drawn from this analysis.

(1) The density of ionization above the F_2 region of the ionosphere falls away with increasing altitude in the range of 400–1,200 km (geocentric), generally on a world-wide scale according to approximate diffusive equilibrium in the earth's gravitational field. This is in agreement with earlier measurements by *Explorer VIII*, vertical sounding rockets and back-scatter radar soundings[2-7].

(2) There are complex variations in the ionization density in this altitude-range, both temporal and geographical, which are comparable in relative magnitude to the corresponding variations in the F_2 region. This result is, of course, just what might have been expected.

(3) Superimposed on (1) and (2) there is very strong evidence for the existence of a further ledge of ionization at very much greater altitudes than F_2. This ledge has been observed regularly on a world-wide scale on every transit of the satellite through the altitude-range which has so far been analysed. These include a total of approximately 60 transits selected at random over a period of 2½ months.

The results obtained on four consecutive orbits on May 13 are quite typical. The form of the orbit on this date in terms of latitude, altitude and local solar times is shown in Fig. 1. Successive orbits will repeat this picture with progressive displacement in longitude of 25° per orbit, thus enabling ionization density at different longitudes to be compared for the same latitude, altitude and local time.

Fig. 2 shows the oblique profile of ionization density through the geocentric altitude-range of 400–1,000 km for four successive orbits in the northern hemisphere in sunlight. Fig. 3 shows the return transit for each of these orbits, which is in the southern hemisphere during local night. It will be noted that the detail of the ionization density profile changes from transit to transit due to the complex dependence on longitude already mentioned, but on each transit an ionization ledge is encountered. This ledge appears as low as at 700 km geocentric altitude during the day, and surprisingly at a higher altitude of 1,000 km near local midnight.

The combined results from all the orbits so far analysed are shown on Fig. 4 in which the altitude of the new layer is shown as a function of local solar time. Fig. 5 shows the ionization intensity of the maximum of the layer also as a function of local solar time. The spread in both these parameters is the result of the geographic and temporal complex variations of ionization, and is not indicative of instrumental errors.

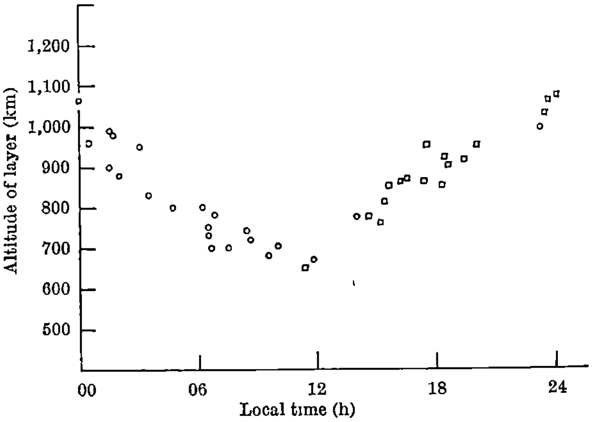

Fig. 4. April 30–July 1, 1962. O, Northern hemisphere; □, southern hemisphere

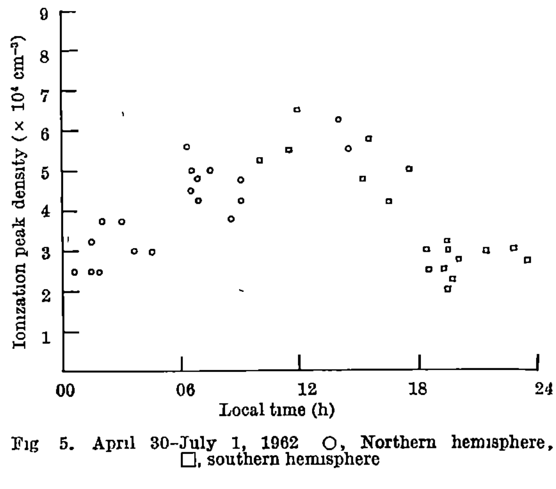

Fig. 5. April 30–July 1, 1962. O, Northern hemisphere, □, southern hemisphere

We conclude, therefore, that the departure from diffusive equilibrium in the Earth's gravitational field which these results indicate is very surprising and indeed it will be difficult to find any mechanism which could produce such a layer. A diligent search has been made for possible instrumental defects which could account for the observations of departure from diffusive equilibrium, but none has been found. Figs. 4 and 5 include data for the full range of latitude and longitude in both northern and southern hemispheres, and so the systematic nature of the results is well established.

A layer of ionization above the F_2 region should be readily detected by a suitable topside sounder experiment using a high-altitude rocket or satellite. The first such experiment has been reported by Knecht, Van Zandt and Russell[8] using a rocket-borne topside sounder from Wallops Island at 18.17 h on June 24, 1961. The topside sounder in this experiment operated at two fixed frequencies, namely, 4.07 and 5.97 Mc/sec. These frequencies are both far above the critical penetration frequency for the new layer and so no regular reflexions could have been recorded. It is, however, very interesting to note that scatter echoes of large intensity were recorded at various altitudes in the range 700–1,000 km and these effects were not at the time understood. The effects observed are consistent with the existence of a layer having deep spatial irregularities as is now proposed. The most intense of the scatter echoes occurred at an altitude of 840 km and this is the altitude at which the peak of the new layer has been found by *Ariel* at 18.00 h local time corresponding to the topside sounder experiment.

A second, similar topside sounder experiment was conducted from Wallops Island by Knecht and Russell at 23.26 h on October 13, 1961. The peak altitude of the rocket carrying this experiment was 1,070 km. This is near to, and may indeed be below, the altitude of the new layer at local midnight since there may be some seasonal variation in the layer altitude. It is therefore not inconsistent that no similar anomalous scatter was recorded on this flight.

We thank the U.S. National Aeronautics and Space Administration (NASA) for providing the satellite, its power supplies, sequence timing, telemetry, data encoding and launching into orbit. We also thank NASA for data decoding and for providing 'quick-look' print-out of data which has made this early presentation of results possible.

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[9] Knecht, R. W., and Russell, S., *J. Geophys. Res.*, **67**, 1178 (1962).

RADIATION HYGIENE IN AND AROUND A NUCLEAR POWER STATION

By DR. J. H. MARTIN

Health and Safety Manager, U.K. Atomic Energy Authority,
Chapelcross Works, Annan

THERE are installed at Chapelcross four of the Calder Hall type of nuclear reactors. Construction of the station commenced in 1955 and the first of the reactors reached power in January 1959 and the fourth in April 1960. Apart from laboratory provisions for technical investigations concerned with the reactors and research in health physics, Chapelcross, in essentials, differs little from the nuclear power stations which are being built for the South of Scotland Electricity Board and the Central Electricity Generating Board. Since also, unlike Calder Hall which is closely integrated with Windscale, it is not associated with other atomic energy operations, the performance of Chapelcross from many aspects, including that of radiation hygiene, serves as a useful pointer to what may be expected from these power stations, and it is the purpose of this article to present the experience of its operation in relation to radiation safety.

Staffing

On the Works control side of the Health Physics Department, as distinct from its research activities, there are four professional physicists, three chemists, and two ancillary staff. These are supported by a team of twenty-four industrial staff who have been selected and trained to operate and interpret health physics instrument readings and to apply the information in the control of operations involving sources of radiation. These men, who are described as health physics monitors, provide, in shifts, round the clock coverage of all operations. To round off the picture, although it is not of direct concern in this article, industrial safety, as distinct from radiation safety, is covered by a safety officer, a safety foreman and five firemen.

Site Control and Experience

The basic cycle of operations at Chapelcross involves charging a reactor with the 10,000 fuel elements which the core contains, then running it at power for a suitable period. At the end of the period selected, some or all of the elements are removed from the core into shielded flasks which form part of a discharge machine. Each flask contains a magazine or 'basket' holding twenty-four elements and these are transferred from the discharge machine on the pile cap, down a shielded shaft to a transport flask, inside which the basket is contained while being transported to the cartridge cooling pond. Here the flask is lowered under water and is unloaded at a depth of some 18 ft. The elements unloaded are then transferred by remote-control grabs into small containers called skips, which hold some 150 elements. The elements are stored in these skips, under water, for a suitable period to allow the more short-lived radioactivity to decay. The skips are then removed from the pond, placed in another transport flask on a large semi-trailer and moved by road to Windscale where the elements are processed for separation of plutonium and recovery of uranium

Over and above these basic operations there is a number of operations which take place, not all of which are connected with the primary functions of Chapelcross for the production of plutonium and power For example, facilities are provided in the Chapelcross reactors for testing of different designs of fuel elements and for experimental work on graphite Many of these experiments require the application of non-standard techniques for inserting and removing from the reactor the materials being tested and for their subsequent handling and examination All these operations, whether they be routine shutdowns or for special purposes, are the subject of careful planning in advance and detailed instructions are prepared to cover each operation. At this stage and particularly for special operations, a professional member of the health physics staff will be a party to the discussions

The removal of items from the reactor, some of which, like fuel elements, contain many thousands of curies of activity, their transport about the site and their movement in and out the pond involve a potential risk of someone being exposed to radiation directly In addition, the accidental release of gas from the reactor, the dropping or scraping of materials such as graphite, the splashing of water in and around the pond, together with maintenance work on apparatus associated with the reactors or with radioactive material, involve the risk of air and surface contamination Elaborate installed alarm systems are in operation to assist in warning personnel of the occurrence of such incidents, but the most effective control is provided by the health physics monitor staff, who make measurements of the radiation-levels at all stages of such operations

While the reactors are in operation there are inevitably, despite heavy shielding, small fluxes of γ-rays, and in some instances neutrons, at various locations around the reactors For example, a quantity of the short-lived argon-41 is produced by (nγ) reaction in argon impurity present in the carbon dioxide coolant gas used in the reactors while by (np) reaction on oxygen nitrogen-16 is also produced The half-life of the latter is only 6 sec, and while therefore there are moderate fluxes due to this isotope at the top of the heat exchanger, by the time the gas has reached the bottom the activity-levels are negligible Because, however, of their fairly energetic γ-rays (1 3 MeV and 6 1 MeV respectively) these isotopes are the principal source of γ-ray background in the neighbourhood of the reactors.

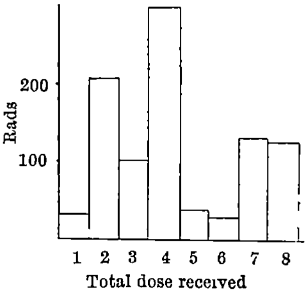
In addition to those present on and around the reactor during the course of their normal duties a good deal of maintenance work, work in preparation for a specific operation and investigations and experimental work is carried out while the reactor is operating Once again these operations or investigations are the subject of careful planning and of surveys and estimates by the health physics department of the radiation-levels in the region concerned

The maximum permissible exposure to external radiation in any one week is 0 3 rads, and the integrated dose received by an individual is governed by the fact that he must not receive more than 3 rads in any period of thirteen weeks, and further, that the dose accumulated in his lifetime must not exceed that expressed by the equation dose = $5(N - 18)$ rads, where N is the age of the individual in years The average exposure-level which this latter equation permits is 0 1 rads a week assuming continuous exposure throughout a working life from the age of eighteen This corresponds to 0 02 rads per day for a five-day week and 2 5 mrads/h for an 8-h day.

When personnel are called on to work in areas where levels are likely to exceed 2 5 mrads/h, monitors exercise special control and they are authorized to determine a working time such that a man may receive up to 0 02 rads A charge-hand monitor can authorize exposure up to the 0 06 rads level Subject to the individual or individuals concerned having satisfactory radiation records a member of the professional health physics staff may authorize an exposure up to 0 2 rads Permission for greater exposures to individuals must be obtained from the Health and Safety Manager This detailed control of operations on site is backed by continuous monitoring of all personnel on the site, who must carry film badge dosimeters

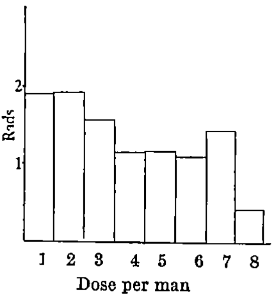
Film badges are normally worn for a period of one month although special issues are made to cover specific requirements In addition, where the radiation-level in which personnel are to work exceeds 2 5 mrad/h, control of the exposure of personnel by stipulation of working times is augmented by the issue of quartz fibre electrometers which can be read directly, and thus the dose received can be checked at intervals while the work is in progress Where the monthly film badge records a dose exceeding 0 4 rads (that is, greater than an average weekly exposure of 0 1 rads) the circumstances surrounding the receipt of this exposure are investigated and where the exposure has been caused by failure of the individual to obey instructions or errors in operating conditions, steps are taken to rectify these The Health and Safety Manager reports these circumstances to the Board of Management monthly

The results of analyses of the personnel dose records for a year with the station operating at full power are shown in Figs 1 and 2 and Tables 1 and 2 Approximately 1,000 people are employed, and the histograms in Figs 1 and 2 show the distribution of the integrated dose per annum and the dose per man per annum in various work categories on the site It is an interesting feature of a more detailed analysis of these records that, contrary to what might be expected at first, operations involving the charge and discharge of a reactor when large quantities of very active material are being moved about the site are not the principal cause of exposure of personnel In fact the charge/



1, Reactor controllers, 2, reactor process workers, 3, charge/discharge team, 4, maintenance dept, 5, pond process workers, 6, monitors, 7, turbine and heat exchanger attendants, 8, miscellaneous

Fig 1



1, Reactor controllers, 2, reactor process workers, 3, charge/discharge team, 4, maintenance dept, 5, pond process workers, 6, monitors, 7, turbine and heat exchanger attendants, 8, miscellaneous

Fig 2

Table 1

No. of people wearing films	989	
No. and per cent of people exceeding annual dose of		per cent
1.5 rads	248	25
3 rads	11	1.1
4 rads	1	0.1
No. of occasions when 13-week limit of 3 rems has been exceeded	Nil	
Total dose received by station (rads)	981.9	
Minimum detectable dose in film (allocated to wearers of all films read as zero)	10 mrads	

Table 2

	1961	1960	1959
No. of people wearing films	989	966	892
No. exceeding annual dose of 5 rads	Nil	Nil	1
Total annual station dose	981.9	729.2	522.5
Average exposure for individual (rads)	0.99	0.75	0.59
Relative exposure per individual per unit of power produced	1	1	2

discharge teams who carry out these operations on the reactor receive lower exposures during the periods of charge/discharge than they do when carrying out other duties around the reactors while they are at power. During these charge/discharging periods, however, workers at the ponds, where all the irradiated fuel elements are being handled, and health physics monitors, tend to receive more radiation. During reactor operation small quantities of radiation are received by staff operating the reactors, by process workers carrying out normal duties around the reactor and by maintenance staff servicing equipment on and about the reactors. As mentioned previously there is in addition to the γ-ray flux around the reactors a possibility of small neutron fluxes. The film badges carried by personnel incorporate a cadmium filter and the (nγ) reaction induced by slow neutrons makes it possible to measure these by use of the blackening of the film under this filter. Appropriate personnel have also been issued with track plates for detection of fast neutrons. The exposures found in both cases have been extremely small. The threshold for the fast neutron plates is 50 mrem and this has been exceeded on one occasion only. The threshold for measurement of slow neutrons is 10 mrem and the highest dose recorded for the year was 130 mrem.

Throughout the history of the station there have been increasing amounts of power generated in succeeding years resulting in an increase in the radiation-level in the vicinity of the reactors. Between 1959 and 1960 this was principally due to the coming on to load of each of the four reactors in turn. However, in the period 1960–61 the increase has largely been due to technical improvements in reactor operation. The Calder Hall–Chapelcross type of reactor was designed for a thermal power output of about 180 MW, and over the years this has been raised to a figure of over 240 MW. It will be seen from Tables 1 and 2 that very satisfactory control of the personnel exposure situation has been achieved and that despite increased power the average exposure of individuals on site per unit of power produced has been held down.

During charge/discharge operations on the pile cap and at the pond there are always slight possibilities of air and surface contamination, and health physics monitors carry out regular air sample surveys as a routine, and monitor personnel on the completion of the work. The protective clothing worn in these circumstances varies a little with the duties being undertaken, but it is simple, comprising, for example, a coverall (boiler-suit), cloth overshoes and cap. There are also other operations which may result in contamination of personnel or of the air they breathe. Examples of this type of work are entry made into the heat exchangers and other parts of the gas circuit for maintenance and inspection work and experimental work carried out on graphite specimens which have been irradiated in the reactors. For such operations more elaborate types of protective clothing, sometimes with separate air supply to the operator, are used, and special air sampling surveys are made. Table 3 shows the results of air sampling for the year 1961, and it will be seen that contamination-levels exceeding maximum permissible concentration are rare. Cases where air contamination occurs unexpectedly are rare and it is unusual for personnel to be contaminated in these circumstances. Contamination of personnel therefore generally arises through disregard of working instructions or through lack of care in undressing from contaminated clothing. During 1961 only twelve cases of contamination had to be sent to the surgery for decontamination, none of these being serious. To ensure that there has been no inhalation and ingestion of radioactivity or absorption through the skin, it is a routine to take urine samples in these instances and to analyse them for fission-product activity. In all cases the findings were negative.

Table 3 AIR SAMPLING RESULTS

	Total No. of samples	No. and per cent above 0.3 m.p.c.	1.0 m.p.c.	10 m.p.c.	Highest result
Routine	15,656	32 0.2 per cent	12 0.08 per cent	Nil	8.9 m.p.c.
Special	34	7 20.6 per cent	3 8.3 per cent	Nil	6.9 m.p.c.
Special (conditions where breathing apparatus has to be worn)	512	261 51 per cent	173 33.8 per cent	46 9 per cent	261 m.p.c.

A high standard of plant cleanliness is maintained by close checking by monitors of the incidence of air and surface contamination and control of the movement of contaminated material. Potentially, contaminated equipment is checked by swabbing, the swabs being assessed for activity. A measure of the success of this control is the fact that both pile cap and pond areas as well as all other regions of the reactors are 'clean' areas when charge/discharge operations are finished and access to them is thus not limited by any considerations of radioactive contamination. Table 4 shows the results of work carried out in maintaining this level of cleanliness.

Table 4 SURVEYS FOR SURFACE CONTAMINATION

Total No. of surveys	1,151
No. showing no contamination above M.P.L.	1,117
No. showing contamination above M.P.L. in a few places	32
No. showing contamination over large areas	2

In maintaining the standards outlined above it is not sufficient to rely solely on the control procedures and personnel alone, and training of operating personnel has an important part to play. All personnel on joining the establishment receive training in radiation safety and supervisory personnel spend a period of time in the Health Physics Department seeing the variety of ways in which the Department assists in maintaining good standards. Regular refresher courses in, for example, "Prevention of Contamination" are run so that personnel act always with a consciousness of the possible existence of a radiation hazard.

Environmental Safety

Small quantities of radioactive effluent are discharged to the environment in two ways. The main radiation absorbing or 'biological' shield of the reactor is cooled by blowing air past it. This air is drawn in near ground-level and is ejected from two 250-ft stacks, one on each side of each reactor. The air passes through a region inside the biological shield where there are substantial neutron fluxes, and a certain amount of induced activity is produced in the air. This is principally the short-lived isotope argon-41. The second source of low-level radioactivity is the cartridge cooling ponds. In the course of operation of these ponds, a small quantity of radioactivity gets into the cooling pond water as a result of corrosion of the canning material of the uranium elements, in which some activity has been induced or by leakage from the uranium to the water through the canning material or occasionally because damaged elements are in the water. The capacity of each of the two cartridge cooling ponds is 400,000 gallons of water, and some 6 per cent of the volume of a pond is discharged to the Solway Firth each day. Water to be discharged is transferred from the ponds to a detention tank, where it is held until discharge time, which is between high tide and high tide plus 2 h. Between the ponds and the detection tanks a filter system is provided for removal of any particulate activity. While in the detention tanks the water is sampled and measured for activity before discharge is permitted. Discharge of liquid and gaseous effluent is carried out under authorizations granted by the Secretary of State for Scotland. That for liquid effluent authorizes discharge, in the period at high tide as stated above, of up to 22.5 curies every 3 months of total $\alpha + \beta$ activity, this activity to contain no more than 112.5 millicuries of strontium-90. A further proviso is that the discharges should be made as uniformly as is reasonably possible. Records of the quantity of radioactive liquid effluent discharged are supplied regularly to the Scottish Home Department.

An example of the performance of Chapelcross in this respect is shown in Fig. 3, where the records for the total $\alpha + \beta$ activity discharged over a period of time are shown. Considerable variations are apparent between periods when all reactors are operating and those when discharge operations are in progress and the ponds contain large quantities of fuel elements, but, overall, the average discharge level is something less than 10 per cent of that authorized.

It is a further requirement of the granting of permission to make these discharges that the station carries out a survey of various biological materials in the region around the site, to ascertain whether any potentially injurious levels of radioactive material are occurring as a result of the discharge operations. In the early period of operation of nuclear energy installations, partly because the scientific information on which to base an environmental sampling programme was somewhat sketchy, but very largely to establish public confidence, considerable amounts and varieties of biological material were collected and assayed for their radioactive content although the scientific justification for a good deal of this work could not be established. Initially, in the land area surrounding Chapelcross, samples of grass, root, mat, soil, milk and reservoir and rain-water were collected at intervals varying from four to six weeks, and analysed for their total α and total β content, and in the cases of milk and water specifically for their strontium-90 content. Measurement of the γ-ray background-level was also made at 31 points within a radius of 9 miles of the site.

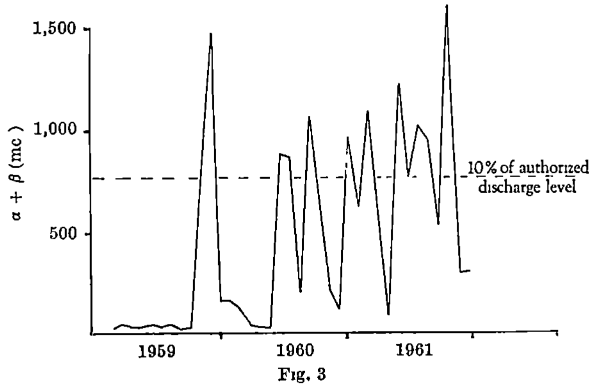


Fig. 3

The primary objective of an environmental monitoring programme is to check that the public health is not endangered, and there is now ample evidence to show that, in most cases, the principal potential hazard to members of the public from radioactive materials released into the environment arises from the ingestion of contaminated food or water. It follows, therefore, that the aim should be to obtain information on the level of contamination of the diet and that the measurements taken should be capable of being related to a maximum permissible level in some item of human diet. In some cases it may be possible to extend the surveys to provide scientific information on the behaviour of radioactive materials in the environment, as has been done by studies of fall-out, but it is important to maintain the distinction between the scientific function and that of assurance of satisfactory operating conditions.

Recently, in consultation with the Department of Health for Scotland, the Agricultural Research Council and the Radiological Protection Division of the Authority Health and Safety Branch, an environmental monitoring programme, based on the foregoing requirement, was agreed.

Accumulation of data has revealed that, in the circumstances pertaining at Chapelcross, the item of the human diet which would convey the largest amount of radioactivity into the human body is milk. Accordingly, although for other reasons γ-ray measurements are still made at a number of points, monitoring cover in relation to gaseous effluent is provided by milk sampling, the samples being analysed for iodine-131 and strontium-90. An example of data from the original surveys is shown in Fig. 4. Large variations, due to variations of the natural activity of the air, will be seen in the measurements of α-activity on grass. Broadly speaking, the activity on grass rises in the winter time when heavy rain brings down the natural α-activity from the air, and since the grass is growing relatively slowly a large quantity accumulates on a small quantity of grass. At other seasons of the year more rapid growth of the grass means less activity per unit weight and, of course, the decreased rainfall means less precipitation. It is, clearly, unrealistic to expect a measurement of the gross α-activity in these circumstances to have any value as an insurance that the Station is not releasing undue radioactivity to the environment. Milk sampling is undertaken from two groups of farms, one group being within a radius of 3 miles

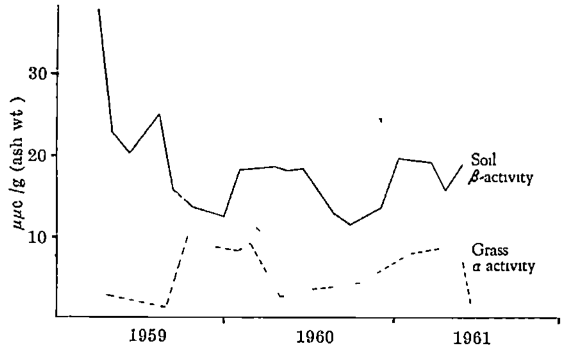
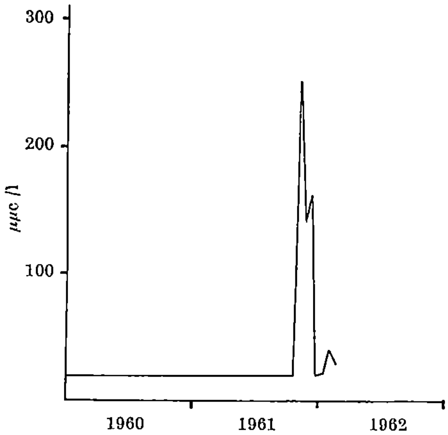


Fig 4

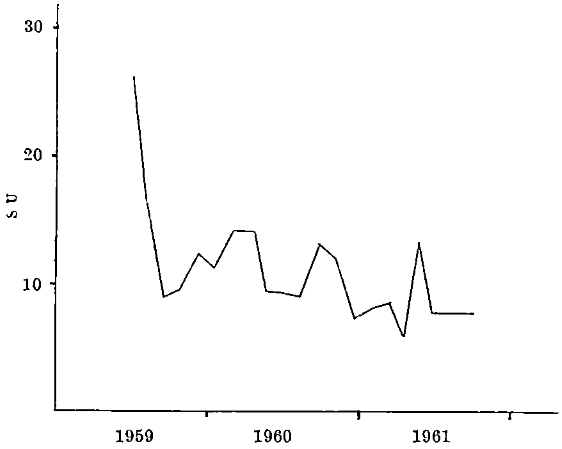
and the other between 3–5 miles from the station The results of analyses of milk for iodine-131 and strontium-90 are shown in Figs 5 and 6 The minimum level of activity of iodine-131 which can be measured is 20 μμc /l, while the maximum permissible concentration as specified by the Medical Research Council is 400 μμc /l

In the case of strontium-90 the maximum permissible concentration specified by the Medical Research Council is 400 μμc /ml, and as the calcium content of the milk in the Chapelcross area varies only a few per cent above and below 1 g/l, the s u values shown in Fig 6 are almost exactly the values in μμc /l Although the actual levels found are affected by the use of supplementary feeding in winter conditions, the rise in iodine-131 activity towards the end of 1961 is due entirely to fall-out from Russian atom bomb testing, and it parallels similar results reported from all over Britain A corresponding rise in the strontium-90 figures is expected in the spring of 1962 Fig 7 shows the results obtained at one of the standard points of γ-ray measurements made regularly since just before the Station commenced operation Once again the only effects which can be detected are those due to fall-out, there being a decrease following the cessation of weapon testing, and a rise at the end of 1961 due to the latest Russian series of tests



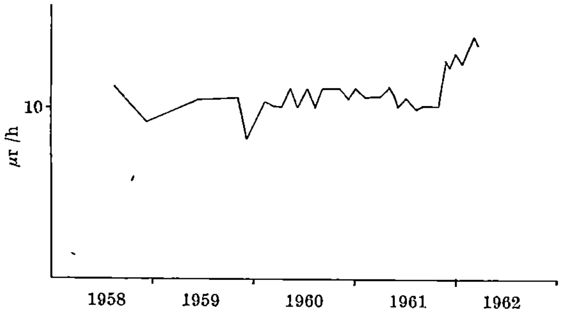
Iodine-131 in milk

Fig 5



Strontium-90 in milk

Fig 6



Gamma-ray background

Fig 7

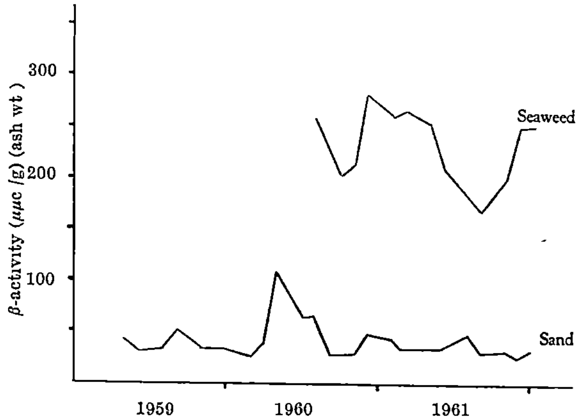


Fig 8

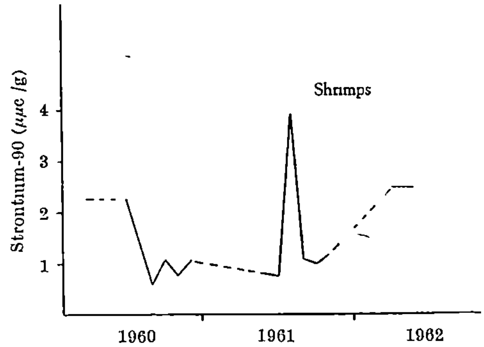


Fig 9

A similar survey programme on and in the Solway Firth is carried out in relation to the discharge of liquid effluent Here samples of sand, seaweed, shrimps, salmon, etc , are collected and analysed for activity

Sand sampling is carried out to ensure that no external radiation hazard to people using the beaches will result, while seaweed is looked at because of its potential use in making lavar bread, although the variety used for this is practically non-existent in the Solway region A large part of the British market for shrimps is supplied from the Solway area, and it is important, therefore, to measure their activity In the grounds fished, shrimps are only available between spring and late autumn Salmon fishing is an important industry in the area of the Solway, and while, because of their wide ranging habit they are of doubtful value as a monitor of the effect of discharge of effluent, salmon are taken for analysis

Examples of the results of such surveys are shown in Figs 8 and 9 In all cases the levels of activity involved are very low, and no variations due to the operation of the station can be detected Because of the difficulties and time involved in separating the flesh of the shrimp from the shell, the whole is ashed and analysed The variations of strontium-90 activity recorded are thought to be associated with the shedding of the exoskeleton, the exact timing and frequency of which are unknown

It will be seen that by proper training of staff and by intelligent use of control measures, a nuclear power station can be operated without personnel receiving unacceptable levels of radiation exposure Further, the small quantities of radioactive effluent which arise from the operation of a nuclear power station and which are discharged to the environment have no detrimental effect on that environment or on those occupying it or using its products

CONVECTION IN THE MOON

By Prof S. K. RUNCORN

Department of Physics, King's College, Newcastle upon Tyne, and Jet Propulsion Laboratory, California Institute of Technology, Pasadena

RECENT investigations of terrestrial continental drift[1] raise the possibility that convection currents exist within the Earth's mantle This supposes that the Earth's mantle, except for a thin surface crust, has zero-strength and also the properties of a Newtonian fluid for stresses of periods of some millions of years, a hypothesis which seems to receive support from solid-state physics[2] A stumbling block, much emphasized by opponents of convection theories, is provided by the fact that the figure of the Moon departs from a sphere by amounts up to thirty times more than if it were in hydrostatic equilibrium This has been held to show that it has considerable long-term strength The non-equilibrium shape of the Moon, however, may simply mean that convection also exists within this body

The hydrostatic theory of the figure of the Moon predicts a bulge towards the Earth due to tidal forces of about 60 m and an equatorial bulge due to its monthly rotation a quarter as large The bulge towards the Earth can be determined from the comparison of the apparent motion of points on the Moon's surface due to the geometrical librations Baldwin[3] shows that the radius towards the Earth exceeds the mean value in the plane of the sky by 1 9 km. Whether the limb of the Moon departs from circular shape has not yet been determined because of difficulties arising from surface relief and irradiation However, the motion of the Moon allows its moments of inertia to be determined about its polar axis, the Earth–Moon axis, and the third axis with high accuracy Their differences greatly exceed those calculated on the hypothesis of hydrostatic equilibrium It is convenient to express this in terms of a triaxial ellipsoid of uniform density The bulge towards the Earth is then found to be 1 km and the polar flattening 0 6–0 7 km Because of the irregular surface, Baldwin's method might be thought to be inaccurate yet he obtains a bulge essentially twice the dynamically determined one whether he relies on points on the maria or on the uplands This strongly suggests that the Moon has a systematically lower density beneath the bulge

It has long been assumed that the departure of the figure of the Moon from that appropriate to a body in hydrostatic equilibrium implies considerable internal rigidity To support a bulge of 1 km, a stress difference of about 20 bars at the centre is required, as was shown by G H Darwin As it was until recently thought that the Moon and Earth were once liquid, and cooled and solidified in the early history of the solar system, it seemed to follow that the non-equilibrium shape was acquired of the order of 10^9 years ago and was frozen in, either, as Laplace thought through inhomogeneous cooling, or as Jeffreys[4] once supposed as a fossil-tide raised on the Moon when it was much closer to the Earth By Kepler's third law, the angular velocity of rotation of the Moon, assuming it to have been then, as now, a synchronous satellite, would be inversely proportional to the three-halves power of the Earth–Moon distance Thus, the bulge due to rotation would have been proportional to the inverse cube power, and therefore proportional to the tidal bulge itself Thus, the explanation of the non-equilibrium shape as a fossil tide must be abandoned, for the ratio of the polar flattening to the earthward bulge is 0 6–0 7, not 0 25

It is easily shown that convective motions cause a slight rising of the surface above the hot uprising current In the theory of convection in a spherical shell under a radial gravitational field[5], it is convenient to develop the velocities of flow and the temperature- and density-perturbations in spherical harmonics From their orthogonal properties, it can be simply shown that only convection described by harmonics of second degree are effective in producing the required differences in the moments of inertia Such a convection pattern, if axially symmetrical, consists of two cells hot fluid rising from the interior of the sphere along its axis and descending, after cooling at the surface, along the great circle 90° away from the axis Therefore, the bulge towards the Earth, determined by Baldwin, can be explained by a second-degree convection current with its axis of symmetry directed towards the Earth Because the moments of inertia about the polar axis and the equatorial axis in the plane of the sky are unequal there must be superposed on this pattern an exactly similar one symmetrical about the latter axis, with velocities 0 6–0 7 of the former

Chandrasekhar[5] shows that the ratio of the inner to the outer surfaces of the spherical shell, in which convection is occurring, and the nature of the bounding surfaces determine the degree of the spherical harmonics describing the convection which occurs at marginal stability. To account for convection of second degree would require the presence of a core with radius lying between about 0.06 and 0.36 of the lunar radius. These values[6] emerge for the case of a spherical shell with a rigid outer boundary and a free inner one. The low temperature and consequent rigidity of the outer crust of the Moon make the former condition realistic. The latter assumes that the core is of iron, which, as it has a lower melting-point than the silicates surrounding it, may be fluid. It seems reasonable to suppose that the iron has been concentrated towards the centre in the Moon as in the Earth by convection[1]. The closeness of the mean density of the Moon to that of olivine suggests that the proportion of iron is lower than in the Earth, a conclusion not in conflict with the existence of this small iron core (0.2–4.6 per cent of the total volume).

Equating the pressure in the uprising and downgoing convective columns, and assuming a coefficient of volume expansion for olivine of 2×10^{-5} per °C, the 2-km bulge towards the Earth implies differences in temperature driving the convection of about 50° C. Such a theory would explain the discrepancy between the dynamical and the optical determinations of the height of the bulge.

In both the Earth and Moon, convection would bring about an equilibrium between the total heat internally generated and that being conducted out through the surface. As the mantle of the Moon and Earth are of the same composition, probably similar to chondritic meteorites, it is reasonable to suppose that the radioactive heat generated per unit volume is the same. The heat-flow out of the Moon will therefore be one-quarter of that through the Earth's surface, that is, 2×10^{-7} cal/cm^{-2}/sec^{-1}. The heat carried by a convection current is proportional to the velocity and temperature differences. As I have shown[6] the latter in the Earth are only $\frac{1}{3}$° C., the velocity of the currents in the Moon must only be 0.02 of those in the Earth, that is, about 2 cm/yr. As the mean density of the Moon and the Earth's mantle are in the ratio of 3:5 and the gravity of the Moon and Earth in the ratio of 1:6, the buoyancy forces driving the convection in the Moon will be fifteen times greater than in the Earth, for the temperature differences are 150 times as great. Similarly, because the Earth is four times as large as the Moon, the viscous forces resisting convection, if the coefficients of viscosity were the same, would be a third of those in the Earth. Therefore, the viscosity of the Moon must be about 50 times that of the Earth's mantle, presumably the result of the lower pressures and temperatures in the Moon.

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OBITUARIES

Sir Ronald Fisher, F.R.S.

SIR RONALD AYLMER FISHER died suddenly at Adelaide on July 29 in his seventy-third year, following an operation. His death is a grievous loss, both to his many friends and to science.

Born on February 17, 1890, the seventh child of G. Fisher, of Robinson and Fisher, auctioneers, of St. James's, he was educated at Harrow and Gonville and Caius College, Cambridge, where he read mathematics, and for a further year held a studentship in physics, studying statistical mechanics, the quantum theory and the theory of errors. He then spent two years as statistician to the Mercantile and General Investment Co., followed by six years teaching in public schools; fortunately for the world, his extremely short sight prevented him from joining the Forces in the First World War.

In 1919 he was selected by Sir John Russell to fill the newly created post of statistician at Rothamsted Experimental Station. Already while at Cambridge he had developed a keen interest in Mendelian theory and its relation to the evolutionary principles of Darwin, and Rothamsted provided a congenial research environment and direct contact with practical biological and agricultural research. This proved a most happy combination, in that he rapidly came to appreciate the real requirements of biological research workers, who have to experiment on highly variable material and are severely limited in the number of experimental units—plants, animals, field plots—that can be included in any one experiment. This demanded, first, that statistical methods should be devised which would enable sound conclusions to be drawn from small samples, and secondly, that the treatments included in an experiment should be so chosen that the maximum amount of information is obtained on the points at issue. On the theoretical side, this quickly led Fisher to the development of exact tests of significance for small samples, and to the development of the theory of estimation, in particular the method of maximum likelihood and the principle of sufficiency. It also led to the recognition of the principles governing experimental design, and to the development of the analysis of variance, which, among its many uses, provides a convenient computational procedure for the estimation of the errors of experimental results and for testing their significance. The modern techniques of experimental design and analysis which flowed from this work have now been almost universally adopted not only in agriculture but also in all fields where experiments on variable material are required. The recent spectacular advances in agricultural production in many parts of the world owe much to their consistent use.

It has been part of Fisher's strength that he always endeavoured to make the statistical methods he evolved available to biologists and others who wish to apply them, and to give adequate insight into the principles without confusing the issue with the mathematics required for their derivation. To this end, after five years work at Rothamsted, he wrote *Statistical Methods for Research Workers*. This book (which has been greatly extended, though little altered, in successive editions) has had an immense influence in introducing modern statistical methods in biological research. *The Design of Experiments*, though not published until 1935, also flowed directly from his work at Rothamsted.

Although at Rothamsted Fisher was not directly concerned with genetics, he pursued his investigations privately, both on the theoretical side and in practical breeding studies on mice, snails and poultry; from the last he confirmed his theory of the evolution of dominance. *The Genetical Theory of Natural Selection*, published in 1930, contains ideas, particularly on human evolution, much in advance of its time. If these have as yet had little practical impact, it may be in part because Fisher's conviction of the power of genetic selection in moulding organisms to fit their environment led him to belittle the importance of reforms, such as improvements in nutrition, designed to improve the environment, and this enabled progressive thinkers to dismiss his theories on human evolution, which in any event they found distasteful, without serious thought. Nevertheless, the book did much to reconcile Darwinian and Mendelian theory.

Fisher's genetical interests led to his appointment in 1933 to the Galton chair of eugenics in the University of London. Here he became interested in blood groups, and in particular resolved the complexities of the rhesus system. In 1943 he became Arthur Balfour professor of genetics in the University of Cambridge, a post which he held until his retirement in 1957. Here he continued his theoretical studies on population genetics and quantitative inheritance, publishing *The Theory of Inbreeding*, and experimental studies on chromosome mapping in mice, and on polyploidy in *Lythrum*. He remained in Cambridge until his successor was appointed in 1959, and then took up work in Adelaide with the Commonwealth Scientific and Industrial Research Organization Division of Mathematical Statistics. After his formal retirement he became interested in the claim that had been made that cigarette smoking was responsible for lung cancer. Whatever the final verdict on this issue, Fisher's re-examination made apparent the weakness of the deductions from the original evidence, and provided an excellent example of the dangers inherent in the statistical analysis of survey material.

Throughout his life Fisher continued to develop his ideas on the fundamental problems of inductive inference and estimation. He early recognized the fallacies of the Baysian approach, and in 1930 introduced the concept of fiducial probability. Other mathematical statisticians have found great difficulty with this concept; but it will, I think, in due course take its place along with likelihood and exact tests of significance of small samples as one of Fisher's outstanding contributions to the theory of statistics. In 1956 he published *Statistical Methods and Scientific Inference*, in which he summarized his current thought on these problems and for the first time put forward the entirely novel concept of reference sets and recognizable sub-sets for defining probability.

Fisher was elected a Fellow of the Royal Society in 1929, receiving the Royal Medal in 1938, the Darwin Medal in 1948 and the Copley Medal, the highest award of the Society, in 1955. He was president of Gonville and Caius College, Cambridge, during 1956–59. He was knighted in 1952, and received recognition from many foreign universities and academies. In 1961 he was elected a member of the Pontifical Academy of the Vatican, an honour which gave him particular pleasure.

To those who knew him well, Fisher was a man of great charm, a brilliant conversationalist, cultured in the widest sense, and appreciative of historical values. He was also a most stimulating scientific colleague, as the many who have worked with him can testify. He did not believe in direction in scientific research—"I wonder how many of us do the work we're paid for '—and this, and his irascible temperament, made him a poor administrator. He was also, I think, unduly sensitive to much of the unjustified criticism which his work, because of its originality, inevitably attracted. This led him into many scientific controversies, which he pursued with vigour and apparent enjoyment, but often in that indignant frame of mind that leads to a partial view of the problem, and leaves unanswered objections that are obvious to the impartial observer. Indeed, his approach to scientific controversy was often that of an advocate rather than a judge, and this did frequent harm to many of the causes he had most at heart.

He married in 1917 Ruth Eileen, daughter of H. Gratten Guinness, and had two sons (the elder was killed in action as a fighter pilot in 1943) and six daughters.

F. YATES

Mr. G. A. Bacon

MR. GEOFFREY ARTHUR BACON, whose untimely death occurred on August 1, 1962, was born at Boxmoor, Hertfordshire, on May 25, 1918. He was educated at Berkhamsted School, and in 1938 joined the laboratory staff of the Royal Free Hospital in Gray's Inn Road, where he worked as personal assistant to Prof. R. A. Webb. The unit in which he worked was moved during the Second World War to the Three Counties Emergency Hospital, near Hitchin, Hertfordshire. In 1944 he joined the staff of Harlow Wood Orthopædic Hospital, Mansfield, where he became technician in charge of the Laboratory Emergency Pathological Service. He entered the Scientific Civil Service in 1947 and was appointed for duty as an experimental officer at the then Microbiological Research Department of the Ministry of Supply at Porton. He was promoted to the post of senior experimental officer in 1955. Throughout his service here he has worked entirely with pathogenic bacteria, initially in investigations of the relationship of nutrition to virulence in *Salmonella typhi* and afterwards, over the past twelve years, on the factors determining virulence in *Pasteurella pestis*. Largely through the devoted work of Mr. Bacon these factors, which initially were completely unknown, have now mostly been identified. This increase in our knowledge of the causative organism should permit a more rational approach to the future prophylactic immunization against plague than has been possible in the past. It is ironical, but by no means unprecedented in bacteriology, that one who had devoted such a substantial part of his working life to this end should have fallen victim to the disease he was helping to prevent in others. His death, however, has made us aware that we do not yet have a complete understanding of virulence and infectivity in this organism and that work on these subjects must continue. There is no doubt that Mr. Bacon was fully cognizant of the risks his work entailed, and these risks he willingly accepted.

Mr. Bacon was held in the highest regard by his colleagues. He may best be described as a gentle man who was always generous, kind and considerate and prepared to help others whenever possible His inventive skill applied to the improvement of laboratory techniques and apparatus was invaluable and possibly irreplaceable A safety pipetting device he designed to eliminate the hazard of aerosol production during pipetting operations has been in use throughout the Microbiological Research Establishment for the past ten years and no doubt has contributed to the excellent safety record of the Establishment

Mr Bacon's main interests outside his work were in his family, his new home and large garden and in the theatre The Salisbury Theatre Club and the Porton Musical and Dramatic Society have lost an ardent supporter, and the social life of the village of Whiteparish and of the Microbiological Research Establishment are the poorer by the death of Geoff Bacon Our sympathy is extended to Mrs Mary Bacon and to his daughters Elizabeth and Susan

T W Burrows

NEWS and VIEWS

U S National Academy of Sciences:

Foreign Associates

The following have been elected foreign associates of the U S National Academy of Sciences Prof E Amaldi, professor of experimental physics, University of Rome, Dr F Lynen, director of the Max-Planck-Institut fur Zellchemie, Munich, Sir William Penney, deputy chairman, U K Atomic Energy Authority, Prof R N Robertson, professor of botany, University of Adelaide

The Second Swinburne Award in Plastics

Dr J C Swallow

Dr John Cuthbert Swallow has been awarded the second Swinburne Medal by the Council of the Plastics Institute for his contribution to the discovery and development of polythene Dr Swallow was educated at the Universities of London and Leyden He joined Brunner Mond as a research chemist in 1924 and was in charge of polythene development work from the discovery to the building of the plant He became research manager of Alkali Division of Imperial Chemical Industries Ltd in 1941 and was appointed research director of the Plastics Division in 1942 Dr Swallow became managing director of the Plastics Division in 1951 and he has been chairman since 1952 Dr Swallow will deliver the Swinburne Award Address at the Royal Institution, 21 Albemarle Street, London, W 1, on November 12, when he will also be presented with the gold medal and the sum of money which accompanies the award The award, which has been endowed by Bakelite Ltd, was instituted in 1959 to commemorate Sir James Swinburne, who died at the age of 100 in the previous year The trust deed specifies the following terms of reference for the award " . to promote the advancement and diffusion of (*a*) the science and technology of plastics and of plastics engineering, and (*b*) science and technology in any field relating to plastics or to plastics engineering by making the award to persons who shall have made an outstanding contribution to such science or technology" The first award was made to Prof G Gee in July 1960

Harry Govier Seeley

Many interesting papers have appeared in the *Bulletin of the British Museum (Natural History)* and not least is one on "Harry Govier Seeley and the Karroo Reptiles" by Dr W E Swinton (3, No 1 Pp 1–39 London British Museum (Natural History), 1962 13*s*) The account of the life and activities of Seeley is an indication of the enormous energy and output of this remarkable man But Dr Swinton was especially fortunate in obtaining permission to publish a series of characteristic letters that Seeley wrote when he was in Africa They constitute a record of great historical and geological interest and throw much light on some of the fundamental fossils in the story of reptilian and mammalian evolution In addition there are interesting glimpses of life in South Africa in the latter part of the past century The paper also contains reproductions of some of the anatomical sketches by Seeley Dr Swinton was the obvious author for such a historical sketch and it was fortunate that he was able to complete the work before he went to Canada

The Panchromatograph

A new instrument for use in research and chemical analysis, the panchromatograph made by W G Pye and Co, Ltd, Cambridge, separates a mixture of liquids or gases into their component parts and gives a measure of the concentration of each The basis of operation of the panchromatograph is that a sample is fed into a column packed with special material and carried through in the stream of inert gas Separation is achieved so that the component parts of the sample emerge at intervals and are measured by a variety of detecting and recording systems The analyser unit is the result of extensive study of modern chromatographic requirements and has been designed to accommodate new advances of technology as they occur It is applicable to all analytical problems Columns and detectors are housed in separate ovens, both temperature-controlled to precise limits by electronic proportional counters The column oven is 42 5 × 28 × 16 cm and is of low thermal inertia construction A wide variety of columns can be accommodated The choice of column size and length is governed by the resolving power required by a given sample For the majority of applications 1 5 metre or 2 7 metre long, 4 mm diameter, glass columns are recommended Metal columns in lengths ranging from 0 3 to 6 m are also available The column units may be rapidly exchanged The detector oven is 23 × 23 × 16 cm and accommodates flame ionization, micro-argon ionization, macro-argon ionization, electron capture, photoionization, cross-section ionization, hot wire gas density and thermal conductivity detectors, together with the experimental triode ionization and electron mobility detectors An important feature is that any two detectors can be accommodated, allowing simultaneous use

The panchromatograph has applications in many fields, including medical research, petroleum refining, drug manufacture and food processing Separate pamphlets, obtainable from W G Pye and Co, Ltd, York Street, Cambridge, give details of the various accessories and the numerous applications

Management Science

DEVELOPMENTS in operational research and other sciences in management have been so rapid that it is not surprising to learn that the abstracts published by the International Literature Digest Service now run to 63 pages (*Operations Research/Management Science*, 2, No 1, 1962 75 dollars annually Published by Interscience Publishers Inc, 250 Fifth Avenue, New York 1, N Y Copies are available in the U K from Interscience Publishers Inc, 88–90 Chancery Lane, London, W C 2) Among the many sections are topics dealing with the management and conduct of operations research and related functions, optimal allocation, queueing, game theory, simulation models and Monte Carlo techniques, miscellaneous mathematical methods, probability and statistics and other methodology or special models

Ultra-sonic Cleansing

THE British Launderers' Research Association has continued work on a laboratory scale on the application of ultra-sonic or sonic energy to the cleansing of soiled fabric With the use of a piezo-electric type of transducer formed in the shape of a hollow roller, it has been found feasible to clean artificially soiled fine cotton fabric It is first soaked in a hot soap/metasilicate solution for 3 sec, mangled to remove excess moisture and then exposed to the sonic radiations for one second in a bath of cold soap/metasilicate solution The exposure times used make it possible to run the fabric round the transducer roll at speeds in the region of 100 ft per min Thicker fabrics require higher power per unit area to achieve the same degree of cleansing The energy supply has been taken from a General Electric Company valve generator and investigations are being made into the alternative use of a motor-driven alternator for producing electrical energy at a higher efficiency and at a lower cost Although at present there is no suggestion that ultrasonic or sonic energy could supplant the methods developed with the continuous processing machine, the results being achieved are sufficiently encouraging to warrant a continuation of this experimental programme It may well be that some form of ultrasonic cleansing will find a useful application in the removal of small areas of resistant soiling that might be left on an article after it has passed through a high-speed continuous-washing processing machine rather than return the article for a normal re-wash (Annual Report for the year 1960–61 Pp 32 Hendon, London British Launderers' Research Association, 1962)

Addiction-producing Drugs

CONTINUING its work of advising on notification, the World Health Organization Expert Committee on Addiction-producing Drugs recommends in its twelfth report exemption from the provisions of international control for oxpheneridine, (−)-3-hydroxy-*N*-propargyl-morphinan, metethoheptazine, metheptazine, and two preparations of diphenoxylate (*Technical Report Series* No 229 Pp 16 Geneva World Health Organization, London H M S O, 1962 1 Swiss franc, 1*s* 9*d*, 0 30 dollars) Four diphenoxylate preparations having now been recommended for exemption, the Committee considers it appropriate to propose a general exemption for such preparations provided they contain not more than a certain concentration of diphenoxylate On the other hand, noracymethadol is considered to be an addiction-producing drug comparable with morphine, while nicocodine and the four compounds designated by the committee as nethadone-intermediate, moramide-intermediate pethidine-intermediate-*A* and pethidine-intermediate-*B* are all found to be convertible into addiction-producing drugs and therefore subject to the relevant provisions of international control Concern is expressed about continuing traffic in heroin In some areas, limitation of the use of opium appears to have encouraged opium addicts to turn to heroin, which has been more readily available The controls on the illicit production of heroin and on the traffic in this drug need to be enforced more strictly Much stress is laid by the Committee on the necessity for providing the medical profession as early as possible with complete and accurate information on the addiction-producing and habit-forming properties of new drugs and on their therapeutic properties The further development of media for disseminating such information should be encouraged. The Committee noted that the Single Convention of Narcotic Drugs, drawn up in 1961, affords the World Health Organization the opportunity to initiate the examination of dangerous drugs and to adapt the extent of control to the degree of hazard to public health

Venereal Diseases and Treponematoses

IN 1961 the World Health Organization undertook a survey on the incidence of syphilis and gonorrhœa Thirty-one countries reported an increase of early infectious syphilis and 30 an increase of gonococcal infections between 1957 and 1960 The increase in syphilis in 1960, as compared with 1959, amounted to 19 per cent in Canada, 30 per cent in England and Wales, 45 per cent in the United States and 85 per cent in Denmark A similar increase was reported in other parts of the world Thus, in many countries, despite settled conditions, good health services and potent drugs, syphilis and gonorrhœa are again becoming a cause of concern to health administrations In the failure to control gonorrhœa, the gradual development of resistance of the gonococcus to penicillin in some countries and to streptomycin in others is significant, but probably not of basic importance Research carried out under auspices of the World Health Organization at the Gonococcus Centre of the Statens Serum-institut, Copenhagen, indicates that cases reported as 'resistant' are more often due to rapid re-infection than to gonococcal resistance to the drugs used (*WHO Chronicle,* 16, No 6, June 1962)

Activities against yaws continue to be aided by the Organization and by the U N International Children's Emergency Fund It is estimated that, since 1948, almost 280 million people have been examined and some 36 million people treated with long-acting penicillin in mass campaigns in Africa, the Americas and Asia, assisted by the World Health Organization The prevalence of clinical yaws has fallen in some countries from more than 10 to less than 0 5 per cent with a relatively small proportion of seroreactors in the younger age-groups By 1963 or 1964 many of the internationally assisted yaws campaigns will have passed into the surveillance phase During 1961, the World Health Organization started an appraisal of the results of the yaws campaigns and the methods used Special attention has been directed to the co-ordination of anti-yaws activities with those of other communicable disease control programmes and of the rural health services

Arterial Hypertension

ALTHOUGH there are, as yet, no effective means of preventing the occurrence of either arterial hypertension or ischæmic heart disease, much may be done in the individual case to delay or prevent the progress and complications of these diseases A report issued by the World Health Organization discusses the various preventive measures that can be applied (*Technical Report Series* No 231 *Arterial Hypertension and Ischæmic Heart Disease Preventive Aspects* Pp 28 Geneva World Health Organization, London H M S O, 1962 1 Swiss franc, 1*s* 9*d* , 0 30 dollars) It describes standard methods for determining blood pressure and classifies both arterial hypertension and ischæmic heart disease into their main clinical categories and stages, with the view of identifying those individuals likely to benefit from regular supervision and treatment A programme of research is suggested, and annexes appended to the report deal with criteria for the differential diagnosis of essential and renal hypertension and with methods for assessing renal function in hypertension

Cancer Research in Budapest

THE report of the Research Institute of Oncopathology, Budapest, for 1958–62 contains detailed accounts of the scientific work carried out at the Institute (Pp 24 Budapest Research Institute of Oncopathology, 1962) Among the main headlines under which the work of different sections is described are pathogenesis and morphogenesis, metastasis formation and how it is influenced, autolysis of tumour cells, chemotherapy, the lipid metabolism of tumours and clinico-pathology The report is published in English, and the Institute's address is Budapest, XII, Rath Gy, u 5, Hungary

Deprivation of Maternal Care

BOWLBY'S monograph, *Maternal Care and Mental Health,* was published by the World Health Organization in 1951, and was at once acclaimed as an unequalled contribution to this subject Its success is attested to by the frequency with which it has been reprinted and the number of languages into which it has been translated It has given wide currency to the term 'maternal deprivation' and has influenced the care given to children in institutions, residential nurseries and hospitals in many countries During the ten years that have elapsed since Bowlby's monograph was published, considerable research has been carried out into various aspects of maternal deprivation Bowlby's views have been subjected to much criticism, some of which is based on the often over-simplified, extreme or distorted interpretations of them prevalent in popular versions, but some of which is constructive and valuable The result has been that the problem of maternal deprivation can now be seen in a wider perspective than it was in 1951 A volume in the series *Public Health Papers* contains a group of papers reviewing the problem of maternal deprivation in this wider perspective (No 14 *Deprivation of Maternal Care a Reassessment of its Effects* Pp 165 Geneva World Health Organization, London H M S O, 1962 7 Swiss francs, 12*s* , 2 25 dollars) It contains contributions by psychiatrists and specialists in allied disciplines, and also by cultural anthropologists and a writer on social science The longest contribution is by a fellow-worker of Bowlby's, and as the latter has read this paper and agreed with it, it may be taken as representing his present views on maternal deprivation

Family Planning Association

THE remarkable progress of the Family Planning Association during the thirty-one years of its existence is emphasized in two notes in its annual report (Pp 26 Family Planning Association, 64 Sloane Street, London, S W 1, 1962) In its revised declaration on the Christian view of marriage and the family, the Methodist Church condemns careless and improvident begetting of children While recognizing that some Christians believe that the only legitimate means of conception control is abstinence from intercourse when a child is not desired, the Methodist Church believes that there are other permissible ways of preventing conception, provided that the means used are acceptable to both husband and wife and that, on the best evidence available, they do neither physical nor emotional harm The declaration goes on to say "When contraception is chosen as the means of family planning competent medical advice shall be sought, such as that provided by the Family Planning Association" Although during the past few years a number of encouraging statements on this subject have been made by responsible ecclesiastical bodies, the Methodist declaration is the first which has specifically mentioned the Family Planning Association The progress of the Association, as reflected by returns from its branches, is as follows

	1960	1961
No of clinics	340	366
No of sessions	20,733	22,246
Individual patients	212,324	284,163
No of visits or references	495,903	698,168
New patients	89,988	90,157
Pre-marital patients	14,120	16,452
Sub-fertility patients	1,567	1,836
Marital difficulty patients	1,329	1,465

The figures do not include the activities of the laboratories at the Association's Central Office Here, during the period under review, 2,132 sub-fertility patients were seen and more than 5,880 pregnancy diagnosis tests were carried out with specimens coming in from as far afield as Bahrein Island and the Persian Gulf In considering the figure of 16,452 patients, it is noteworthy that 380,000 women were married in England, Scotland and Wales during 1961 The number seeking advice is still very small

Planning the Future Metropolis

THE winter 1961 issue of *Daedalus*, the journal of the American Academy of Arts and Sciences, was devoted to "The Future Metropolis", including articles by R Vernon on the economics and finances of the large metropolis, A Fleisher on the influence of technology on urban forms, E C Banfield on the political implications of metropolitan growth, K W Deutsch on social communication and the metropolis, and J Dyckman on the changing uses of the city About a third of the *Proceedings of the American Philosophical Society* for June 29, 1962, is devoted to the allied theme of metropolitan planning Economic aspects of metropolitan planning are discussed by B Harris, transportation problems and their solution by R B Mitchell, the legal basis for reorganizing metropolitan areas in a free society by C Abrams, and urban form by G H Perkins

Research Careers in the Ministry of Defence, India

A BOOKLET issued by the Indian Ministry of Defence has the primary object of inviting graduates in technology and engineering, postgraduate research workers and other scientists and engineers, to consider the opportunities for taking up a research and development career in the Defence Research Laboratories/Establishments of the Research and Development Organization, as members of the Defence Science Service, India Research and development activities of the Organization cover a wide field and embrace subjects as different as, for example, explosives and psychology The researches are carried out either individually or by small teams in the Research and Development Laboratories/Establishments which are spread throughout India and have considerable resources The work is carried out in an atmosphere in which any enthusiastic person with a flair for research and inventive bent of mind would feel at ease The defence scientists get opportunities of giving lectures, attending conferences in India and abroad, participating in symposia, exchanging ideas through contact with scientists in universities and other establishments, and of becoming known in the scientific world generally. They are encouraged to publish papers and also to submit theses for postgraduate degrees on their research work when this is not of a secret nature, patents are taken out whenever permissible They may also be selected for attachment to establishments in India or abroad Copies of the booklet are obtainable from the Research and Development Organization, Ministry of Defence, "*G*" Block, Hastings Road, New Delhi-11

Analytical Methods for Fruit Juices

A VALUABLE compilation on *Analytical Methods for Fruit Juices* has recently been produced in Paris by the International Federation of the Producers of Fruit Juices It consists of methods of analysis acceptable to members from all countries affiliated to the Federation The project has been the responsibility of the Scientific and Technical Commission of the Federation, and the collaborative work has been organized by the Sub-Committee on Unification of Analytical Methods, of which Prof W Diemair of the University of Frankfurt is the chairman The brochure just published contains seven methods of analysis accompanied by drawings of all apparatus, graphs and tables necessary for the determination of specific gravity, alcohol, titratable acidity, sugar (before and after inversion), volatile acidity and total sulphur dioxide The methods are presented in French, German and English The complete compilation can be obtained, postage paid, for N F 15, from Fédération Internationale des Producteurs de jus de fruits, 16 rue de la Chaussée d'Antin, Paris (9°)

Excavation in New Zealand

ANY attempt at getting a relative chronology in New Zealand is always welcome, even when the various layers are of no great antiquity C D Smart and Roger C Green have recently been excavating a stratified dune site at Tairua, Coromandel, New Zealand (*Dominion Museum Records in Ethnology*, Vol 1, No 7 *A Stratified Dune Site at Tairua, Coromandel* By C D Smart and Roger C Green With a specialist report by J C Yaldwyn Pp 243–266 Wellington, N Z Dominion Museum, 1962) There is a specialist report on the fauna found, by John Yaldwyn The site is on the rocky islet of Paku at the mouth of the Tairua harbour There are no less than 9 layers, but only two are of archæological interest While there is no reason to think that any of the finds are earlier than the fourteenth century, the study of the so-called Archaic phase is much advanced as a result of this excavation The artefacts include drills, obsidian knives, scrapers, etc Among the bones, those of the moa have been identified

Australian Rock Carvings

IN Australia the archæologist has gone ahead of the quaternary geologist Much detailed study of the many rock carvings which occur has been made, but very little in the way of a relative and actual chronology has yet been attempted Among field workers the name of C P Mountford is well known In the July issue of *Man*, together with Robert Edwards, he describes a number of engravings and carvings allegedly representing marine animals from sites very remote from the sea It is suggested that they are of great antiquity, and date to a time when the sea was nearby If the identifications are correct it is now up to the geologist to say when the sea actually was nearby, or whether folk-memory must be invoked

Preserving Rural Wales

TESTAMENT to the work of so many devoted people in preserving the amenities of Wales, the combined annual reports for 1959–60 and 1960–61 of the Council for the Preservation of Rural Wales contain accounts of activities promoted to see that electricity transmissions are sited with discrimination, that town and country planning is carried out with a thought to the future, that historic buildings are not wantonly destroyed, that mineral undertakings are not only concerned with costs, that the beauty of the coasts are preserved and that adequate precautions are taken to prevent the spread of litter (Pp 93+2 plates Machynlleth, Montgomeryshire Council for the Preservation of Rural Wales, 1962) The reports also contain accounts of the best-kept village competitions A sad feature of the reports is the record of the small number of members in Monmouthshire

The Weizmann Institute of Science

THE report of scientific activities of the Weizmann Institute of Science for the two years 1960–61 gives short accounts of the work in progress in the various departments (Pp 151 Rehovoth The Weizmann Institute of Science, 1962) These include Applied Mathematics, Biophysics, Nuclear Magnetic Resonance, Nuclear Physics, Organic Chemistry, Plant Genetics, Polymer Research, X-Ray Crystallography, and the new Departments of Virology and Genetics, Cell Biology, Biodynamics and Carbohydrate Metabolism formed out of the Department of Experimental Biology during the period, as well as of the sections of Biochemistry, Electronics, Infra-red Spectroscopy, Isotope Research and Photochemistry and Spectroscopy To each of these is attached a list of publications, and there are also reports from the Graduate School in the Natural Sciences and the Library with a list of scientific staff of the Institute Among the numerous subjects under investigation it must suffice to mention the work on the biological synthesis of peptides and proteins, the catalysis of photophosphorylation by chloroplasts, on bacterial ribosomes, the physical chemistry and synthesis of ribonucleic acid, the synthesis and properties of low molecular weight peptides and polyamino-acids, polypeptidyl proteins, viruses and bacteria, synthetic polypeptide antigens, the structure and biological activity of proteins, biochemical aspects of urethane carcinogenesis, mechanism of epileptic behaviour, the chemistry of sphingolipids, effect of gibberellins on morphogenesis of *Cucumis sativis* and the properties of polyelectrolyte-salt solutions.

Organizing Scientific Research in Poland

The *Review of the Polish Academy of Sciences* for April–June 1962 contains an article by J Malecki describing the purpose and objects of the long-term plan for the Development of Science in Poland, the way work on the plan is being organized, and the measures being taken to develop a supply of the necessary scientific personnel and to organize the scientific research involved The plan envisages an increasing use of 'directed' research during the next twenty years, but although in this context the word 'directed' is used in much the sense in which 'applied' is used in Britain, it appears to imply a certain amount of direction at least to the extent of ensuring effective use of expensive equipment and the avoidance of duplication of effort, as is done by the grants from the Department of Scientific and Industrial Research Particular attention is also given to the encouragement of interdisciplinary effort It is proposed to rely on relatively small but highly specialized teams The same issue also includes an article by W Nowacki "Links between Science and Life" This discusses the general principles of scientific policy and the contribution of the Polish Academy of Sciences in particular, including the organization of fundamental and applied research and the problem of encouraging the application of results

Gordon Dean Memorial Fellowship Fund for Nuclear Science

A MEMORIAL fellowship fund has been created to honour the late Gordon Dean, former chairman of the U S Atomic Energy Commission and co-founder of the Nuclear Science and Engineering Corporation Former President Harry S Truman has been elected honorary chairman of the Gordon Dean Memorial Fund, which will grant scholarships and fellowships for study and research in nuclear science Until his untimely death in 1958 in a plane crash, Mr Dean served as chairman of the board at Nuclear Science and Engineering, which he founded with Mr Brightsen in 1954 At the time of his death, he was also senior vice-president of General Dynamics Corporation and an adviser on atomic energy to the investment banking firm of Lehman Brothers Further information can be obtained from the Nuclear Science and Engineering Corporation, P O Box 10901, Pittsburgh 36, Pennsylvania

The Night Sky in October

FULL moon occurs on Oct 13d 12h 33m U T and new moon on Oct 28d 13h 05m The following conjunctions with the Moon take place Oct 2d 09h, Venus 11° S, Oct 8d 11h, Saturn 0 9° S, Oct 10d 11h, Jupiter 1° N, Oct 21d 01h, Mars 1° N In addition to these conjunctions with the Moon, Mercury is in conjunction with Spica on Oct 30d 21h, Mercury being 4° N Mercury is a morning star, visible low in the south-east before sunrise, and may be favourably observed during the later part of the month when it rises nearly two hours before the Sun Venus is too close to the Sun for easy observation Mars rises at 22h 55m, 22h 40m and 22h 25m on Oct 1, 15 and 31, respectively It is at a distance of 129 million miles on October 15, and its stellar magnitude is +0 9 Jupiter sets at 2h 50m, 1h 50m and 0h 50m at the beginning, middle and end of the month, respectively It is in Aquarius, its stellar magnitude is −2 3 and its distance from the Earth in the middle of the month is 396 million miles Saturn is in Capricornus, setting at 0h 00m, 23h 05m and 22h 05m on Oct 1, 15 and 31, respectively Its stellar magnitude is +0 7 and its distance from the Earth on October 15 is 898 million miles Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich Oct 9d 18h 09 4m, δ Cap (*R*), Oct 14d 22h 12 5m, μ Cet (*D*), Oct 14d 22h 53 6m, μ Cet (*R*), Oct 21d 00h 26 6m, θ Cnc (*R*) *D* and *R* refer to disappearance and reappearance, respectively The Orionid meteors are active during October 15–25 but conditions for observation are unfavourable

Announcements

OPEN DAYS will be held at the National Chemical Laboratory, Teddington, on September 26 and 27 Further information can be obtained from the Chief Press Officer, Department of Scientific and Industrial Research, State House, High Holborn, London, W C 1

THE second annual meeting of the American Society for Cell Biology will be held at the Jack Tar Hotel, San Francisco, during November 5–7 Further information can be obtained from Dr M Alfert, Department of Zoology, University of California, Berkeley 4, California

A SYMPOSIUM on "Fungi and their Metabolites" is to be held under the auspices of the Plant Phenolics Group and the British Mycological Society in the School of Pharmacy, London, W C 1, on October 1 and 2 Further information can be obtained from Dr J G Manners, Department of Botany, The University, Southampton

THE third Rapkine Memorial Lecture of the Institute of Biology will be delivered by Prof F Morel, of the French Centre for Nuclear Studies, Saclay, on October 8, at the French Institute, Queensberry Place, London, S W 7 Further information can be obtained from the General Secretary, Institute of Biology, 41 Queen's Gate, London, S W 7

THE twelfth Dalton Lecture of the Royal Institute of Chemistry will be delivered by Sir Lawrence Bragg on October 24 in the Town Hall, Manchester The title of Sir Lawrence's lecture will be "Living Molecules" Further information can be obtained from Mr H H Armstrong, Science Department, College for Further Education, Wellington Road South, Stockport

DR J H VAN DER WAALS, Koninklijke/Shell Laboratorium, Amsterdam, will deliver the following Bourke Lectures of the Faraday Society "Study of the Phosphorescent State of Organic Molecules by Electron Resonance" (University of Nottingham on October 23 and University of Sheffield on October 26), "Clathrates as Elementary Solutions" (University of Oxford on October 30) Further information can be obtained from the Faraday Society, 6 Gray's Inn Square, London, W C 1

ERRATUM In the communication entitled "Stomatal Sensitivity to Low-Intensity Illumination in *Xanthium pennsylvanicum*", by Dr T A Mansfield, which appeared on p 514 of the August 4 issue of *Nature*, the second sentence of the first paragraph should read, "The rhythm was shown 10 lux given instead of darkness was sufficient to delay its progress"

SCIENTIFIC AND TECHNICAL RESEARCH IN ARGENTINA

THE annual report (February 1, 1961—January 31, 1962) of the National Council for Scientific and Technical Research of the Argentine Republic has recently been published

One of the main activities of the Council continues to be the award of fellowships, with the object of training for research In the period covered by the report, 146 fellowships were granted, 53 of them to carry out research projects in scientific institutions abroad and 93 for the same purpose in Argentina, apart from numerous extensions of fellowships granted the previous year The total amount of fellowships awarded by the Council during the four years of its existence is 571, of which 319 were awarded for research carried out in Argentina and 252 abroad Reports received from universities and research centres of the United States, Great Britain, France and other countries which have received Council Fellows emphasize the high standard and achievements of these men

During the period covered by the report, the Council has intensified its aid to different research projects by means of grants The total amount granted exceeded 80 million Argentine pesos

Grants are awarded primarily for the purchase of scientific equipment and to finance research projects In some cases they have been awarded for the building of new laboratories, for example, the Institute of Computing, Faculty of Exact and Natural Sciences, University of Buenos Aires, the Radiocarbon Dating Laboratory for Archæological and Geological Research, being installed at La Plata University as a joint project of the Council and the former, the Laboratory of Neurophysiology, already operating in the Institute of Experimental Biology and Medicine, located in Buenos Aires The Council has also contributed towards equipping and staffing the Town Planning Institute of the Universidad del Litoral, in Rosario In all such cases and other similar ones, the Council, while awarding the funds for the creation of new research centres, resolved that they should operate under the ægis of other institutions

Lately, however, the Council has decided to create some research centres under its own jurisdiction This is considered a fitting way to establish new research facilities for Argentine scientists, and will permit a better co-ordination of efforts in certain fields, apart from filling some scientific gaps The aim is to organize a few model institutes, and to this end numerous projects are actually under investigation by the Council

In another section of the report it is mentioned that during 1961 the 'Research Scientist's Career' was put into operation This programme has been established by the Council with the object of fostering full-time dedication to original research, offering investigators better conditions than heretofore from the point of view of financial security and advancement, while tending to making research an attractive proposition for young graduates with a scientific vocation Registration was opened by mid-1960, and applications filed up to the end of March 1962 totalled 494 of which 276 applicants were admitted. Of these, about 200 scientists have already complied with the necessary formalities and are actively engaged in research work within the scheme Applications continue to be filed in considerable numbers, revealing a healthy interest in scientific research among young university graduates

The Council has continued with its programme of inducing qualified Argentine research scientists settled abroad to return to the country The report points out that it has been possible to retrieve Argentine men of science who held important positions in well-known foreign institutions by using funds earmarked for repatriation purposes Increasingly favourable conditions for scientific research which have been created in the country during the past few years have, of course, played a predominant part

During 1961, the Council considerably stepped up the programme aiming at the improvement of teaching scientific subjects at high-school level With this object in mind, the Council has made available to secondary school science teachers the opportunity of improving their knowledge by attending refresher courses in the country and also abroad A physics summer course for secondary school teachers, selected from all over the country, was held at the beginning of 1961 Four more courses were organized for the summer of 1962, in mathematics, physics, chemistry and biology A total of 123 high-school teachers attended these courses

During 1961, two Argentine high-school teachers (mathematics and physics) attended the Oak Ridge Institute of Nuclear Studies, in Tennessee, while six others attended summer school courses organized by the U S National Science Foundation It has also been possible to send some Argentine high-school teachers to refresher courses organized by the Pan-American Union in other Latin-American countries

Simultaneously, efforts are being made by the Council to bring up to date science teaching at secondary school level in Argentina and also to foster the interest of high-school graduates in scientific careers

Mention is made of the different services rendered to research scientists by the Council, such as making available scientific information by means of microfilm, photostats, publications, etc

Another chapter of the report refers to international scientific relations and contacts established by the Council It is stated that the Council is the national member of the International Council of Scientific Unions (ICSU), the International Council for Medical Sciences (CIOMS), the International Committee on Space Research (COSPAR) and the International Federation of Documentation (FID)

Another aspect of contacts with scientific circles abroad are the invitations extended by the Council to foreign scientists of international standing in order to lecture in Argentina or to act in an advisory capacity on research projects Argentine delegates are also sent by the Council to many international scientific meetings It is pointed out that the Council finances the attendance of Argentine scientists at international meetings when certain conditions are fulfilled by the candidates, such as authorship of a paper on original research work which has gained acceptance by the organizing committee of the meeting Due emphasis is given to the generous aid received from the Ford and Rockefeller Foundations of the United States which has enabled the Council to extend considerably its fellowship programme and has helped to finance some of the activities already mentioned

A brief analysis of the Council's budget for the fiscal year which ended on October 31, 1961, shows that the amount budgeted was Argentine pesos 211,386,009,90 (about £1 million at the then prevailing rate of exchange) Expenditures for the same period did not amount to the total budgeted, and it is noteworthy that administrative expenses amounted to only 8 per cent of the total expenditure

During 1961, the Council resolved to buy a building in order to have more adequate premises The future address is Rivadavia 1917, Buenos Aires, Argentina, where the Council expects to move before the end of the year, once the necessary alterations have been completed

In the final part of the report, overall aspects of the Council's task are given consideration They can be summed up by the considerable increase of young scientists adequately trained for research, the larger volume of research work undertaken in Argentina with the aid of Council funds granted to institutions and scientists, the establishing of new research teams and research centres and the higher level attained by research work in different fields It is emphasized that the activity of the Council, combined with that of some other institutions carried out along similar lines in the past few years, has established a most favourable environment for original and creative scientific research by fostering the general interest in scientific activity and obtaining increased aid for its continuation from official quarters as well as from the general public

CYTOCHEMICAL PROGRESS IN ELECTRON MICROSCOPY

THE symposium on "Cytochemical Progress in Electron Microscopy", which was held by the Royal Microscopical Society in Oxford during July 2–4, was a success both in the size of the attendance and the interest of the topics The majority of those attending were British, but several papers were submitted from France and others from Belgium, the United States, Holland and Sweden

The information presented fell into four categories (1) Effects of different fixatives, embedding media and electron-absorbing 'stains' on morphological detail (2) Sub-microscopic localization of enzymes utilizing electron-absorbing cytochemical reaction products (3) The effects on morphological detail of extraction by chemical or enzymatic reagents (4) Autoradiography at electron-microscope magnifications It is a pity that the symposium was not organized to present consecutively the papers covered by these or similar headings As it was, the fragmentation of discussion of any one topic prevented the important points resulting from it from being clearly presented to the non-specialist in the audience

Under the first of the headings which I have chosen here, Dr W Bernhard and Dr Elizabeth Leduc (Villejuif, France) presented beautiful pictures of the striking difference in appearance produced in tissues embedded in water-soluble glycol-methacrylate, compared with those embedded in media soluble only in organic solvents These results were enlarged on by Dr V Marinozzi (Villejuif), who presented much useful information on differences produced by fixation in various solutions and embedding in different non-water-soluble media Much technical information will be available as a result of the investigations of Dr J Sikorski (Leeds) on keratinous cells, of Dr J R Casely-Smith (Villejuif) on fixation and staining of lipids, and of Dr E H Mercer (London) and Drs W Th Daems and J P Persijn (Leyden, Holland) on 'stains' for electron microscopy The papers of Drs E L Benedetti and B Bertolini (Amsterdam), on the use of phosphotungstic acid as a stain for the plasma membrane, and Dr R L Tapp (Cambridge), on the fine structure of desmosomes after a variety of techniques, illustrated the necessity of studying cell structure after using a variety of methods of preparation before attempting to interpret the functional significance of the details detected

The value of using light-microscopy to investigate material and the changes which occur in it during preparation for electron microscopy was underlined by the papers of Prof N A Barnicott (London) and Dr H E Huxley (Cambridge) on their investigations of chromosomes and Dr H G Davies (London) on his experiments with erythrocyte nuclei It is to be hoped that the structureless nature of the chromosomes which was so beautifully displayed may be due to fixation artefacts as was suggested by the information provided by Dr B M Richards (London) in the discussion

The most striking results in cytochemical investigations at the electron-microscope level have been those of the American and German workers using enzyme reaction products containing heavy metals In the absence of these original contributors Dr A G E Pearse (London) gave a lucid account of the possibilities of this approach He pointed out the incompatibility of good osmium fixation and the preservation of some enzymes, but also indicated that a method giving a bad picture at the light-microscope level might give a good one in the electron microscope He also warned that much shorter incubation of tissues in substrates would probably be required if good electron micrographs devoid of diffusion of reaction product were to be obtained Drs S J Holt and Marian Hicks (London) illustrated many of these points with their paper on the localization of acid phosphatase in lysosome-like particles Drs G de Thé, A B Novikoff and J W Beard (North Carolina) showed among other things that virus particles on entering and leaving a cell not only acquired a covering of part of the cellular membranes but also the adenosine triphosphatase activity associated with these membranes It is this sort of result which makes the detailed work of cytochemistry at the electron-microscope level worth while Dr B Boothroyd (Liverpool), in his paper on the effect of electron irradiation on glycogen, showed pictures of this polysaccharide stained by the periodic acid–Schiff technique, which illustrates that deposition of sufficient organic stain at a locus may sufficiently raise its electron-absorbing properties as to give good contrast in the electron microscope This has already been found to be the case at ultra-soft X-ray wave-lengths, and some preliminary work suggests that heavy metals need not be incorporated into enzyme reaction products in order to make them absorb electrons sufficiently

The use of extraction methods, and biochemical and cytological control of the extraction process, were well illustrated by Drs S and M Nass (Stockholm), who postulated that a deoxyribonucleic acid-like material was present in mitochondria Drs R R Dourmashkin, R M Dougherty and R J C Harris (London) presented their superb pictures of the pits or holes in virus and cell membranes after these had been extracted with saponin and digitonin

The introductory review by Dr S R Pelc (London) on the theory of autoradiography presented the background to the limitations of the use of conventional photographic emulsions in studying isotope localization with the electron microscope Drs G A Meek (Oxford) and M J Moses (North Carolina) presented excellent pictures of the localization of ^{3}H-thymidine in HeLa cells Dr P Granboulan (Villejuif) discussed the resolving power and sensitivity of some new emulsions and showed autoradiographs with extremely small grains (0 1μ) Dr H Firket (Liège), working in conjunction with Dr Granboulan, showed the result of the use of these emulsions in studying the incorporation of ^{3}H-uridine in cells A lively discussion followed all these papers and the questions of resolution (spatial relation between developed grain and emitting particle) and the probability of biological significance of a few developed grains overlying cellular structures were widely debated It appears that progress in this branch of electron microscope cytochemistry will depend on the exploitation of photosensitive layers other than silver halide–gelatin emulsions and the use of less-conventional methods of development These methods have been described in the literature of photographic processes

Presentation of the use of the electron microscope for studying the localization of antigens in cells by staining them with antibody conjugated to ferritin was made by Miss Jane Baxandall and Drs P Perlmann and B A Afzelius (Stockholm) and by Dr Margaret Mott (Edinburgh) These methods appear to permit localization of substance on the surface of the cells, but the contrast of the conjugate is so low as to give a rather unconvincing picture This is another case where 'chromaticity' in the electron microscope would be useful in identifying the iron component of the conjugate

The contrast of the modern approach used by the contributors and the near-medieval surroundings in which they were lodged during the conference was even greater than that between the excellent new lecture theatre in which the scientific sessions were held and the fabric of the University Museum which contains it It is to be hoped that the Royal Microscopical Society will before long present another symposium of such topical interest

The communications will be published in detail in the Society's *Journal* by the end of this year A J H

RADIO RESEARCH 1961

IN the previous report of the Radio Research Board, it was noted that Mr J A Ratcliffe took over his duties as director of the Radio Research Station on October 1, 1961 (see *Nature*, 185, 354, 1960 191, 1255, 1961) The report for 1961 has now become available*

This report describes the action that has been taken to implement earlier decisions to devote half the effort available to the Radio Research Station to a programme of space research and by this is meant the use of sounding rockets and satellites to carry out fundamental investigations of the upper atmosphere and ionosphere, and of the factors which determine the behaviour of these media for transmitting radio waves

For many years past the work of the Radio Research Station has included detailed measurements of the properties of the ionosphere and the application of the results to the study of the propagation of high-frequency radio waves over long distances Charts are now available giving detailed characteristics of the *F* region of the ionosphere for the conditions of maximum and minimum solar activity By sensible extrapolation, these can be used to calculate the transmission characteristics required for various point-to-point communication services Experiments are in hand to check these calculations by a pulse transmission technique between Slough and New Delhi—a distance of some 7,000 km

In parallel with this work, which is based on investigations of the ionosphere from below, the facilities provided by radio transmissions from rockets and satellites are now being used to explore the ionosphere above the height of peak ionization Two methods are in use for observing the waves transmitted downwards from an artificial satellite at a height of several hundred kilometres In the first, measurements are made at Slough and Singapore of the Faraday rotation of the plane of polarization of a wave transmitted through the ionosphere The second method in use at Slough records the Doppler shift on two harmonically related frequencies due to the motion of the satellite The result is used to calculate the total electron content in the ionosphere

This work makes use of the facilities now availabe at Winkfield, near Slough, for the tracking of American satellites and the recording of the telemetry signals received from them The same equipments, together with the corresponding installations at Singapore and Port Stanley, are being used for co-operating in the British experiments now in progress with the American satellite *S*-51, which was planned for investigations organized by the British National Committee on Space Research Preparations are also well advanced to enable all three of the above stations to participate in the reception of the telemetry signals from the United States and Canadian 'topside sounding' satellites The purpose of these satellites, expected to be launched in the near future, is to study the characteristics of the upper part of the ionosphere by radio-wave sounding from heights well above that of the peak ionization

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* Department of Scientific and Industrial Research Radio Research 1961 The Report of the Radio Research Board and the Report of the Director of Radio Research Pp iv+27+4 plates. (London H M S O , 1962) 3s net

Investigations of the characteristics of the troposphere continued to be conducted by staff of the Radio Research Station by several different methods The micro-wave radio refractometer carried in an aircraft, as described in previous reports, has been used to record the gradient of refractive index at various heights and in several cases these have been correlated with the existence of layer-type echoes observed by vertical-sounding radar technique The process of the scattering of radio waves by discrete layers or other types of discontinuity in the troposphere has been studied by measuring the variations in direction of transmission of a narrow beam of waves To some extent, the results have been related to the prevailing meteorological conditions, taking advantage of information, when available, obtained from radio-sonde ascents

A programme of international intercomparison of reference standards for measuring power at microwave frequencies has been in progress for several years A wire bolometer transfer standard for use at a frequency of about 9 Gc/s, which had been calibrated by microcalorimeter reference standards in the United States and in Japan, was checked against a film bolometer standard developed at the Radio Research Station The results were in agreement with the American and Japanese reference standards to within ± 0 9 per cent At a lower frequency of 300 Mc/s, a coaxial transfer standard submitted by the National Bureau of Standards, Boulder Laboratories, was compared with two instruments at Slough a coaxial thermionic diode and a thermistor milliwattmeter The measurements made in the United States and United Kingdom agreed to within 0 3 per cent, which is well within the limits of error assigned to any one of the reference standards The various techniques now available in the United Kingdom make it possible to carry out measurements of power above a few milliwatts at frequencies up to 30 Gc/s Power measurement at higher frequencies probably depends on the development of new techniques

As in the case of previous reports, appendixes give details of the Committees of the Radio Research Board, the staff of the Radio Research Station and the papers published by them during 1961.

SATELLITE METEOROLOGY

SINCE the first observations commenced to be received from *Tiros* 1 there has been much speculation concerning the optimum utilization of meteorological satellites in meteorological operations and research In particular, it is reasonable to inquire whether the enthusiasm and novelty which these spectacular technical advancements have aroused will gradually fade away with the realization that, however spectacular they may be, their real value is limited, or whether the observations will represent a true potential for meteorological operations and research

It has always been appreciated that the main practical value of the observations lies in the use which can be made of cloud picture mosaics in regions where the supply of weather reports from the normal station network is inadequate This is especially so over the huge extent of the oceans of the globe, much of which is only sparsely threaded with ships' trading routes, assisted at a few strategic places by ocean weather stations The identification of incipient, or even fully developed, tropical cyclones before they have been picked up by the conventional observational network represents an outstanding and much publicized example of the great value which cloud observations from satellites can be to national meteorological services charged with giving timely preventative warnings to communities likely to be affected by these devastating storms But such occurrences are relatively rare in the spectrum of meteorological events, and it is hoped that more lasting and regular returns will be received from these expensive observations

There are many less-obvious uses of observations from meteorological satellites The photographs themselves can reveal the distribution of snow and ice-fields in addition to cloud cover Photographs of snow-covered land masses can, for example, help to provide information about water resources At night, when clouds are not properly visible, an approximate distribution of the cloud pattern may be obtained from infra-red radiation measurements Such radiation suffers little absorption during its passage through the atmosphere and enables an estimate to be made of the temperature of the radiating surface Clouds are thus revealed as cold areas against the warmer background of the Earth's surface The temperature pattern of the upper cloud surface may yield an index of the contours of that surface

Future developments may enable measurements to be made in the centimetre wave-length band which would identify precipitation areas and freezing-levels by radar techniques It should also be possible for observations of lightning to be made, either by visible flashes or by sferics discharges Finally, it has been suggested that the satellites might be used for global communications to relay pressure, temperature and wind data made by automatic weather stations in oceans and remote land areas

The United Nations General Assembly has shown an awareness of the international aspects of the question in passing a resolution dealing with International Co-operation in the Peaceful Uses of Outer Space A section of this resolution of December 1961 includes a request that the World Meteorological Organization should report to its member Governments on the appropriate organizational and financial arrangements (1) to provide a greater knowledge of the basic physical forces affecting climate and the possibility of large-scale weather modifications, (2) to help members to make the utmost use of existing weather forecasting possibilities

The report has now been prepared*, it lists several basic requirements to translate the potential of weather satellites into a working scheme

There is first a need for an internationally coordinated plan to achieve agreed decisions concerning satellite orbits, types of observations to be made collection and dissemination of data and standardization of accuracies The plan should provide for research on the use of the data received The most important recommendation in the report is no doubt the call for a 'world weather watch', a global meteorological observing and prediction system designed to avoid duplication of effort in the preparation of analysed charts and forecasts, yet providing all meteorological services with the basic data needed to carry out their responsibilities The report also proposes that the scope and quality of both conventional and satellite observations should be improved and emphasizes that the latter supplement but do not supplant the former Optimum results can be achieved by means of a combination of the wide scanning ability of the satellites with the capabilities of the usual vertical sounding instruments Improvements should also be made to the communication facilities to permit the rapid transmission of a greatly increased volume of meteorological traffic throughout the world The hope is expressed that the satellites themselves may serve as communication systems for this purpose

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* *WMO Bulletin*, 11, No 3, July 196

The evaluation of the optimum utilization of meteorological satellites is an important issue that must be faced The United Nations and its specialized agency, the World Meteorological Organization, have taken a big step forward The working group which has been established to formulate an atmospheric sciences programme will keep the matter under active review until the World Meteorological Organization Congress constitutes an advisory committee at its fourth session in April 1963 There is a long road to tread before the full potential of meteorological satellites will come to fruition A H GORDON

FILARIASIS

DURING the past few years, filariasis control has progressed to the point where it has become possible to look forward to the introduction of eradication campaigns like that organized by the World Health Organization for malaria In contrast to the malaria programmes, however, the first line of attack would have to be mass chemotherapy, mainly because of the long life of the parasite in the human body To keep transmission at a low enough level to ensure eradication, chemotherapy will have to be backed up by adequate vector control This may not prove easy, since the culicine mosquitoes, the main vectors of filariasis, rapidly acquire resistance to the commonly used insecticides and since the habitats of some of the mosquitoes make it difficult to attack them by conventional methods

The World Health Organization Expert Committee on Filariasis has reviewed recent advances in the epidemiology of *Wuchereria* and *Brugia* infections and directed attention to the many problems on which research is still needed* It is important to improve methods of identifying developing forms of filariæ in mosquitoes, so that carriers of animal filariæ should not be mistaken for vectors of the human infection Expert taxonomic, ecological and other studies are needed to establish the strains with the highest infective potential and the most effective means of destroying them Rapid urbanization has enabled *Culex fatigans* to breed apace in some areas with only primitive sanitation, and the situation has been made worse where insecticide spraying campaigns for malaria eradication have eliminated competitors of this mosquito The Committee recommends that, wherever possible, *Culex fatigans* should be attacked by improvements in sanitation aimed at suppressing man-made breeding sites

Diethylcarbamazine appears to be still the most satisfactory drug available for mass chemotherapy, but a number of new compounds containing arsenic or antimony are at present under trial Further research is needed to establish the best dosage schedule for diethylcarbamazine—one that, in order to ensure a high acceptability, combines effectiveness with ease of administration and relative freedom from side-effects

Among the other subjects suggested for research, the Committee advocates studies on transmission and on the life-cycle of the parasite in relation to clinical manifestations, serological and immunological studies on filariasis, and an investigation of the effect of malaria eradication activities on the local epidemiology of filariasis

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* World Health Organization Technical Report Series No 233 Expert Committee on Filariasis (*Wuchereria* and *Brugia* Infections)—Report Pp 49 (Geneva World Health Organization, London H M Stationery Office, 1962) 2 Swiss francs, 3*s* 6*d*, 0 60 dollars

INHERITANCE IN BACTERIA

AMONG the wide variety of research activities described in the report of the Governing Body of the Lister Institute of Preventive Medicine for 1962*, that of the investigations of the Guiness–Lister Unit into inheritance in bacteria is of particular interest. The Unit continued its investigation of inheritance in bacteria of the *Salmonella* (food poisoning and enteric fever) groups, using two different kinds of fertility factor to induce conjugation the colicine factors, which determine the production of antibiotics called colicines, and the *F* factor and some variants of it, transferred from *Escherichia coli*

S Smith continued to map the chromosome of *Salmonella typhimurium*, obtaining conjugation (and hybridization) by the use of colicine factors *col I* and *col EI* Additional hereditary characters were introduced into the strains used, by the isolation of mutants unable to synthesize particular amino-acids, to ferment particular sugars or to produce the fraction of the polysaccharide component of the O antigen factor 5 Many of these mutants were obtained by treating bacteria with the mutagen ethyl methane sulphonate The genes regulating all the characters concerned were mapped in the single closed-loop linkage map already tentatively established Dr B A D Stocker, E Subbaiah, and E. Dubnau, using the same system, investigated the segregation of additional characters resulting from mutation in the laboratory or pre-existing in strains isolated from natural sources

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* The Lister Institute of Preventive Medicine Report of the Governing Body 1962 Pp 29 (London The Lister Institute of Preventive Medicine, 1962)

From analogy with the situation in mating of *Escherichia coli* resulting from activity of the fertility factor *F*, it was suspected that, in conjugation of *Salmonella* induced by colicine factors, the partner carrying the colicine factor acts as a 'male' and injects a part or perhaps the whole of its chromosome into the cytoplasm of the other, 'female', partner Some data on frequency of various recombinant classes cast doubt on this idea, and suggested that perhaps the partner which was lacking the colicine factors sometimes acted as 'male' S Smith, in a further investigation of crosses in which a reversal of sexual roles was suspected, has now shown that if in such a cross the partner carrying the colicine factors is resistant to streptomycin and the other partner sensitive, exposure to streptomycin prevents the appearance of nearly all the streptomycin-resistant recombinants which develop in the absence of the antibiotic This is the result expected if only the colicogenic partner can inject its chromosome into its partner, for sensitive 'female' bacteria which had received a chromosome fragment from the streptomycin-resistant colicinogenic partner would be killed by the antibiotic, even when the injected chromosome fragment carried the gene for resistance to streptomycin, because of the streptomycin-sensitivity of their own cytoplasm

Subbaiah and Stocker established that two genes, regulating, respectively, ability to ferment rhamnose and ability to produce the filamentous bacterial appendages, called fimbriæ, are well separated in the chromosome and functionally independent, despite the almost complete correlation of the two characters in 'wild' strains of *Salmonella typhimurium* Now that the chromosome of *Salmonella typhimurium* has been reasonably well mapped, new investigations of the genetics of virulence in this species have been begun by Subbaiah As a start he has isolated a series of 'rough' mutants, in strain *LT*2, as the 'rough' character entails loss of virulence

GROWTH OF A CLONED STRAIN OF HAMSTER KIDNEY CELLS IN SUSPENDED CULTURES AND THEIR SUSCEPTIBILITY TO THE VIRUS OF FOOT-AND-MOUTH DISEASE

By P. B CAPSTICK, R C TELLING, W. G. CHAPMAN and DOREEN L. STEWART

Research Institute (Animal Virus Diseases), Pirbright

MACPHERSON and Stoker[1] have described the origin of the baby hamster kidney cell strain 21 (*BHK 21*), and Mowat and Chapman[2] the susceptibility of the strain to the virus of foot-and-mouth disease (*FMD*) in monolayer cultures All experiments so far reported with this cell strain have utilized cells grown in monolayer cultures The advantages of suspended cell culture techniques are obvious if the cell is to be used for large-scale vaccine production or even for extensive laboratory use in plaque assay systems The experiments described here demonstrate the growth of a cloned strain of these cells (clone 13) in suspended cell culture

Culture of cells in suspension was carried out in cylindrical 'Pyrex' glass vessels, 3 in in diameter, 10 in in height, with two glass entry ports in the top end The volume of culture used was 200 ml, which occupied about one-third of the vessels The cultures were stirred by central 'Teflon'-coated bar magnets rotated at 335 r p m by mechanically propelled external magnets The vessels were held in a 37° C copper water bath They were sealed with glass stoppers after seeding and no aeration was attempted Slight gas exchange with the atmosphere occurred when the cultures were sampled

The medium consisted of Eagle's basal medium modified to contain twice the normal concentration of amino-acids and vitamins with 10 per cent Seitz-filtered bovine serum and 10 per cent tryptose phosphate broth Antibiotics were incorporated to final concentrations per ml of 100 units penicillin and streptomycin, 25 units mycostatin, and 70 units neomycin

BHK 21 clone 13 cells which had had 110 monolayer transfers were suspended in the foregoing medium and added to the vessels, the contents of which were collected every 2 days or when the cell concentration in the vessels ceased to increase During the first 21 days in culture, growth was irregular with considerable clumping of cells and growth on the sides of the vessels This was counteracted by collecting the cells every 24 h and resuspending them in 'Versene'–trypsin (0 01 per cent) mixture in the vessels for 1 h The 'Versene'–trypsin was then removed and fresh medium substituted After this initial period, regular growth occurred and we were able to collect cells from the vessels every 48 h with a constant cell yield of 2 to 3 6 × $10^{8\ 0}$ cells per vessel Cell clumping was still a problem although there was little growth on the sides of vessels that had been in use for six days or more Better assessments of the number of cells in a vessel were obtained by collecting the contents and subjecting both cells and vessel to the 'Versene'–trypsin treatment described above

The results of a typical growth-cycle are shown in Fig 1, from which it can be seen that from a seed of 3 5 × $10^{5\ 0}$ cells per ml a final concentration of 1·65 × $10^{6\ 0}$ per ml was achieved in 40 h This figure also demonstrates that the growth-curve was linear in character, and this is presumptive evidence that a rate-limiting factor or factors was operating Further investigations on the nature of these limiting factors is being carried out

The work of Hayflick and Moorhead[3] on human diploid cell strains suggests that, to achieve successful growth in suspended culture, a diploid cell strain must change its chromosome karyotype from diploid to heteroploid and that a change may occur in the range of viruses to which the cell is susceptible We have not yet observed these changes with *BHK 21*

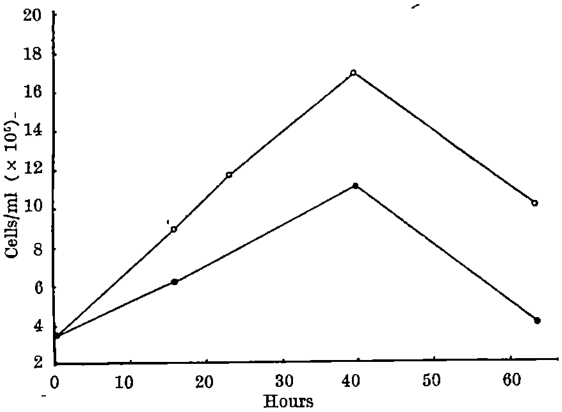

Fig 1 Growth of *BHK 21* clone 13 cells in suspension ●, Mean cell counts from replicate vessels before 'Versene'/trypsin action, ○, mean cell counts from replicate vessels after 'Versene'/trypsin action

clone 13 cells in suspended culture Table 1 shows the chromosome analyses of cells grown in monolayers and of the same cells after a further 66 days growth in suspended culture

Table 1 CHROMOSOME KARYOTYPE AND CELL SIZE OF *BHK 21* CLONE 13 CELLS GROWN IN MONOLAYERS AND AFTER A FURTHER 66 DAYS GROWTH IN SUSPENDED CULTURE

Cell passage history	Nuclei of diploid karyotype (per cent)	Nuclei of heteroploid karyotype (per cent)	Total mitotic nuclei determined	Modal cell size
110 monolayer transfers	93 1	6 9	350	13μ
110 monolayer transfers plus 66 days in suspended culture	91 8	8 2	777	13μ

Comparisons of *FMD* virus titres assayed in mice and on monolayers produced from suspended cells and monolayer-passaged cells are shown in Table 2 in which it can be seen that cell sensitivity has altered for some strains of *FMD* virus, although plaques of the same size were formed on both types of monolayers This change may be due to the fact that we have not yet determined the best method for using suspended cells in assay systems At the moment, these cells are being used under conditions which were found to give maximum sensitivity with cells produced in monolayers We have noted, however, that infection of roller tube monolayers prepared from suspended cells always results in the destruction of all the cells in the monolayer Thus, while cell sensitivity may be reduced, the cells appear to remain susceptible to higher concentrations of virus and are probably suitable for vaccine production Further work is in progress on the yields of virus per cell

Table 2 TITRATION OF FOUR STRAINS OF *FMD* VIRUS IN MICE AND CULTURES PRODUCED FROM MONOLAYER CELLS AND SUSPENDED CELLS

Virus strain	*Virus titre in Petri dish culture produced from cells grown in Monolayers	Suspension	†Virus titre in roller tube cultures of cells grown in Monolayers	Suspension	Titre in mice‡
Rho 1 *m* 28 *mo* 89 *m* 3	$4 \times 10^{4\ 0}$	$1\ 5 \times 10^{3\cdot0}$	$10^{3\ 0}$	$10^{3\ 0}$	$10^{5\ 5}$
Kemron O' 119 egg *m* 13	$2\ 7 \times 10^{6\ 0}$	$7 \times 10^{5\ 0}$	$10^{5\cdot8}$	$10^{5\cdot9}$	$10^{7\ 0}$
RV 11 *mo* 34 *m* 2	$1\ 8 \times 10^{6\cdot0}$	$3\ 8 \times 10^{5\ 0}$	$10^{5\ 0}$	$10^{4\ 5}$	$10^{6\ 2}$
RV 11 25 *c*, *PK* 10	$4\ 4 \times 10^{5\cdot0}$	$9\ 6 \times 10^{5\cdot0}$	$10^{4\ 5}$	$10^{4\ 1}$	$10^{5\ 2}$

m 28 *mo* 89 *m* 3 = 28 passages in unweaned mice + 89 passages in adult mice + 3 passages in unweaned mice

119 egg *m* 13 = 119 passages in eggs + 13 passages in unweaned mice

mo 34 *m* 2 = 34 passages in adult mice and 2 passages in unweaned mice

25 *c PK* 10 = 25 passages in cattle + 10 passages in pig kidney monolayers

* Titre in plaque-forming units per ml

† Titre in tissue culture ID_{50} per ml

‡ Titre in mouse LD_{50} per ml

Cells propagated in the small vessels for 37 days have been transferred to a 5-l bacterial culture vessel[4] from which the baffles were removed From initial concentrations of about $3 \times 10^{5\ 0}$ cells per ml, maximum concentrations of about $1\ 5 \times 10^{6\ 0}$ cells per ml have been obtained in culture volumes of 2 5 and 4 1 with stirrer speeds of 400 and 480 r p m, respectively

The vessel is fitted with *p*H electrodes, and this allowed continuous monitoring of the *p*H of the culture A degree of aeration was achieved by continuously passing a mixture of air and carbon dioxide, at an arbitrarily selected flow-rate of 400 c c /min, across the top of the stirred culture By manual adjustment of the relative flow-rates of carbon dioxide and air, *p*H of the culture was maintained between 7 0 and 7 2 In practice we found that approximately 10 per cent carbon dioxide air mixture is required to keep within this range

We thank Prof M G P Stoker and Dr I A Macpherson for supplying the *BHK* clone 13 cells, and Dr A Jones for his assistance with the chromosome analyses

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REVERSIBLE ENERGY TRANSFORMATION WITH FILAMENTS PREPARED FROM A NATURAL MUSCLE

By Prof W. KUHN, I. TOTH and G EBNER
Institute of Physical Chemistry, University of Basle

(1) *Energy transformation with artificial filaments* It has been possible to obtain filaments made from macromolecular artificial substances which, embedded in a aqueous solution, dilate and contract under the influence of reversibly produced chemical changes[1], for example, dilation of a filament containing polyacrylic acid (and polyvinyl alcohol) on addition of sodium hydroxide and contraction on addition of hydrochloric acid ('*p*H-muscle')

It has been shown, furthermore, that a mechanical stretching of the filament will in the case of all these gels be associated with an increase in activity in the embedding medium of the reactants producing the contraction and a decrease in activity of the reactants producing the dilation ('temochemical' principle[2]).

The chemically induced contraction and the activity shift produced by mechanical stretching are related one to the other in such a way that the realization of equation (1) assures a quantitative transformation of free chemical into mechanical energy by use of these systems. The actual existence of a change in chemical equilibrium produced by mechanical deformation was demonstrated for the first time with the artificial cross-striated '*p*H-muscle' in 1958[3]. A change in *p*H in the embedding medium is in this case produced by a mechanical stretching of the filament. Analogous equilibrium changes were afterwards shown to occur with the homogeneous *p*H-muscle[4], the redox-muscle[1,2], the ion-precipitation-[1,2] and the $Cu^{\cdot\cdot}$-polyvinylalcohol-complex-formation-muscle[5].

In the case of the $Cu^{\cdot\cdot}$-polyvinylalcohol-muscle a mechanical stretching of the filament (shortened by the addition of $Cu^{\cdot\cdot}$-ions) produces a reversible increase of the $Cu^{\cdot\cdot}$-ions in the embedding medium (which may in this case be a buffer of $pH = 5\cdot9$).

(2) *Filaments prepared from a natural muscle.* It was interesting to find whether the temochemical principle is limited to the various kinds of artificial fibres mentioned or whether it is valid in a direct way also for filaments prepared from natural muscles. Strips made from the muscles of the earthworm (*Lumbricus terrestris*) were used for these experiments. In a first series of experiments (started in collaboration with D. H. Walters) the behaviour with copper ions was investigated, in another series, analogous tests, using calcium ions and hydrogen ions, were carried out.

(*a*) *Experiments with Cu.* For these experiments the earthworm was opened, washed, and the muscular system containing both longitudinal and transversal fibres treated for about 5 min. with *N* sodium hydroxide and subsequently with distilled water at 20° C. A muscle thus prepared was embedded in, for example, 5 c.c. of a 0·1 mol. sodium acetate buffer $pH = 5\cdot9$, the muscle's dimensions were then in a particular case $4\cdot57 \times 0\cdot5 \times 0\cdot2$ cm. On addition of $Cu^{\cdot\cdot}$-ions (copper sulphate or acetate) to the embedding solution the $Cu^{\cdot\cdot}$-ions are nearly completely absorbed by the muscle which thereby contracts. In the example mentioned the absorption by the muscle of $5\cdot25 \times 10^{-6}$ moles of $Cu^{\cdot\cdot}$ was associated with a change in length from 4·57 to 4·07 cm., that is, with a contraction by 11 per cent. The $Cu^{\cdot\cdot}$-ion concentration of the embedding liquid was then 9×10^{-3} moles/l., it was measured by means of a metallic copper electrode introduced into the embedding fluid and a reference saturated calomel electrode. When mechanically stretching the contracted filament from 4·07 to 5·58 cm. (that is, by 30 per cent) an increase of the $Cu^{\cdot\cdot}$-ion concentration in the embedding liquid was observed and quantitatively determined from the potential shift shown by the copper electrode. The potential shift mentioned indicates in a particular case a relative increase of the $Cu^{\cdot\cdot}$ activity of

$$\frac{d\,a_{Cu}}{a_{Cu}} = 0\cdot165$$

The reversible increase of the $Cu^{\cdot\cdot}$ activity ascertained when stretching the earthworm muscle (partly contracted by $Cu^{\cdot\cdot}$) shows qualitatively the applicability to this system of the temochemical principle.

We mention that the activity changes of $Cu^{\cdot\cdot}$ and the contractions and dilations produced by $Cu^{\cdot\cdot}$ are completely reversible and that when $Cu^{\cdot\cdot}$ is removed, for example, by dilute hydrochloric acid at the end of a series of experiments, the earthworm muscle has the same properties, for example, the same contractility with various reagents, as it had before being used for $Cu^{\cdot\cdot}$ experiments. In spite of this it was felt that experiments using calcium instead of copper ions would be nearer to 'natural' conditions.

(*b*) *Experiments with Ca.* For these experiments the earthworm muscular system was treated[6] with water at 80° C. for 1 min., washed three times at 20° C. with distilled water for 20 min. each time and then, still at 20° C., with 0·3 *N* acetic acid. The treatment with 0·3 *N* acetic acid produces a marked elongation of the muscle, for example, from 6·49 to 8·50 cm. The major part of the acetic acid absorbed is then removed by washing the muscle three or four times, each time with a new quantity of about 5 c.c. of distilled water. This removal of the acetic acid is accompanied by a partial contraction.

For the final observation the muscle was embedded in a 0·133 *N* solution of acetic acid. In a particular case its length was then 8·35 cm. After establishment of the equilibrium the volume of the embedding liquid was reduced to 1 c.c., and $1\cdot6 \times 10^{-5}$ mol. calcium acetate was added to the system. A part of the $Ca^{\cdot\cdot}$ added was absorbed by the muscle, producing a contraction by 0·62 cm. (8·35–7·73 cm.). In the embedding liquid the calcium concentration left was $0\cdot8 \times 10^{-2}$ mol. $Ca^{\cdot\cdot}$/l. and the *p*H was 3·78.

When this equilibrium was reached, the muscle was mechanically stretched in contact with the embedding fluid (of 1 c.c.) by a force $\mathfrak{K}$ of 13·5 gm. $= 1\cdot35 \times 10^{4}$ dynes. It produced an increase in length of 0·75 cm. An increase of the $Ca^{\cdot\cdot}$ concentration in the embedding liquid was found to occur from $0\cdot80 \times 10^{-2}$ to $0\cdot813 \times 10^{-2}$, that is, the relative increase was $\Delta(Ca^{\cdot\cdot})/(Ca^{\cdot\cdot}) = 0\cdot13/8\cdot0 = 0\cdot016$ or $1\cdot6 \pm 0\cdot2$ per cent. This concentration shift was measured with a flame photometer. The shift is reversible and can be repeated with the same muscle sample any number of times. Besides the increase of the $Ca^{\cdot\cdot}$-concentration a reversible increase of the *p*H by 0·04 units (from 3·78 to 3·82) was associated with the stretching mentioned.

The further analysis of these results shows that the stretching is associated (besides the shifts in the $Ca^{\cdot\cdot}$ and $H^{\cdot}$ concentrations mentioned) with an increase of the acetate ion concentration from $1\cdot60 \times 10^{-2}$ to $1\cdot626 \times 10^{-2}$, that is, with a relative acetate ion increase by $1\cdot6 \pm 0\cdot2$ per cent (practically equal to the relative $Ca^{\cdot\cdot}$-ion increase). The analysis shows, furthermore, that the stretching produces a release from the muscle of $1\cdot3 \times 10^{-7}$ gm. mol. or $2\cdot6 \times 10^{-7}$ gm.-equivalents of calcium acetate and an uptake by the muscle from the embedding liquid of about 10^{-5} gm. mol. of acetic acid.

The release of $Ca^{\cdot\cdot}$ and the uptake of acetic acid corresponds to the statement of the temochemical principle saying that a mechanical stretching of the filament is associated with an increase in the embedding medium of the activities of the substances producing a contraction ($Ca^{\cdot\cdot}$) and a decrease in the embedding medium of the substances (acetic acid) producing a dilation of the filament.

It is essential in the $Ca^{\cdot\cdot}$ experiment that the change in length of the filament produced by mechanical stretching does not greatly exceed the contraction which had been produced by the uptake of $Ca^{\cdot\cdot}$

For high mechanical stretching a change of the sign of the Ca effect is observed.

(3) *Quantitative.* It has been mentioned that the contraction of a filament produced by a chemical reagent and the chemical equilibrium change produced by a mechanical stretching of the filament are in the case of the artificial contractile systems quantitatively connected one to the other.

The quantitative formulation of the temochemical principle, adapted to the conditions prevailing in our experiments, reads

$$-\left(\frac{\partial n_{1,\text{tot}}}{\partial L}\right)_{\mathfrak{K}=\text{constant}} = \left(\frac{\partial \mathfrak{K}}{\partial L}\right)_{n_{1,\text{tot}}=\text{constant}} \frac{1}{RT\left(\frac{\partial \ln a_{1,\text{tot}}}{\partial L}\right)_{n_{1,\text{tot}}=\text{constant}}} \qquad (1)$$

In this equation $n_{1,\text{tot}}$ is the number of gm. mol. of the temochemical substance considered (Ca acetate or Cu acetate in our case) present in the total system (consisting of the gel and the embedding liquid), L the length of the filament. $\left(\frac{\partial n_{1,\text{tot}}}{\partial L}\right)_{\mathfrak{K}=\text{constant}}$ is the number of gm. mol. of the temochemically active substance (Ca acetate) added to the system (to gel plus embedding liquid), divided by the change in length of the filament, whose charge $\mathfrak{K}$ is kept at a constant value. In our experiment where the addition of 1.6×10^{-5} mol. Ca acetate produced a contraction by 0.62 cm. of the uncharged filament we had obviously

$$-\left(\frac{\partial n_{\text{Ca acetate}}}{\partial L}\right)_{\mathfrak{K}=0} = +2.6 \times 10^{-5}\ \text{mol cm}^{-1} \qquad (1a)$$

$\left(\frac{\partial \mathfrak{K}}{\partial L}\right)_{n_{1,\text{tot}}=\text{constant}}$ on the right-hand side of equation (1) is the quotient of the increase $\partial\mathfrak{K}$ of the force acting on the filament and the change in equilibrium length observed under the condition that the filament is in each stage of the experiment in equilibrium with the embedding liquid and that the number of gm. mol. of all substances present in the system (filament plus embedding liquid) is constant.

In our experiment where a force of 1.35×10^4 dynes produced an increase in length $dL = 0.75$ cm., we had:

$$\left(\frac{\partial \mathfrak{K}}{\partial L}\right)_{n_{1,\text{tot}}=\text{constant}} = 1.8 \times 10^4\ \text{dynes cm}^{-1} \qquad (1b)$$

R and T in equation (1) are the universal gas constant and the absolute temperature respectively.

$\left(\frac{\partial \ln a_1}{\partial L}\right)_{n_{1\,\text{tot}}=\text{constant}}$ is the activity change, divided by the increase in length of the temochemical substance (in our case of calcium acetate) produced, if the filament is mechanically stretched at a constant composition of the total system. In our experiment where calcium acetate is the temochemical substance, we have

$$\frac{\partial \ln a_1}{\partial L} = \frac{\partial \ln a_{\text{Ca}}}{\partial L} + \frac{2\partial \ln a_{\text{Ac}^-}}{\partial L}$$

When stretching the muscle in our experiment we had, with an increase in length $(dL) = 0.75$ cm.

$\frac{d\,C_{\text{Ca}}}{C_{\text{Ca}}} = \frac{d\,C_{\text{Ac}^-}}{C_{\text{Ac}^-}} = \frac{dc}{c} = 0.016$ and therefore, if $\bar{f}$ is the mean ion activity coefficient of the Ca - and acetate-ions

$$\frac{\partial \ln a_{1,\text{tot}}}{\partial L} = 3\frac{d\ln c\bar{f}}{\partial L} = \frac{3 \times 0.016}{0.75}\left(1 + \frac{\partial \ln \bar{f}}{\partial \ln c}\right)$$

An estimate of $\frac{\partial \ln \bar{f}}{\partial \ln c}$ by help of the theory of electrolytes gives for the embedding solution $\frac{\partial \ln \bar{f}}{\partial \ln c} \simeq -0.2$ to 0.4. Using the latter value we get

$$\frac{\partial \ln a_{1,\text{tot}}}{\partial L} = 0.038\ \text{cm}^{-1} \qquad (1c)$$

If we use the numerical values indicated in equations (1b) and (1c) we obtain with $RT = 2.4 \times 10^{10}$ ergs for the right-hand side of equation (1)

$$\frac{\left(\frac{\partial \mathfrak{K}}{\partial L}\right)_{n_{1,\text{tot}}=\text{constant}}}{RT\left(\frac{\partial \ln a_{1,\text{tot}}}{\partial L}\right)_{n_{1,\text{tot}}=\text{constant}}} = 2.0 \times 10^{-5}\ \text{mol cm}^{-1} \qquad (2)$$

The agreement of this value with equation (1a), that is, the experimental verification of the temochemical equation (1), is obvious. The moderate deviation is accounted for by the inaccuracy of the measurements, the uncertainty with respect to the activity coefficients used, and, in particular, to the fact that finite values for $d\mathfrak{K}$, dL, $d\ln a_{1,\text{tot}}$ had to be used instead of infinitely small differences for the determination of the differential quotients in (1a), (1b) and (1c). They should all have been determined in the limes $\mathfrak{K} = 0$ (or all in the neighbourhood of another definite value of $\mathfrak{K}$ chosen). It has been mentioned that even a change of sign may occur if large instead of small deformations are produced.

The thermodynamical consideration leading to equation (1) is similar to the derivation given in a recent paper[4] of a relation which is substantially and formally analogous to equation (1) but somewhat less adapted for the direct use in our experiments.

(4) *Reversible and quantitative energy transformation.* As the derivation of equation (1) is based on the second law of thermodynamics, applied to a cycle involving mechanical and chemical dilations and contractions of the filament, the possibility to transform free chemical energy (or osmotic energy) quantitatively into mechanical energy is assured for each system which obeys equation (1).

As we have mentioned in the introduction, the validity of equation (1) has been confirmed for the cross-striated and the homogeneous *p*H-muscle, the polyvinylalcohol-2-methylnaphthochinon-hydrochinon-redox-muscle and the polyvinylalcohol-Cu -complex formation muscle. We can state now that the validity also exists for the system consisting of the earthworm muscle in a dilute acetic acid–calcium acetate solution, that is, the teinochemical principle holds both in its qualitative and its quantitative form also for filaments made from a natural muscle The structure and properties of these filaments are such that they are able to transform reversibly into mechanical energy the free energy of dilution of Ca - (or Cu -) solutions or the free energy of chemical reactions leading to a formation or elimination of these substances After that the possibility of a quantitative energy transformation in the elementary process of muscular contraction has to be reckoned with As our experiments were carried out in the absence of adenosine triphosphoric acid (ATP) they prove that the structure of the earthworm muscle realized in our experiments permits the contractions, dilations and transformation of Ca -ion dilution energy into mechanical energy without participation in any way of ATP On the other hand, this does not exclude that ATP plays an important part in the natural muscle contraction as well in the elementary contraction process as in ion transport phenomena preparatory to the contraction

(5) *Generalization* The observation reported when describing the earthworm muscle-Ca -acetate-acetic acid system, that a mechanical stretching is associated with a release of Ca acetate and simultaneously with an uptake of acetic acid from the embedding liquid (and that the muscle correspondingly contracts on addition of Ca acetate and dilates on addition of acetic acid) shows that the same contractile filament is teinochemically active at the same time for more than one kind of substance present in the system It is therefore to be expected that the same natural muscle filament will be able to transform into mechanical energy also the free dilution energies of further substances, for example of Na^{+}, Mg^{++} and K^{+}-ions and possibly of ATP. A teinochemical equation of the form of equation (1) will be valid for each substance, for example for potassium chloride, sodium chloride, etc , by the addition of which a contraction or dilation of the filament can be produced

It will be seen, furthermore, that the nominator

$$\left(\frac{\partial \mathfrak{R}}{\partial L}\right)_{n_{1,\mathrm{tot}}} = \text{constant}$$

on the right-hand side of equation (1) will be the same for all these expressions. There will therefore be an interdependence of equation (1) valid for the individual teinochemical substances occurring in the system

So far as the contraction mechanism is concerned no direct conclusion can be drawn from the validity of equation (1) The validity proves the quantitative transformation into mechanical energy of the free dilution energy of the substance involved but nothing about the mechanism

The parallelism in the general behaviour between the strips made from the earthworm muscle and the artificial fibres made of polyvinylalcohol, polyacrylic acid, etc , all obeying equation (1) suggests only in a non-conclusive way, that is, as a possibility, some parallelism of the contraction mechanisms

However this may be, the establishment and experimental proof of equation (1) prove also, for filaments prepared from a natural muscle, the possibility of a quantitative and reversible energy transformation Due to the verification of this equation the observation of the activity changes produced by mechanical stretching should be an efficient tool to ascertain or to exclude the significant participation in the mechanical energy creation of any given muscle constituent

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HELICAL MODELS OF FEATHER KERATIN STRUCTURE

By R. D. B. FRASER and T. P. MACRAE

Division of Protein Chemistry, Commonwealth Scientific and Industrial
Research Organization, Parkville, Victoria, Australia

IN a recent publication dealing with the structure of feather keratin[1] it was suggested that many features of the elaborate X-ray diffraction diagram given by this material could be explained in terms of linear aggregates of small β-crystallites A sheet-like arrangement of crystallites similar to that proposed by Bear and Rugo[2] was shown to account for certain features of the low-angle diffraction pattern, but it was pointed out that a helical arrangement was equally possible

Although our analysis of helical arrangements is not yet complete, the recent discovery by Filshie and Rogers[3] that feather keratin contains fibrillar elements (microfibrils) about 30 Å in diameter (Fig 1) prompts us to direct attention to the possibility that these microfibrils may be helical aggregates of β-crystallites This view contrasts with that of Schor and Krimm[4], who consider that it is the β-chains rather than β-crystallites which are arranged in a helical manner. These authors have described a detailed model for feather keratin and consider that it is the only one which satisfies the requirements of the experimental data. We have examined the diffraction properties of this model, and have not been able to reconcile it with such features as the strong 10 Å group of equatorial and near equatorial reflexions, the 'net' diagram obtained by Bear and Rugo[2], the very considerable intensity of the 24 Å meridional reflexion and the strong group of reflexions about 2 Å The latter are more indicative of a conventional pleated-sheet chain arrangement than the polar pleated-sheet envisaged by Schor and Krimm We have thus been led to consider an alternative possibility derived as follows

The great majority of the reflexions in the X-ray diagram of feather keratin may be indexed on an axial period *b* of 95 Å and at lower angles of diffraction meridional reflexions are, with minor exceptions, confined to layer lines with k equal to a multiple of four This suggests that the primitive helix has a pitch of 95 Å with approximately 4 nodes per turn, as illustrated by the circles and full line in Fig 2*a* The appearance expected in cross-section in electron micrographs would depend on the distribution of scattering matter about each node, and in the example shown in Fig 2*a* would give the appearance of five sub-fibrils within each microfibril as in Fig 2*d* The distribution could equally well have been drawn to give any number of sub-fibrils

At wider angles of diffraction meridional reflexions occur on layer lines other than those with values of k which are multiples of 4 and in addition a strong meridional reflexion with $k = 1$ appears when feather keratin is stained with mercuric acetate or silver nitrate It follows, therefore, that the helix shown in Fig 2*a* is only a pseudo-structure, that is to say, the crystallites within one turn of the helix are not identical The pattern of residues and precise location must differ from crystallite to crystallite in such a way that the axial projection of the structure repeats only after 95 Å or some multiple of this distance

Two features of the X-ray diagram which appear to be related are the restriction of diffraction to even layer lines in the near equatorial reflexions around 10 Å and the occurrence of a 'net' diagram with the restriction $k/2 + h = 2n$, where n is integral, in samples which have been partially heat denatured[2] or stained with certain heavy atoms[1] Two explanations of these features appear possible They may be due to external interference between microfibrils or alternatively to the presence of two crystallites about each node of the primitive helix as, for example, in Fig 2*b* and *c* The fact that the crystallites are not identical has been emphasized by numbering them in two of the many possible ways consistent with the result of staining with mercuric acetate or silver nitrate

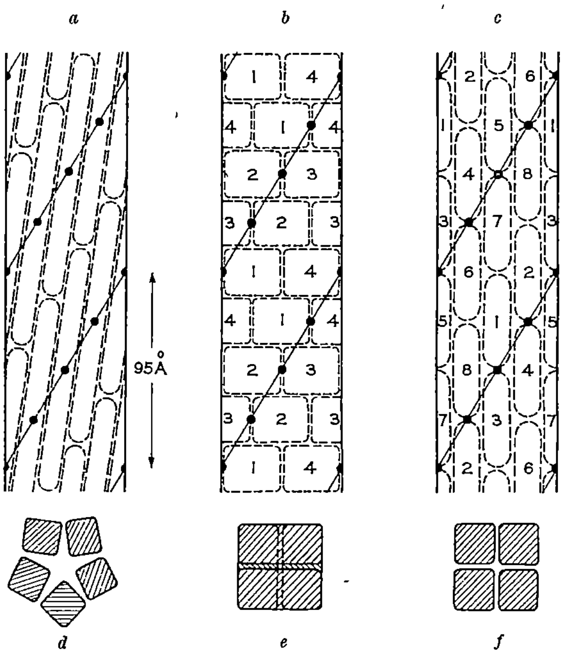

Fig 2 *a* *b*, *c*, Radial projections of helical models for feather keratin, the black circles joined by a continuous line represent the primitive helix In *a* one β-crystallite is arranged about each node, in *b* and *c* there are two crystallites about each node, *d*, *e*, *f*, the appearance of *a*, *b*, *c* respectively in cross-section (not to scale)

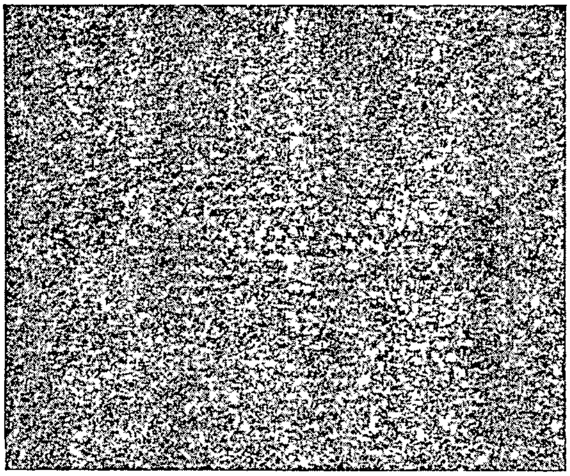

Fig 1 Electron micrograph showing microfibrils about 30 Å in diameter in a cross section of feather keratin (ref 3)

It is not possible to decide at present whether the polypeptide chain of the protein sub-units in feather keratin is confined to one crystallite or passes through many crystallites The crystallites in Fig 2*a* have quite arbitrarily been drawn in such a way that they are in contact with their neighbours The considerable intensity of the 24 Å meridional reflexion would be well explained if in the direction of the fibre axis the crystallites were separated by regions of specific but irregular configuration

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ELECTRON TRANSPORT SYSTEMS IN PURPLE BACTERIA

Hydrogenase and Light-stimulated Electron Transfer Reactions in Photosynthetic Bacteria

CHROMATOPHORES isolated from purple bacteria are capable of producing adenosine triphosphate (ATP) from adenosine diphosphate and inorganic phosphate by an anaerobic light-dependent process[1] and, in addition, these pigmented particles catalyse a number of oxidation-reduction reactions, some of which are activated or stimulated by illumination[2]. For example, chromatophores can effect a net 'photo-reduction' of nicotinamide adenine dinucleotide (NAD) in the presence of succinate[3,4], reduced flavin mononucleotide ($FMNH_2$) (refs 3 and 4), or a mixture of ascorbic acid + 2,6-dichlorophenolindophenol (DCPIP) (ref 5) serving as electron donor Similarly, 'photoreduction' of fumarate in the presence of ascorbic acid + DCPIP has been reported[6] These light-stimulated oxidation-reduction reactions have been usually[4,5,7] interpreted in terms of 'non-cyclic' electron-transfer mechanisms Thus, it is postulated that the action of light on the photochemical apparatus causes production of a 'photoreductant' and a 'photo-oxidant', presumably, the acceptor (NAD or fumarate) is reduced by the photoreductant, while the added electron donor (succinate, $FMNH_2$, etc) is oxidized by the photo-oxidant

Thus far, there is no unambiguous direct evidence for the 'non-cyclic' electron-flow interpretation of the oxidation-reduction reactions cited, and we believe that the present available facts are not incompatible with other possible mechanisms One such alternative is that the added electron donor reduces the acceptor by a direct (enzymatic) reaction which is promoted by ATP, or energy-rich precursors of ATP, produced by illumination[8] The concept of energy-dependent electron flow from succinate to NAD (in mammalian mitochondria), has been advanced by Chance *et al* [9], who have also proposed that a similar process can account for kinetic data, obtained by dynamic spectrophotometry, on light-stimulated NAD reduction in intact cells of purple bacteria[8] In this communication, we summarize new experimental results which support the view that energy-dependent electron transfer is the basis of the light-stimulation of certain oxidation-reduction reactions observed in the purple bacterium *Rhodospirillum rubrum*, or in chromatophores derived from this organism

During photosynthetic growth in a medium[10] containing malate (or succinate) and glutamate as the nitrogen source, *R rubrum* produces large quantities of molecular hydrogen and the collected cells contain an active hydrogenase. The enzyme in cell-free extracts (made by sonication, passage through a French pressure cell, or grinding cells with alumina) is associated with the particulate chromatophore fraction[11], is remarkably stable toward oxygen, and is capable of reducing a variety of electron acceptors with hydrogen in darkness (namely, benzyl viologen, janus green *B*, nile blue *A*, methylene blue, brilliant cresyl blue, thionine, *N*-methylphenazonium methosulphate, toluylene blue, DCPIP, *p*-benzoquinone, ferricyanide) Frenkel and Cost (following communication) have shown that the hydrogenase in *R rubrum* chromatophore preparations also reduces mammalian cytochrome *c* Metal-chelating agents (*o*-phenanthroline, α,α′-dipyridyl, diethyldithiocarbamate) and *p*-chloromercuribenzoate, do not inhibit the oxidation of hydrogen with artificial electron acceptors and the hydrogenase activity is not significantly diminished by (anaerobic) dialysis[12]. Although FMN is not reduced at an appreciable rate by the particles, treatment of the latter with sodium lauryl sulphate or sodium deoxycholate leads to significant hydrogenase activity with this nucleotide (Fig 1) and also with flavin adenine dinucleotide It thus appears that added flavin nucleotides are not readily accessible to the hydrogenase enzyme in the usual type of chromatophore preparation

Untreated particles do not reduce NAD with hydrogen, but after exposure to deoxycholate the pyridine nucleotide can be reduced as shown by Fig 1 and Table 1 The reaction requires the presence of FMN and a trapping system (lactic dehydrogenase (LDH) and pyruvate) for continual reoxidation of the $NADH_2$ produced Control experiments with the complete system showed that lactate formation under a gas-phase of helium was approximately one-tenth that observed under hydrogen In a number of experiments, the molar quantity of hydrogen utilized after addition of the trapping system (subsequent to reduction of the FMN) corresponded closely to that of lactate formed For unknown reasons, the total amount of hydrogen consumed by the complete system in the experiment of Table 1 was lower than that expected (that is, approximately 6 5 µmoles) In any event, the present results indicate that NAD can be reduced in darkness by chromatophore preparations through the following sequence

$$H_2 \rightarrow FMNH_2 \rightarrow NADH_2$$

Participation of flavins as intermediary carriers in the reduction of NAD by hydrogen in other microbial preparations has been reported by several investigators[13]. The one-electron dye benzyl viologen ($E'_0 = -0\ 359$ V) can apparently substitute for flavins, and possibly other carriers, in certain of these systems[14] and we have observed a similar reaction in untreated chromatophores of *R rubrum*

Although the FMN redox couple has a more positive standard potential ($E'_0 = -0\ 22$ V) than that

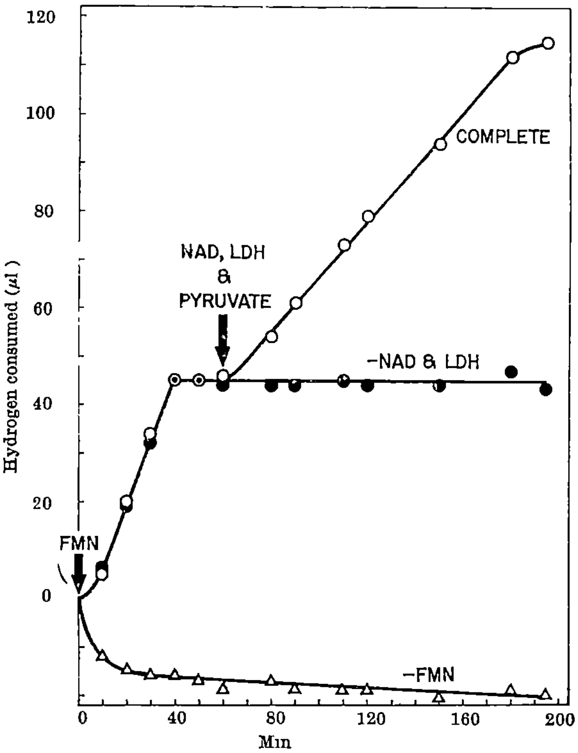

Fig 1 FMN-dependent dark reduction of NAD with molecular hydrogen by deoxycholate-treated particles from *R rubrum* Complete system potassium phosphate buffer *p*H 6 8, 100 µmoles, FMN, 2 µmoles, NAD, 1 µmole, sodium pyruvate, 5 µmoles, lactic dehydrogenase (LDH), 200 µg, sodium deoxycholate, 6 mg, *R. rubrum* particles containing 0 99 mg bacteriochlorophyll, water to make 2 0 ml final volume, 0 1 ml of 20 per cent potassium hydroxide in the centre-well, gas phase, hydrogen, temperature, 30° C FMN and other additions were introduced at the times indicated by the vertical arrows NAD, LDH and pyruvate were added at zero time to the control vessel not containing FMN The stoichiometry of hydrogen consumption and NAD reduction (as measured by lactate formation) in this experiment is shown in Table 1

Table 1 HYDROGEN UTILIZATION AND NAD REDUCTION BY *R rubrum* PARTICLES

Conditions	H_2 utilized (µmoles)	Lactate formed (µmoles)
Complete system	5 1	5 0
FMN omitted	0	0
FMN and NAD omitted	0	0
NAD and LDH omitted	1 9	0 2

Experimental conditions as described for Fig 1 The experiment was terminated at 190 min and lactate determined by a modification of the Barker–Summerson method (ref 20)

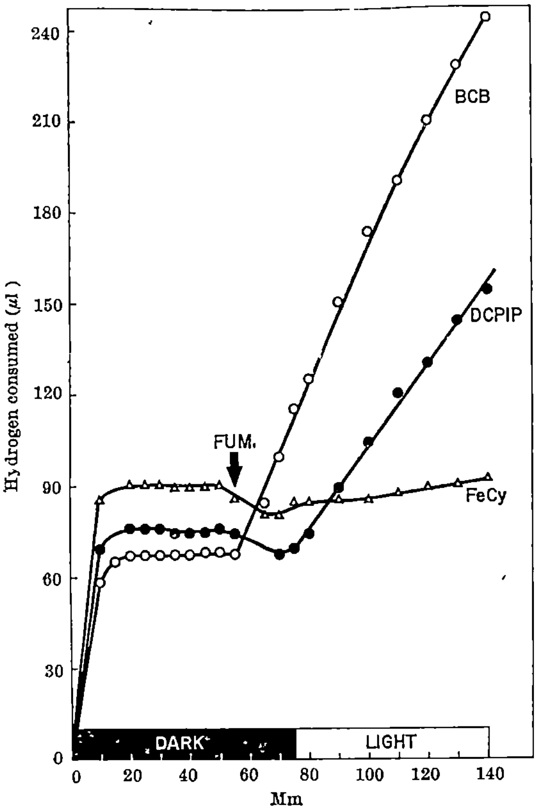

Fig 2 Oxidation of hydrogen with fumarate by *R rubrum* particles in the presence of redox carriers, light-stimulated catalysis by DCPIP

Reaction mixture *tris* (hydroxymethylaminomethane) *p*H 8 0, 150 μmoles, potassium phosphate *p*H 7 6, 3 5 μmoles, sodium fumarate, 40 μmoles, washed *R rubrum* particles (from alumina-ground cells) containing 0 98 mg bacteriochlorophyll, water to make 2 0 ml final volume, 0 2 ml of 20 per cent potassium hydroxide in centre-well, gas phase, hydrogen, temperature, 30° C, light intensity, 1,000 foot-candles, redox carriers (approx 4 μmoles) were added at zero time and fumarate at 55 min, brilliant cresyl blue (BCB), potassium ferricyanide (FeCy)

of the NAD couple ($E'_0 = -0\ 32$ V), it is apparent that the potentials of the two systems can overlap, particularly if the FMN is maintained in the reduced state by hydrogen, and NAD in the oxidized state by the trapping system Frenkel[3,4] has observed a light requirement for reduction of NAD by chromatophores with chemically reduced FMN (or with succinate) as the electron donor, since a trapping system was not used in his experiments, it seems possible that light was required to provide energy for overcoming an unfavourable potential difference between the two redox couples Presumably, a similar light-stimulation of NAD reduction by hydrogen (mediated by FMN) may be demonstrable under appropriate experimental conditions

Chromatophore preparations from *R rubrum* do not reduce fumarate with molecular hydrogen unless a suitable electron carrier is added As is illustrated in Fig 2, the dye brilliant cresyl blue ($E'_0 = +0\ 047$ V) catalyses the reduction of fumarate by hydrogen in darkness, and illumination has no significant effect on the rate of the reaction Similar results are observed with mediators of low redox potential (for example, with janus green *B*, and with FMN in deoxycholate-treated preparations) Although the rate of fumarate reduction by hydrogen (using untreated particles) varies widely, depending on the electron-carrier added, it is clear that chromatophores show a potent fumaric reductase activity which can be readily coupled to the hydrogenase Furthermore, the general properties of the coupled system in *R rubrum* particles, including lack of inhibition by malonate, closely resemble those described earlier[15] for preparations derived from certain non-photosynthetic anaerobes

Reduction of fumarate by hydrogen also occurs in the presence of catalytic quantities of the other dyes (but not ferricyanide) noted to be reducible by the hydrogenase In many instances, a definite acceleration in rate is caused by illumination, particularly when the mediating dye has a redox potential significantly higher than that of the fumarate–succinate couple ($E'_0 = +0\ 031$ V) This is especially clear-cut with DCPIP ($E'_0 = +0\ 22$ V) The latter is rapidly reduced by the hydrogenase of washed chromatophore preparations, but the dye is unable to act as an efficient catalyst for fumarate reduction in the dark Addition of 5×10^{-3} M Mg^{++} induces appreciable activity in darkness, but in the absence of such ions dark activity is usually negligible From Fig 2 it can be seen that when Mg^{++} is not added, utilization of hydrogen with fumarate (mediated by DCPIP) is observed if the system is illuminated Owing to the presence of excess hydrogenase, the dye remains in the reduced (leuco) form during the course of the reaction Suitable controls, containing sufficient added hydrogenase to generate $DCPIPH_2$, showed that the light-activated reduction of fumarate is abolished by heating the chromatophore preparations

The 'photohydrogenase' activity catalysed by DCPIP appears to be insensitive to antimycin *A* and amytal, and is appreciably stimulated by atebrin Relatively high concentrations of 2,4-dinitrophenol are required to inhibit photophosphorylation by chromatophores[16], and this uncoupler at levels of 0 01–0 02 M causes marked inhibition of the 'photoreduction' of fumarate by hydrogen Another inhibitor of phosphorylation, 2-nonyl-4-hydroxyquinoline-*N*-oxide, also was found to be effective, but variable results were obtained using different particle preparations Details of experiments with the foregoing and other inhibitors of electron transfer and photophosphorylation will be given elsewhere

Since the dye-mediated reduction of fumarate by hydrogen can occur in darkness, it is evident that the overall reaction does not necessarily require light, or entities produced by illumination The fact that illumination stimulates when the mediating electron-carrier has a high redox potential (relative to the fumarate–succinate couple) suggests to us that the action of light is concerned with providing energy for driving hydrogen transfer against an unfavourable thermodynamic gradient (for example, from $DCPIPH_2$ to fumarate) Washed chromatophores also exhibit a light-stimulated hydrogen consumption with fumarate in the presence of catalytic amounts of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine ($E'_0 = \sim +0\ 26$ V) The latter has been used as an electron donor for energy-linked reduction of NAD and ubiquinone in mammalian mitochondria[17].

Recent experiments in this laboratory have shown that *R rubrum* can convert organic substrates to carbon dioxide and hydrogen by means of an anaerobic light-dependent citric acid cycle, and this process has been interpreted as a physiological regulatory mechanism for controlling 'energy balance'[18,19] It is significant, in this connexion, that photoproduction of H_2 is markedly inhibited by a variety of agents (antimycin *A*, 2,4-dinitrophenol, redox dyes) known to interfere with photophosphorylation[19] Alternative hypotheses concerning the precursor of the hydrogen evolved visualize either a photoreductant created by the photochemical apparatus or NADH$_2$ produced by reactions of the citric acid cycle[18,19] According to our present views, the light-dependency of hydrogen formation by the hydrogen-evolving enzyme system of intact cells could be accounted for by supposing that energy-rich intermediates created by light facilitate a 'dark' enzymatic conversion of NADH$_2$ to NAD + H$_2$

In summary the concept of light-dependent 'non-cyclic' electron transfer, as a means of generating net reducing power of hydrogen, is invoked in hypotheses of the mechanism of bacterial photosynthesis largely on the basis of comparative biochemical argument, but lacks critical experimental support A more plausible conception, suggested by the present and other observations, is that 'photoreduction' of NAD (or fumarate) and photo-evolution of hydrogen occur through promotion of 'dark' oxidation-reduction reactions by energy-rich intermediates, produced by the interaction of light with the photochemical apparatus Accordingly, the 'accessory hydrogen donor', characteristically required for growth of photosynthetic bacteria, may in some instances (for example, succinate) serve as a direct source of hydrogen for reduction of pyridine nucleotides only if light energy is provided

This investigation was supported by a training grant (Division of Medical Sciences, 2*G*–714) and a research grant (National Institute of Allergy and Infectious Diseases, *E*–2640), from the U S Public Health Service Support was also provided by a grant (*G*–9877) from the National Science Foundation

SUBIR K BOSE
HOWARD GEST

The Henry Shaw School of Botany
and
The Adolphus Busch III Laboratory
of Molecular Biology,
Washington University,
St Louis, Missouri.

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Reduction of Mammalian Cytochrome *c* by *Rhodospirillum* hydrogenase

GEST[1] originally described a particulate hydrogenase from *Rhodospirillum rubrum* and now Bose and Gest have reported on a number of reactions mediated by this enzyme system (preceding communication).

In the search for light-induced reactions of molecular hydrogen with naturally occurring electron transport systems mediated by *Rhodospirillum* chromatophores, it was observed that isolated chromatophores readily reduce mammalian cytochrome *c* with molecular hydrogen in the dark Well-washed preparations, in the absence of molecular hydrogen or of suitable electron donors, show little or no activity in reducing added cytochrome *c* (Fig 1) Hydrogenase systems isolated from other organisms have been shown to be capable of reducing mammalian cytochrome *c* like that isolated and purified from *Clostridium pasteurianum*[2], nevertheless, the reported activity of *Rhodospirillum* chromatophores may be of possible interest for further investigations of light and dark reactions in this organism involving molecular hydrogen

A typical reaction course followed spectrophotometrically is given in Fig 1 With higher concentrations of cytochrome *c* and of chromatophores the reaction can be followed manometrically The initial rates are about 5 μM of cytochrome *c* reduced per μM bacteriochlorophyll per hour, or 0 05–0 1 μM of cytochrome *c* reduced per hour per milligram dry weight of material precipitable by cold trichloroacetic acid Thus far it has not been possible to increase this rate appreciably by the addition of a number of co-factors including flavin mononucleotide or flavin adenine dinucleotide Carbon monoxide (1 atm CO + 20 mm (Hg) H$_2$) does not inhibit the reported hydrogenase activity, nor does it inhibit the activation of the enzyme

The enzyme preparation is stable for several months at —10° C when stored in dilute aqueous buffers Under the same conditions the ability of the chromatophores to carry out photo-phosphorylation or light-induced electron transport reactions involving nicotinamide adenine dinucleotide (NAD) have been

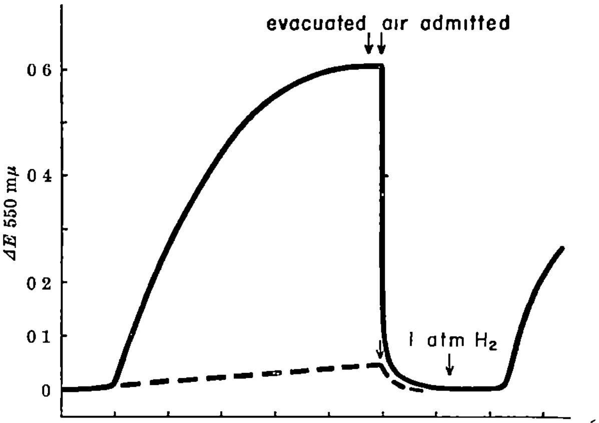

Fig 1 Reduction and oxidation of cytochrome *c* by *Rhodospirillum* chromatophores

Chromatophores prepared from three-day-old bacterial cultures grown anaerobically in the light Method of preparation described in ref 6 Chromatophores suspended in 0 1 M potassium glycylglycine *p*H 7 7 Optical density of chromatophore suspension at 880 mμ = 1 72 Additions 0 08 μM cytochrome *c* (from horse heart, Sigma) Anaerobic cuvettes evacuated (- - -), or evacuated and flushed with hydrogen (——)

lost Nevertheless, even with fresh preparations which can carry out such light-induced reactions, we have not been able to demonstrate a light-induced electron transport from reduced mammalian cytochrome *c* to NAD as reported by Vernon[3], or a transfer from reduced *Rhodospirillum* cytochrome *c* (cytochrome c_2 (ref 4)) to NAD Studies on the reduction of cytochrome c_2 by *Rhodospirillum* hydrogenase are in progress

Reduced mammalian cytochrome *c* is rapidly re-oxidized by molecular oxygen[5] (Fig 1), a reaction which is far more rapid than the hydrogenase reaction Manometrically it is possible to establish the stoichiometry of these reactions for a given amount of cytochrome *c* leading to a net oxidation of molecular hydrogen by oxygen In molar ratios

Hydrogen consumed cytochrome *c* (Fe^{+++}) reduced = 2 4 (hydrogenase)

Oxygen consumed cytochrome *c* (Fe^{++}) oxidized = 1 4 (oxidase)

Overall reaction $2 H_2 + O_2 = 2 H_2O$

This reaction sequence of alternate reduction and oxidation of cytochrome *c* can be continued many times at 25° C before appreciable irreversible inactivation of the hydrogenase takes place

The picture of the chromatophore which emerges from other studies and these observations is that of a complex photochemical and dark electron transport system The latter, however, is studied here with a 'foreign' electron carrier. It will be of considerable interest to find the natural carrier in this system which readily can react with molecular hydrogen in the chromatophore under physiological conditions

We thank Dr H Gest for a generous sample of cytochrome c_2 and Drs Gest and E Lindstrom for advice

This investigation was supported by grants from the National Institute of Allergy and Infectious Diseases (*E*–2218) and the U S National Science Foundation (*G*–9888)

ALBERT W FRENKEL
KONSTANTINE COST*

Department of Botany,
University of Minneapolis,
Minnesota

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* Holder of fellowship from Charles F Kettering Foundation for Photosynthetic Research

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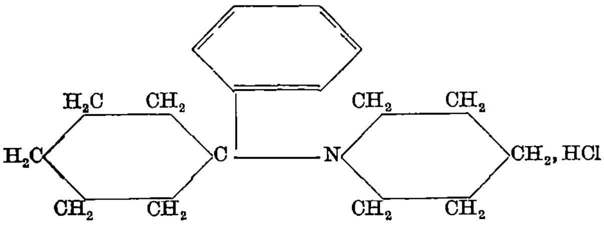
EFFECTS OF PHENCYCLIDINE ON THE RADIOSENSITIVITY OF MICE

By LIEUT -COLONEL J H. WILKINS and J H BARNES

Medical Research Council, Radiobiological Research Unit, Harwell, Berks

PHENCYCLIDINE (Cl-395) I-(I-phenylcyclohexyl) piperidine monohydrochloride is a white solid, freely soluble in water and ethanol It was synthesized in the Detroit Laboratories of Parke, Davis and Co about four years ago



The drug has undergone investigation in several animal species[1–6], revealing potent analgesic properties It can be administered orally, intramuscularly or intravenously The drug completely blocks somatic sensory perception[7] and causes vasomotor and respiratory stimulation It acts on the central nervous system by stimulation or depression, the effect varying according to the species of animal and dosage used excitement is predominantly shown in rodents whereas in most other species depression is the principal effect Phencyclidine cannot be regarded as a hypnotic or a sedative, and is best classified as a psychotropic drug

Previous studies with goats[5] showed promising results As it was intended to use the drug as an induction analgesic agent in certain radiation experiments it was necessary to discover whether phencyclidine possessed any protective effects With this objective in view experiments with mice were undertaken

Experiments I and II were designed to discover the general effects of the drug on mice before and after irradiation All injections were given intraperitoneally. Female mice of *R* stocks, weighing between 20 and 25 g, were used at the age of 14 weeks

Experiment I Effects at various dose-levels in unirradiated *R* mice

2 mg/kg and below no apparent effect

4–14 mg/kg after a few minutes disoriented In lower part of range, some signs of excitement In higher part of range, often unable to stand Effects lasting about 1 h

20 mg/kg after a few minutes, very unsteady and depressed There was recovery after about 2 h

80 mg/kg and higher convulsions, collapse and death

At the higher dose-levels, œdema of the lungs was noticeable at post-mortem

The dose giving the greatest degree of depression was 20 mg/kg

Experiment II Effects at various dose-levels in lethally irradiated *R* mice

Groups of ten mice each were given respectively 2, 5, 20 and 60 mg/kg of the drug intraperitoneally 15 min later they were exposed, in groups of five, to 1,025 r X-irradiation, which normally gives more than 95 per cent mortality in controls within 30 days Ten controls were included

The conditions of the X-irradiation were as follows 250 kV, 15 m amp , half-value layer, 1 2 mm copper, distance to underside of partitioned box, 60 cm dose-rate, 73 6 r /min

All five treatments (that is, controls, 2, 5, 20 and 60 mg/kg) were represented in each box during each irradiation

Controls	all died 8–13 days
2 mg/kg	all died 9–13 days
5 mg/kg	all died 5–12 days
20 mg/kg	all died 6–14 days
60 mg/kg	one died on first day, remainder died 7–24 days

The reaction to the drug of irradiated mice differed from that of the unirradiated Generally speaking, the irradiated mice showed more depression, which increased in extent as the dose of the drug was increased

2 mg/kg	showed little effect
5 mg/kg	caused disorientation with difficulty in standing
20 mg/kg	partial collapse with depression
60 mg/kg	more collapsed with greatest depression

It was decided that to detect any protective effect LD_{50} doses of X-irradiation would be necessary

Experiment III Effect of phencyclidine on the $LD_{50(30)}$ of mice exposed to X-irradiation

The mice were randomized with regard to sex, weight and age Groups of ten mice were irradiated at a dose-rate of 54 5 r /min so as to receive a total dose of 650 r , 725 r , 800 r , 875 r and 950 r respectively in one fraction

Control mice received 0 3 ml of saline intramuscularly and the treated groups 0 3 ml of a diluted solution of phencyclidine, so that each mouse received an equivalent of 4 mg/kg

Irradiation took place 0 5 h after the injections and the X-ray beam was directed upwards towards the partitioned boxes Treated and control mice were irradiated simultaneously

The conditions of irradiation were as follows 250 kV , 14 m amp , half-value layer, 1 2 mm copper, distance to underside of boxes, 72 cm, dose-rate, 54 5 r /min

Table 1 and Fig 1 give the mortality response of control and treated groups

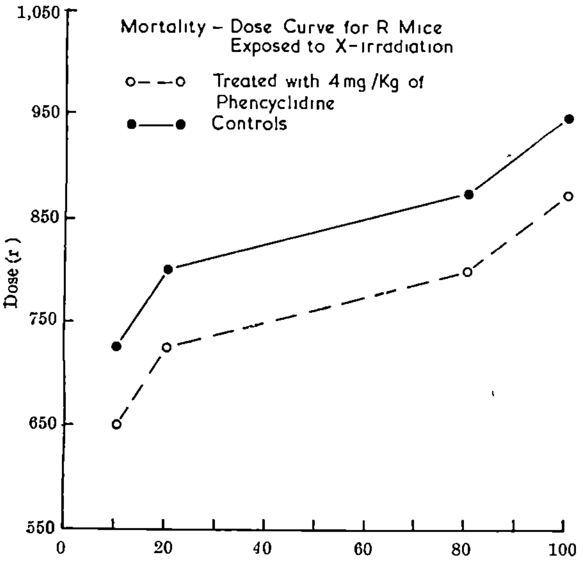


Fig 1

Table 1 30-DAY MORTALITY RESPONSE

Dose (r)	Controls	Phencyclidine-treated
650	1/10	0/10
725	2/10	1/10
800	8/10	2/10
875	10/10	8/10
950	10/10	10/10
$LD_{50(30)} \pm S\ E$	752 5 r ± 15 8 r	828 0 r ± 15 9 r
Approx 95 per cent limits of $LD_{50(30)}$	724 r 785 r	799 r 861 r

Table 2 gives the cumulative mortality over the period of 30 days

Table 2 30-DAY CUMULATIVE MORTALITY

Days after irradiation	Controls No dead	Treated with phencyclidine (Cl-395) No dead
8	5	—
10	3	—
11	1	1
14	16	13
15	1	3
16	2	2
18	1	—
21	1	1
28	—	1
30	1	—
Total	31	21

The $LD'_{50(30)}$ for mice for this experiment was calculated to be as follows

Controls	752 5 r
Phencyclidine-treated	828 0 r

The LD_{50}s are significantly different ($P < 0\ 001$)

It was concluded that phencyclidine at 4 mg/kg increased the $LD_{50(30)}$ by 75 5 r and had a slight protective effect on the mortality response of *R* mice

We thank the Director, Army Veterinary and Remount Services, the War Office and the Director, Radiobiological Research Unit, Harwell, for permission to publish this article We also thank Messrs Parke, Davis and Co , who kindly supplied the drug, and Mr M J Corp, who arranged the irradiations

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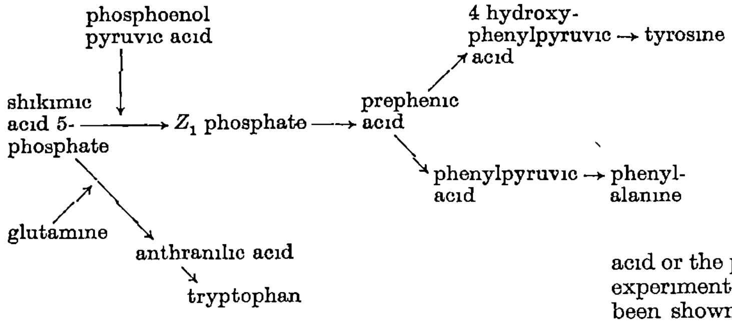
THE BRANCH POINT IN THE BIOSYNTHESIS OF THE AROMATIC AMINO-ACIDS

By DR MARGARET I. GIBSON, DR F GIBSON, DR C. H DOY and PAULINE MORGAN

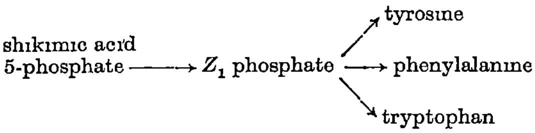
Bacteriology School, University of Melbourne

IT has recently been suggested by Sprinson[1] that 5-phospho-shikimic acid is a branch point in the biosynthesis of aromatic amino-acids (scheme 1), being metabolized on one hand through the compound 3-enolpyruvyl shikimic acid 5-phosphate (Z_1 phosphate), by the addition of phosphoenolpyruvic acid and thence through prephenic acid to phenylalanine and tyrosine Alternatively, nitrogen from glutamine is added to yield anthranilic acid

This scheme differs from that proposed by Davis[2] which is based essentially on the observation[3] that certain mutant strains of bacteria which required



Scheme 1 (after Sprinson, ref 1)



Scheme 2 (Davis, ref 2)

phenylalanine, tyrosine and tryptophan for growth accumulated 3-enolpyruvyl shikimic acid (compound Z_1) in their culture media

The recent observation that phosphoenolpyruvic acid stimulates the synthesis of both anthranilic acid and the phenylpyruvic acids from shikimic acid[4] is better in accord with the scheme advanced by Davis

The problem of the branch point has now been further examined It has been found possible to separate the conversion of shikimic acid to anthranilic acid or to the phenylpyruvic acids into two stages Cell extracts of *Aerobacter aerogenes* incubated with shikimic acid plus adenosine triphosphate (or shikimic acid 5-phosphate), magnesium and phosphoenolpyruvic acid (PEP) in *tris* buffer, form a compound with the same properties as had that assumed by Levin and Sprinson[5] to be Z_1 phosphate From the reaction mixture containing this compound either anthranilic acid or the phenylpyruvic acids can be formed under suitable conditions using mutants such as *A aerogenes* poly 3 blocked in the conversion of shikimic acid 5-phosphate to Z_1 phosphate These mutants are unable to form anthranilic acid or the phenylpyruvic acids from shikimic acid 5-phosphate An experiment in which anthranilic acid is produced in two stages is illustrated in Fig 1 This shows the rate of formation of precursor in the first stage as measured by the amount of anthranilic acid formed in the second stage Phenylpyruvic acids may be formed by the omission of glutamine in the second stage but the yields are relatively low owing to the presence of enzymes which destroy the acids

Treatment of the reaction mixture after the first stage with alkaline phosphatase followed by acid treatment yields a substance supporting the growth of a mutant strain of *Escherichia coli* which responds to shikimic acid Neither treatment alone produces a growth factor, but either destroys the substrate for anthranilic acid formation This substrate also disappears, as does the substance assaying as Z_1 phosphate, during prolonged incubation of the first stage The yield of anthranilic acid from the substrate formed is improved by the addition of potassium fluoride This is consistent with the observation[5] that fluoride inhibits the breakdown of Z_1 phosphate to Z_1, which would also be the product, already shown to be inactive as a substrate, of alkaline phosphatase treatment

The precursor which appears to be Z_1 phosphate was at first made by crude extracts or ammonium sulphate precipitated protein fractions of tryptophan auxotrophs, using as substrates shikimic acid 5-phosphate, magnesium and PEP in the absence of added diphosphopyridine nucleotide (DPN), its reduced form (DPNH) or glutamine The omissions prevent formation of anthranilic acid or the phenylpyruvic acids in the first stage of the experiment The *A aerogenes* mutant *A*170–44 has been shown[3] to accumulate compound Z_1 in culture media Extracts of this mutant fail to form anthranilic acid or the phenylpyruvic acids, but will do so when mixed with an extract of *A aerogenes* poly 3 In the presence of potassium fluoride, mutant *A*170–44 has also been shown to produce the substrate required by mutant poly 3 in a two-stage experiment

These observations suggest that the branch point is beyond shikimic acid 5-phosphate Scheme 3 represents our present concept of reactions around the branch point

The evidence for this scheme is as follows

(1) There is strong presumptive evidence that the same substrate, having the properties of Z_1 phosphate, serves as a precursor both for anthranilic acid and for the phenylpyruvic acids

(2) An extract of a mutant unable to convert shikimic acid 5-phosphate to Z_1 phosphate fails to form either anthranilic acid or the phenylpyruvic acids from shikimic acid 5-phosphate, as does an extract of a mutant accumulating Z_1 By mixing the two extracts it is possible to form these aromatic compounds

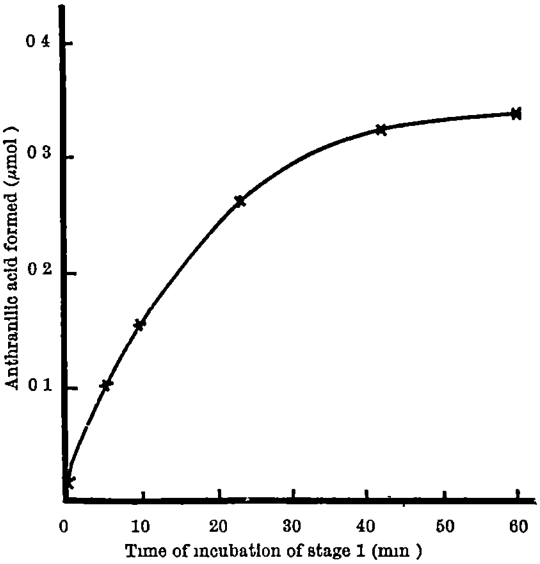
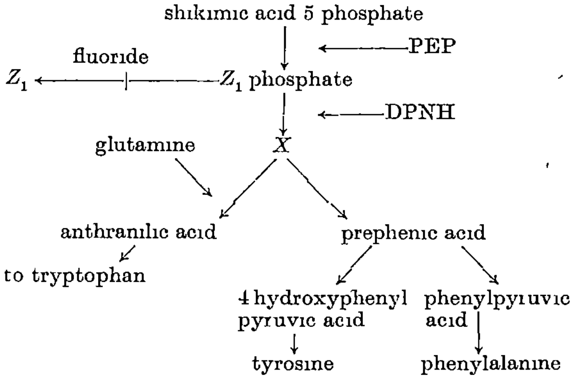


Fig 1 Production of anthranilic acid in a two-stage experiment Stage 1 Added per ml *tris* buffer *p*H 8 2, 100 μmol, $MgCl_2$ 10 μmol , ATP 2 μmol , shikimic acid 2 μmol , PEP 2 μmol , KF 20 μmol , *NC*$_3$ crude extract, 4 mgm protein Incubated at 37° C 0 5 ml samples taken at times shown and heated 5 min in boiling water bath
Stage 2 To each heated sample the following were added in 0 5 ml ribose 5-PO_4 2 5 μmol , glutamine 5 μmol , DPNH 1 μmol , poly 3 crude extract 2 7 mgm protein Incubated 45 min at 37° Tests acidified with 0 1 ml of *N* HCl and extracted with 4 ml ethyl acetate Anthranilic acid measured by optical density at 336 mμ
Strain *NC*$_3$ is a tryptophan auxotroph of *A aerogenes* responding to anthranilic acid Strain poly 3 is a 'polyaromatic' auxotroph of *A aerogenes* unable to form Z_1 phosphate from shikimic acid 5-phosphate



Scheme 3

(3) DPNH is not required for Z_1 phosphate formation, but is required for both anthranilic acid and phenylpyruvic acid formation

(4) Most mutants accumulating Z_1 require for growth tryptophan, phenylalanine, and tyrosine[3]

The existence of mutants which accumulate Z_1 and require for growth phenylalanine, tyrosine, tryptophan, 4-aminobenzoic acid and 4-hydroxybenzoic acid[3] suggests that the common pathway extends beyond Z_1 phosphate Evidence that in yeast shikimic acid 5-phosphate is the branch point for 4-aminobenzoic acid[6,7] was based on the observation that compound Z_1 did not serve as a substrate for 4-aminobenzoic acid synthesis As already seen, Z_1 phosphate rather than Z_1 is required for the formation of anthranilic and the phenylpyruvic acids and may also be the true intermediate in the formation of 4-aminobenzoic acid and 4-hydroxybenzoic acid

This work was supported by grants from the U S Public Health Service (*A*4632) and the National Health and Medical Research Council of Australia We thank Dr D B Sprinson for a culture of *Aerobacter aerogenes* 170–44

Note added in proof While this paper was in the press it was reported (Rivera, A, and Srinivasan P R, *Proc U S Nat Acad Sci*, 48, 864, 1962) that Z_1 phosphate can be converted to anthranilic acid in the presence of a DPNH generating system These observations support the general scheme advanced in the present article

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IMMUNE OPSONINS IN *SCHISTOSOMA* INFESTATIONS

By Dr J. NEWSOME

Medical Research Council Bilharzia Research Unit, St Albans

SOME experimental schistosomicides stimulate an intense phagocytic reaction to schistosomes in the liver[1] During a trial of active compounds related to lucanthone[2] we believed that potentiation of drug action may have occurred, a very heavy *Schistosoma mansoni* infection (50,000 cercariæ) was cured in a baboon which had received thirteen previous infections but a similar infection was not cured in an animal infected only twice before The contrast between the two baboons at post-mortem was very striking This kind of drug potentiation has also been described in malaria[3], only trivial doses of antimalarials are needed for permanent cure of falciparum and malarial infections in hyperendemic countries When we began to examine the effects of antimony and lucanthone on *S mansoni* in whole blood and in media containing serum and white blood cells, it seemed advisable to repeat some of the experiments with blood from infected subjects in case immunological reactions should potentiate the drug effects

Accordingly, adult flukes were incubated in blood media from baboons infected five times in seven years, with and without small amounts of antimony or lucanthone, they were all rapidly attacked by leucocytes and killed within a day in the centre of large masses of white cells The drugs did no more than speed up the early stages of this lethal phagocytic response, in normal blood their effect had been slow, and a small amount of leucocytic sheathing only appeared after three days when the flukes were moribund The effect of immune blood was clearly much greater than that of the drugs, and this article describes some of the characteristics of the phagocytic reaction

Adhesion of leucocytes to parasites was first described in detail early in this century, Laveran and Mesnil[4] gave a full account, with fine illustrations of the opsonizing effect of antitrypanosome sera in 1901 They worked with *Trypanosoma lewisi* and were able to watch the attachment of leucocytes to trypanosomes removed from the peritoneal cavity followed by 'englobement' and phagocytosis The active cells in these preparations from the peritoneum were monocytes Mesnil and Brimont[5] in 1909 mentioned this reaction again and pointed out that immune serum by itself had no effect on trypanosomes They urged that the protective effect of antitrypanosome immunity must be due to the opsonin and depend on phagocytosis In 1927, Davis and Brown[6] described their work with *T equiperdum* They found that immune sera made many things adhere to trypanosomes leucocytes and leptospira sometimes became attached, but platelets were always attracted The platelet adhesion factor, first described by Rieckenberg[7] in 1917, was heat-stable and did not depend on complement There is a considerable literature on phagocytosis of malaria parasites by immune hosts[8] A paper by Zuckerman[9] refers to this literature and suggests that the controlling factor in acquired immunity to malaria is likely to be the production of opsonins which stimulate phagocytosis Zuckerman demonstrated phagocytosis of avian malaria parasites by agranulocytes in immune serum The opsonin was heat-stable, rendered normal and malaria-infected red cells sticky and stimulated agranulocytes from immune or normal animals Its activity was greatly reduced if the preparations were kept in motion

The most interesting parallel to the phagocytic reaction is that to microfilariæ of *Wuchereria bancrofti*[10] Tests were run on four types of sera from (1) patients with active elephantiasis, (2) patients with circulating microfilariæ but no clinical disease, (3) patients with a history of filariasis but no circulating microfilariæ, (4) normal people and patients with leucocytosis as controls A suspension of microfilariæ and red and white cells from *bancrofti* patients was added to these sera Within 12 hr , large numbers of leucocytes became attached to the microfilariæ, sheathed them and soon killed them in the sera from elephantiasis patients but not in the other classes of sera Figs 1–4 show clearly the equivalent of a 2 + antischistosome phagocytic reaction, as described here It was believed that the presence of this heat-stable antibody only in elephantiasis patients explained why such patients very seldom have microfilariæ in their blood A positive test was not obtained with any sera from patients with circulating microfilariæ It is interesting to remember that the prompt action of the microfilaricidal drug diethylcarbamazine is thought to be due to an opsonizing effect which causes the microfilariæ to be held up and destroyed by phagocytes in the liver[11]

The usual sterile precautions were taken when carrying out the phagocytic reaction test Blood was defibrinated in a conical flask containing glass beads and either filled into 6 × ¾ in test tubes or centrifuged at 3,000 r p m for 15 min to obtain serum and buffy coat For routine use 2 5 or 5 ml of the following media were set up in the 6 × ¾ in tubes whole blood, serum with buffy coat, and equal parts of serum and Tyrode's solution with buffy coat Streptomycin and penicillin, 100 units of each per ml , and glucose, 2 or 4 mgm /ml , were added A micromethod also gave satisfactory results 1 or 2 ml of blood was drawn into a 5-ml syringe containing glass beads and the needle occluded by pushing it into a small rubber bung The blood was defibrinated in the syringe by shaking The needle was changed and the blood delivered into a 11 × 0 5 cm glass tube, capped at both ends After centrifuging, most of the red cells were removed with a syringe and fine needle through the cap, leaving serum and buffy coat Antibiotics, glucose and Tyrode's solution were then added Adult *S mansoni* flukes were taken from the portal veins of hamsters into Tyrode's solution and introduced into the tubes, which were incubated upright at 37° C

Special reaction tubes in which the media were dialysed against serum were used in some experiments Cellulose sacs were attached to one end of 6 × ¾ in glass tubes by rings of silicone rubber which also served to close the 3 × 1 in rinsing tubes in which the dialysis sacs were suspended The rinsing tubes, filled with 18 ml of reconstituted dried human serum or fresh baboon serum containing glucose and antibiotics and stored in the refrigerator, were changed daily in a bacteriological hood

In the reaction tubes the flukes settled at the interface between cells and serum and could be seen with a lens For examination under a dissecting microscope, they were twitched up on to the tube wall by a rolling movement. The ensheathed flukes of a positive phagocytic reaction were preserved, either on the tube wall or at the interface, by removing the serum and replacing with formol saline. Paraffin sections and fresh smears were stained with Giemsa's stain and hæmatoxylin and eosin

The degree of leucocytic adhesion and lethal action was scored by one to four plus signs as follows

+	Flukes a little slow, some with small collections of white blood cells or very thin partial coating, often rather loosely adherent
+ +	Most flukes affected, movements and peristalsis slow, definite white blood cell collections round head and/or tail, or thin overall or thicker partial sheathing which made them stick to the tube wall
+ + +	Little movement, thicker complete or partial sheaths firmly attached, all flukes with some white blood cell coating, most appearing twice the normal thickness and sticking to the tube wall
+ + + +	Unmistakable large irregular masses of white blood cells with flukes in the centre, all affected and movement rare, but slow cæcal peristalsis occasionally present

The stages of a good 4+ positive test in serum with buffy coat medium were

Up to 2 hr the flukes were much slowed and cæcal peristalsis and sucker movement was reduced

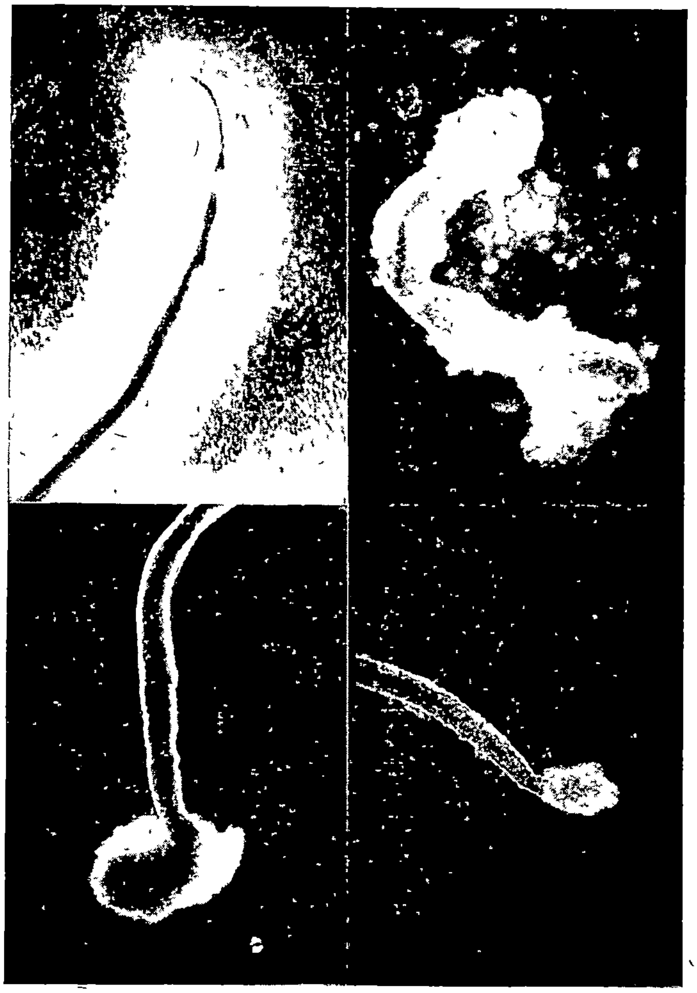
2–6 hr collections of leucocytes were firmly attached at head and tail and a coating of leucocytes, about as wide as the breadth of the fluke, slowly covered the whole surface, the flukes were barely moving

15–18 hr each fluke was in the centre of a large mass of leucocytes, but slight movement and weak peristaltic movement of cæcal pigment were sometimes seen

24–36 hr the flukes were dead and opaque, with clotted cæcal pigment, and the leucocyte masses round them had increased in size and sometimes contained pigment

The stages of weaker reactions took longer to appear, a final reading of 1+ did not usually occur until the third day and was considered doubtful unless repeated, the 2+ and 3+ results were evident by the second day Routine readings were made at approximately 18, 24, 40 and 64 hr and the quickest reaction was seen in the serum with buffy coat medium, followed by that in serum and Tyrode's solution with buffy coat medium and in whole blood Control preparations in normal blood supported flukes for 4–6 days, in normal serum with buffy coat medium for 8 days and in normal serum and Tyrode's solution with buffy coat medium for a fortnight Small collections of leucocytes and occasional thin loose overall sheathing were sometimes seen on dying flukes in normal media damaged by handling or by drugs

The influence on the phagocytic reaction of anæsthetics, antibiotics, heparin, additional glucose and the metabolic products of the blood cells was investigated Anæsthetics good positive reactions in media from unanæsthetized animals were not altered by giving the animals normal doses of ether, nembutal or phencyclidine, chance heavy dosage of these anæsthetics, however, sometimes reduced the adhesion of leucocytes Antibiotics the phagocytic reaction was the same whether streptomycin and penicillin (100 or 200 units per ml) were added to the



Figs 1–4 Leucocyte sheaths round adult *S mansoni* flukes (1) Fresh specimen showing large sheath round female fluke, (2) fixed specimen of male with 3+ reaction, (3) and (4) fixed specimens of small terminal sheaths on females with a 2+ reaction

media or not Heparin it was thought at first that good reactions often occurred when the hamster supplying flukes had been heparinized Comparison of heparinized and unheparinized flukes in a strongly positive serum with buffy coat preparation showed that heparin in trace amounts did not affect leucocytic attraction Glucose the initial glucose concentration in the media was brought up to well over 400 mgm per cent, but when glucose was omitted a good phagocytic reaction was still obtained, although sometimes it was less intense Metabolic products of blood cells whole blood, after 3 days' incubation, contained no glucose, 37 mgm per cent of urea, 200–230 mgm per cent of potassium and had a *p*H of 6 94 Flukes lived in these abnormal conditions for 4–6 days The speed and degree of the phagocytic reaction were, naturally, often less in blood than in serum with buffy coat and serum and Tyrode's solution with buffy coat media. However, the metabolic products of cells cannot have made much difference to the reactions, which remained the same in immune media kept reasonably healthy by dialysis against daily changes of serum In dialysed media made from normal blood, flukes lived for a fortnight, remained paired and laid eggs, some of which matured and hatched lively miracidia This was the first time that eggs laid *in vitro* had matured[12], and the dialysed media were clearly better than any yet used If, however, the cells were kept in motion by rolling the dialysis tubes, the phagocytic reaction in immune whole blood was reduced or prevented

Phagocytosis was first seen in blood from six adult *Papio hamadryas* baboons repeatedly and heavily infected with *S mansoni* The phagocytic reaction was stronger in two animals which had received four or five yearly infections of 1–4,000 cercariæ, weaker reactions being produced by animals infected two or three times. In addition, three animals infected once with 500–2,000 cercariæ 1½ years before examination gave no reaction

It was then found that uninfected baboons injected with fresh whole fluke material emulsified in complete Freund's adjuvant gave a strong phagocytic reaction Two animals were given 200 male and 200 female flukes on two occasions a week apart, and two received 120 of each sex at the first injection and 220 at a second injection a week later A 4+ phagocytic reaction was recorded 12 days after the second injection and, five months later, it was still graded as a good 3+

Nine adult monkeys (*Macaca mulatta*), belonging to Dr R Smithers at the National Institute for Medical Research, were also tested Media from an uninfected monkey and from one infected with 1,000 cercariæ of *S mansoni* 8 weeks before testing were negative. The infection dies out in rhesus monkeys within a year and they become resistant to re-infection, two such animals were examined twice at 15 and 16 months after 2,000 cercariæ, when they were no longer passing eggs One was negative and one gave a weak 1+ phagocytic reaction on both occasions

The remaining five uninfected monkeys all received an injection of fresh schistosome material with complete adjuvant and were challenged by an infection of 1,500 cercariæ. The phagocytic reaction was checked twice, at 5½ and 8 weeks after challenge, with the same results (Table 1) The adult *S mansoni* material always gave a positive phagocytic reaction Intramuscular injection was more effective than intradermal, and the best reaction was produced by monkey No 22 which received adult and egg antigens intramuscularly The cercarial antigens gave negative results and it may be supposed that adjuvant alone would also be inactive

Table 1 PHAGOCYTIC REACTION IN RHESUS MONKEYS INOCULATED WITH FRESH FLUKE MATERIAL AND ADJUVANT BY THE INTRADERMAL AND INTRAMUSCULAR ROUTES

Monkey No	Antigen	Route of injection	Phagocytic reaction
25	Cercariæ	I D and I M	−ve
12	Adult flukes	I D	++
24	Adult flukes	I M	+++
13	Adults and eggs	I D	+++
22	Adults and eggs	I M	++++

The test has only been performed in man on 18 patients, 6 with *hæmatobium* and 12 with *mansoni* infections As no *S hæmatobium* flukes were available, media from five of the *hæmatobium* patients were set up with *S bovis*, only two patients gave weak positive reactions (1+ and 2+) All five were adult males with histories of chronic infection The sixth *hæmatobium* patient, who had a long history and active disease, was tested with *S hæmatobium* flukes, but there was no reaction The clinical condition of the twelve *mansoni* patients varied from symptomless infection to severe diarrhœa with hepatomegaly Ten of them were negative One youth, with diarrhœa and numerous eggs in the stools, gave a 3+ reaction during a course of treatment, three other patients with less-acute infections received the same treatment but their phagocytic reactions were negative Of two more patients in an acute phase, one was negative and the other only gave a weak but definite 1+ reaction It was impossible to see any connexion between clinical condition and the ability to produce a positive phagocytic reaction

It had been hoped that repeatedly infected small animals which were highly resistant hosts for *S mansoni* might give positive phagocytic reactions in less time than the 3–5 years apparently required for immunizing baboons Cavies and rabbits, however, remained negative, and the later results with rhesus monkeys, the infections of which had died out naturally, confirmed that infection of naturally resistant species was unlikely to stimulate the phagocytic reaction

Because of the observation that *S bovis* flukes were sometimes attacked by leucocytes in human *hæmatobium* blood, it seemed that the phagocytic reaction might not be species-specific The effects of serum with buffy coat and serum and Tyrode's solution with buffy coat from two baboons, immunized with *S mansoni* material, on adult *S mansoni*, *S hæmatobium* and *S japonicum* flukes, were compared No difference in the degree or speed of leucocytic sheathing between the three species could be seen It is likely that *S bovis* flukes, had they been available, would also have been rapidly killed The test was performed in serum with Tyrode's solution with buffy coat medium on eggs, miracidia, cercariæ and a preparation of 20-day-old schistosomulæ which contained all sizes of pigment-containing larvæ The cercarial envelope test was positive but leucocytes were not attracted to cercariæ or to eggs Miracidia vanished from the preparation within 24 hr and were thought to be attacked by leucocytes Schistosomulæ of all sizes were killed and ensheathed by 18 hr but in normal blood they lived for 5 days

The serology of the reaction has not yet been fully investigated, but there is some preliminary information When immune and normal baboon sera were crossed with thoroughly washed cells from immunized and normal baboons, a positive phagocytic reaction appeared only in preparations containing antiserum Normal serum and 'immune' cells were inactive, except in some early experiments when the 'immune' cells had only been briefly washed and the residual antiserum allowed a weak reaction Removal of protein from antiserum by graded heating prevented the phagocytic reaction Inactivation of antiserum by heating at 56° C for 30 min did not prevent the reaction but usually reduced it by 1+ The addition of complement (neat cavy serum) to inactivated baboon antiserum did not improve the phagocytic reaction but prevented it Dilution of the mixture of sera with Tyrode's solution restored the phagocytic reaction The results with media containing dilutions of the two sera and well-washed 'immune' cells are shown in Table 2 The phagocytic reaction did not occur in inactivated serum diluted with more than three parts of Tyrode's solution and it was far from completely dependent on complement Flukes could not be made attractive to leucocytes by incubating them for 48 hr in strongly positive serum and, after a brief rinse, introducing them into normal serum with buffy coat, there was no sign of any phagocytic reaction after 3 days

Table 2 PHAGOCYTIC REACTION IN IMMUNE BABOON SERUM WITH CAVY SERUM DILUTION WITH TYRODE'S SOLUTION

Time (hr)	Cavy serum diluted times	Inactivated pooled immune serum diluted times			
		2	4	8	16
	2	0	0	0	0
18	4	±	0	0	0
	8	+	+	0	0
	2	0	+	0	0
28	4	+ + +	+ +	0	0
	8	+	+	±	
	2	0	0	0	0
40	4	+ + +	+	0	0
	8	0	0	0	0

The histology of the ensheathed flukes was examined fresh and in paraffin sections stained with hæmatoxylin and eosin The sheaths consisted of leucocytes in approximately the same proportions as in the buffy coat, the majority of the cells were neutrophil polymorphs and there was no evidence of differential attraction of any one type of cell Some neutrophils and monocytes entered the fluke tissues and the fluke cuticle was ragged in places It seems that the flukes died before the phagocytes had been able to inflict much cellular damage The leucocytes were examined fresh at 37° C and the majority remained alive and moved normally up to 72 hr

The phagocytic response of white cells from immunized primates to schistosomes *in vitro* has, so far as we know at present, these characteristics It appears in baboons repeatedly infected at yearly intervals with *S mansoni*, but it is weak after two infections and absent in baboons and rhesus monkeys after one infection It is absent in infected resistant hosts, such as cavies, rabbits and rhesus monkeys, the infections of which have died out It appears in baboons and rhesus monkeys within 20 days after injection of fresh fluke material with complete adjuvant, the most effective antigen is made from adult flukes with eggs It is rare and weak in man (but this is based on only 18 patients) In vaccinated baboons the phagocytic reaction lasts for 6 months and probably much longer It is not species-specific; all three human schistosomes are sheathed by leucocytes and killed in media from baboons immunized with *S mansoni* material It does not occur with cercariæ or eggs, is improved with miracidia, but is positive with all stages from the equivalent of 8-day larva to adult

A strong positive phagocytic reaction greatly weakens the flukes within 2 hr, before leucocytes can be seen to adhere to the cuticle The earliest collections of cells are commonly at head and tail, and in weak reactions these are often the only sites affected (Figs 3 and 4) Very large leucocytic sheaths can appear within 24 hr and all flukes die in 20–40 hr Movement of blood cells, for example, when the preparations are rolled, reduces the number of leucocytes that can gain attachment and an excess of moving red cells, as in whole blood media, can prevent the phagocytic reaction The reaction is not affected by anæsthetics, antibiotics, traces of heparin, additional glucose, high potassium concentration and metabolites of blood cells present in the preparations, it occurs when these abnormal substances are removed by continuous dialysis against serum

The attraction of leucocytes to the flukes depends on a serum fraction which is stable at 56° C but is precipitated with serum proteins at 85° C The fraction probably has no need of complement for its activity and it does not react with the fluke to make the cuticle attractive to leucocytes It remains active to a dilution of 1 in 4 and will produce a positive phagocytic reaction when added to leucocytes from normal uninfected hosts The phagocytic reaction system greatly potentiates the *in vitro* action of drugs A concentration of 0 1 mgm per cent of trivalent antimony (as potassium antimony tartrate) in media from uninfected subjects takes 3 days to kill *S mansoni* flukes, but in media with a strong positive phagocytic reaction, flukes die in less than 24 hr

The experiments performed under the good conditions prevailing in media dialysed against serum and rolled showed that the phagocytic reaction will only appear in almost stationary blood, fast-moving red cells prevent leucocytes from attaching firmly to the flukes, even in blood with a 4+ reaction The less the movement and the fewer the red cells, the quicker was the leucocytic sheathing[9] This suggests that hosts with a strong positive phagocytic reaction are unlikely to be protected against a new schistosome infection, but are in a good state of readiness to kill any adult flukes which, from old age, bad judgment, drug action, malnutrition or other deleterious changes in the *milieu* provided by their host, let go their hold on peripheral veins and are swept into the liver or lungs Here, as in the livers of rodents treated with diphenoxyalkanes[1], the flukes can block vessels and produce ideal conditions for rapid leucocytic sheathing

It is possible that the variable results of standard courses of antischistosome treatment in man reflect different degrees of phagocytic reaction activity as well as different rates of drug detoxication and excretion If so, frequently re-infected adults would show more positive phagocytic reactions and be easier to treat than children It is a common clinical observation, usually ascribed to efficient drug excretion by young healthy kidneys, that children can resist treatment which cures adults As the phagocytic reaction can be produced in a week or so by vaccination with whole-worm material, purified antigen may one day be given to uncured patients, with the object of alerting the reticulo-endothelial system ready for a second course of treatment a week later Before thinking of any practical applications, however, much more needs to be known Fractionation of the antigens and of the antibody globulins may give clues to the source of the antigen and the type of globulin that contains the antibody Other adjuvants less toxic than Freund's complete preparation must be used The lethal effect has to be investigated, it may be due to inability to absorb foodstuffs through cuticle and pharynx or to increased permeability of the cuticle to toxic antibodies With this new information the phagocytic reaction can be compared with the phagocytic systems described in filariasis and in other diseases due to helminths and protozoa which have contact with the blood

All the information we have at the moment suggests that immunity in parasite diseases is seldom mediated by antibodies which directly kill the parasite It seems more likely that the parasite's normal defences against immediate phagocytic attack are weakened and finally overcome Recent experiments, however, have not shown any protective effect of the phagocytic reaction system against challenge infection in monkeys The possibility of potentiation of drug action by the phagocytic reaction antibodies is under investigation

I thank Dr Janet Niven, Dr J H Humphrey and Dr R Smithers, of the National Institute for Medical Research, Mill Hill for their co-operation in these investigations

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KINETICS OF MITOCHONDRIAL SWELLING: EVIDENCE FOR SPECIFIC RECEPTOR SITES FOR INDUCER COMPOUNDS

By HANS H GOSCH*, PROF JOSEPH C ARCOS and MARY F ARGUS

Department of Medicine, Tulane University School of Medicine, New Orleans

A VARIETY of organic compounds and inorganic ions are known to induce the swelling reaction of isolated mitochondria, a phenomenon that has been related to variables such as the level of adenosine triphosphate (ATP)[1,2], the coupling of oxidation to phosphorylation[3], the oxido-reductive state of the respiratory carrier[4], and the presence of oxidizable substrates[5] Previously, investigations on the swelling of mitochondria from the livers of rats administered hepatic carcinogens indicated[6,7] the existence of receptor sites for the inducer compounds (cf ref 8) It has now been found that the kinetics of induced mitochondrial swelling follows the Michaelis–Menten equation providing direct evidence for the existence of these sites

Male Sprague–Dawley rats, fed *ad lib* a dextrose–casein base semi-synthetic diet[7], called hereafter the basal diet, were used The rats which were administered 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) received the hepatic carcinogen incorporated in the basal diet at the level of 0·06 per cent for 4 weeks. The initial weight-range of all the rats was 180–220 gm. Isolation of the liver mitochondria and the swelling tests (by the photometric method, at 23°–25° C) were carried out as previously described[6,7], and the values of percentage swelling at 40 min were used. Phosphate (as disodium hydrogen phosphate), calcium chloride and *p*-chloromercuribenzoate (PCMB) were assayed in 0·15 *M* potassium chloride, buffered with 0·02 *M* *tris*-hydroxy-aminomethane, at *p*H 7·4. Thyroxine and diphosphopyridine nucleotide (DPN), however, were tested in identically buffered 0·30 *M* sucrose since these inducers failed to give consistent results in the potassium chloride system. Extensive preliminary testing over a wide concentration-range was required with each inducer to establish the experimental concentration-range (8–11 points) showing typical Michaelis–Menten kinetics when plotted in the direct manner. The amount of mitochondria isolated from a single liver was sufficient for swelling tests covering the whole experimental range plus a control in the respective system without added inducer. The experiments with each inducer were carried out on ten non-pooled livers. The control values were subtracted from the experimentals and the net percentage swelling values averaged.

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* Recipient of a Student Research Stipend from the Tulane Educational Fund

The swelling reaction velocities versus the inducer concentrations were plotted in the double reciprocal manner according to Lineweaver and Burk[9] for calculation of the Michaelis–Menten K_m values and the extrapolated maximum velocities V_{max} (Fig. 1 and Table 1). The best fit of each curve was determined by the method of least squares. The correlation coefficients calculated for all double reciprocal plots are given in Table 1. This latter statistical treatment indicated that for PCMB (on basal diet and on 3′-Me-DAB) and for DPN the experimental values fit two lines with different slopes much better than one line, the two lines intersecting at the transition concentration S_{trans}. Accordingly for these two inducers two K_m and two extrapolated V_{max} values have been calculated. Similar kinetics was observed with phosphate and mitochondria from 3′-Me-DAB-fed rats, showing on the double reciprocal plot zero increase of the swelling reaction velocity at concentrations below 2·4 × 10^{-5} *M*. This inducer with normal mitochondria gave a plot fitting one line throughout the whole experimental range. The data obtained with mercuric chloride, also assayed in this investigation, are not included in Table 1 as this inducer gave considerable scattering of values with both test systems (K_m = 3·1 × 10^{-6} *M* to 7·1 × 10^{-5} *M*). Experiments now being carried out indicate that the swelling reaction of isolated rat heart sarcosomes induced by DPN, PCMB and pentachlorophenol also follow the Michaelis–Menten equation.

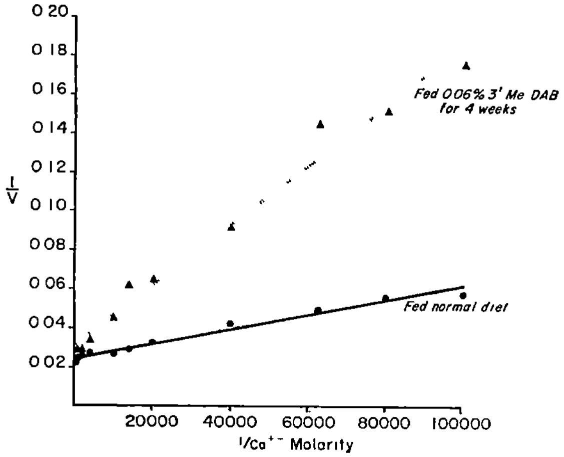

Fig. 1. Double reciprocal plots showing the effect of increasing inducer concentration (calcium chloride) on the velocity of the swelling reaction of isolated liver mitochondria

Table 1. Kinetic Values of the Swelling of Rat Liver Mitochondria Induced by Different Agents

	Inducer	K_m (*M*)	V^*_{max} (per cent)	S_{trans} (*M*)	Exp. range (*M*)	Correlation coefficient
Fed basal diet	$CaCl_2$	1·5 × 10^{-5}	40·0	—	1·0 × 10^{-5} to 5·0 × 10^{-3}	0·99
	PO_4^{3-}	6·5 × 10^{-5}	37·0	—	1·0 × 10^{-5} to 4·0 × 10^{-4}	0·92
	PCMB	7·3 × 10^{-5} (1·3 × 10^{-4})†	25·0 (200·0)†	2·0 × 10^{-5}	1·5 × 10^{-5} to 7·0 × 10^{-5}	0·99 (0·96)†
	Thyroxine	3·3 × 10^{-6}	52·7	—	2·0 × 10^{-6} to 3·0 × 10^{-5}	0·98
	DPN	2·7 × 10^{-2} (6·4 × 10^{-3})†	54·1 (145·0)†	3·8 × 10^{-3}	3·4 × 10^{-3} to 5·0 × 10^{-3}	0·99 (0·99)†
Fed 3′-Me-DAB	$CaCl_2$	5·2 × 10^{-5}	32·3	—	1·0 × 10^{-5} to 1·0 × 10^{-3}	0·91
	PO_4^{3-}	4·1 × 10^{-5}	9·5	—	1·0 × 10^{-5} to 4·0 × 10^{-4}	0·76
	PCMB	7·8 × 10^{-5} (1·3 × 10^{-4})†	18·5 (143·0)†	2·0 × 10^{-5}	1·5 × 10^{-5} to 7·0 × 10^{-5}	0·97 (0·99)†

* Calculated V_{max} as percentage swelling. † Higher concentrations.

The present results provide strong support for the hypothesis[6–8] that the various agents that induce the ATP-reversible swelling[2] of mitochondria act in the particulate membrane[10,11] on specific loci which are receptor sites in the pharmacological or enzyme kinetic sense. That the relative affinities of the inducers to the receptor sites are estimated from the final extent of terminal effector action (that is, swelling) versus inducer concentration has the advantage that it excludes the obscuring of the kinetics by the rates of membrane penetration (possibly responsible for the initial lag period observed in the time-curve of certain types of swelling) and by non-specific adsorption, which may well be the case when determining the total fixation of inducers in the particles.

During the feeding of various hepatic carcinogens to rats the swelling ability of the liver mitochondria undergoes drastic alterations[6,7] which correlate with the time of appearance of gross tumours[6]. With 3′-Me-DAB the onset of irreversibility of these alterations is at about 4 weeks under similar dietary conditions as used in the present investigation[6]. The double reciprocal plots obtained with calcium chloride, phosphate and PCMB, following feeding of this dye for 4 weeks, show displacement of the curves akin to a non-competitive inhibition (for example, Fig. 1) resulting in decrease of the calculated V_{max} and no or relatively small change in the K_m values (Table 1). Since the initial amounts of mitochondria used in these tests were maintained strictly constant, the data are consistent with the decrease of the amount of receptor sites per particle due to feeding of the dye. Hence, further evidence is provided for the thesis[6,7] that one aspect of the azo carcinogen-induced cellular alterations may be the 'micro-denaturation' of control sites of the mitochondrial membrane. This is in agreement with other results from this laboratory that various water-soluble carcinogens are powerful agents of protein denaturation[12] A detailed report of the results here presented and of related investigations now in progress will be published at a later date together with further statistical analysis.

We thank Mrs Joyce B Mathison for her assistance in carrying out the determinations with DPN and mercuric chloride These investigations were supported by the U S Public Health Service research grant *CA* 05431

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NEW SEROLOGICAL TEST FOR ALLERGY IN MAN

By Prof WALTER B. SHELLEY

Department of Dermatology, University of Pennsylvania, Philadelphia

PREVIOUSLY we have demonstrated that immediate allergic hypersensitivity can be detected in both patients and sensitized animals by means of the basophil leucocyte degranulation response[1,2] The test consists in exposing fresh heparinized blood to a given antigen for 15 min *in vitro*[3] Concentration of the basophils is achieved by liquid fixation and filtration, and is followed by specific toluidine blue staining for examination of the degree of degranulation During the past two years, practical experience with this test in many clinical conditions has revealed it to be an accurate and sensitive one Significantly, it was found that in acute allergic states the basophil undergoes degranulation not only *in vitro* but also *in vivo* Thus, the concept of the critical role of the basophil in immediate hypersensitivity reactions was confirmed, but the usefulness of the diagnostic test was dimmed at times for lack of basophils to observe

This article describes a new laboratory method which requires of the patient only serum Basophils as well as the antigen are supplied by the laboratory Such an indirect technique considerably expands the usefulness of the basophil degranulation response Simple enough for the smallest laboratory, it nevertheless lends itself to large central laboratory mass testing of diverse antigens on specimens of serum from patients at any distance No longer must the patient present himself to the laboratory for testing within minutes after the blood is drawn The serum may now be repeatedly tested any time or in any laboratory As many as 200 tests may be conducted on 1 ml of serum Furthermore, the test procedure is considerably shortened and simplified Finally, the donor basophil permits accurate readings on patients who have few or no circulating basophils

Venous blood is drawn from the patient into a sterile vacutainer, allowed to clot, and the serum separated for storage in deep freeze (–4° C) The serum may be used fresh or after repeated thawings, but plasma is not routinely satisfactory

Simple aqueous (0 9 per cent sodium chloride solution) dilutions of pure antigens, for example potassium salt of penicillin G or soluble extracts of complex antigens, for example, pollens, are used These may be prepared in the local laboratory or are available commercially as used in intradermal skin tests (Hollister-Stier Laboratories, Yeadon, Pennsylvania) The degree of reaction will vary with the concentration of the antigen used In the case of penicillin, screening tests have been made with an initial test antigen concentration of 1/100 (w/v). The sensitivity titre has then been ascertained by serial dilution of this antigen Positive reactions are rare at a concentration less than 1/10,000, but note that the effective final concentration is far less than this due to the dilution in performing the test The antigen concentration must not be so high as to induce basophil changes by itself as shown in the antigen control test The penicillin antigens are stored frozen, whereas most of the commercial antigens are simply kept in the refrigerator Precautions are taken to prevent bacterial contamination

Antigens not soluble in saline or buffer solutions may be dissolved in alcohol, ether or acetone, placed on the test slide, allowed to dry, and then used.

Although the donor basophils may be from a normal healthy human subject, we have found that for routine work the greatest number of test basophils are to be secured from rabbit blood. Using albino New Zealand rabbits, 1 ml heparinized blood is freshly drawn from the lateral ear vein Keeping it chilled in the refrigerator such blood maintains a physiologically and anatomically satisfactory basophil for about 2 h For each test, a drop of blood is introduced into a 7-cm length of transparent polyethylene tubing (Clay–Adams *P E* No 90, 0 86 mm internal diameter, end, heat sealed), through a long No 22 needle on the end of a tuberculin syringe If capillary blood is desired, an open polyethylene tube may be filled with a drop of blood from the finger-tip and the end then heat sealed by match flame and hæmostat The tubing is placed in a 2-mm glass tubing for support and spun in a micro-hæmatocrit centrifuge (for example, 30 sec, 11,500 rev/min) The resultant buffy coat is isolated by clamping and cutting with a razor blade The micro-drop of buffy coat is then ready for immediate addition to the serum on the specially prepared slides

Flame clean microscope slides, cool until only slightly warm, flood with freshly prepared stain rapidly, and avoid overlaying. Drain excess stain back into bottle and air-dry Stain should not be applied before an open window, or in hot, humid atmosphere, 5 ml of staining solution are adequate for 75-100 slides The slides may be stored indefin-

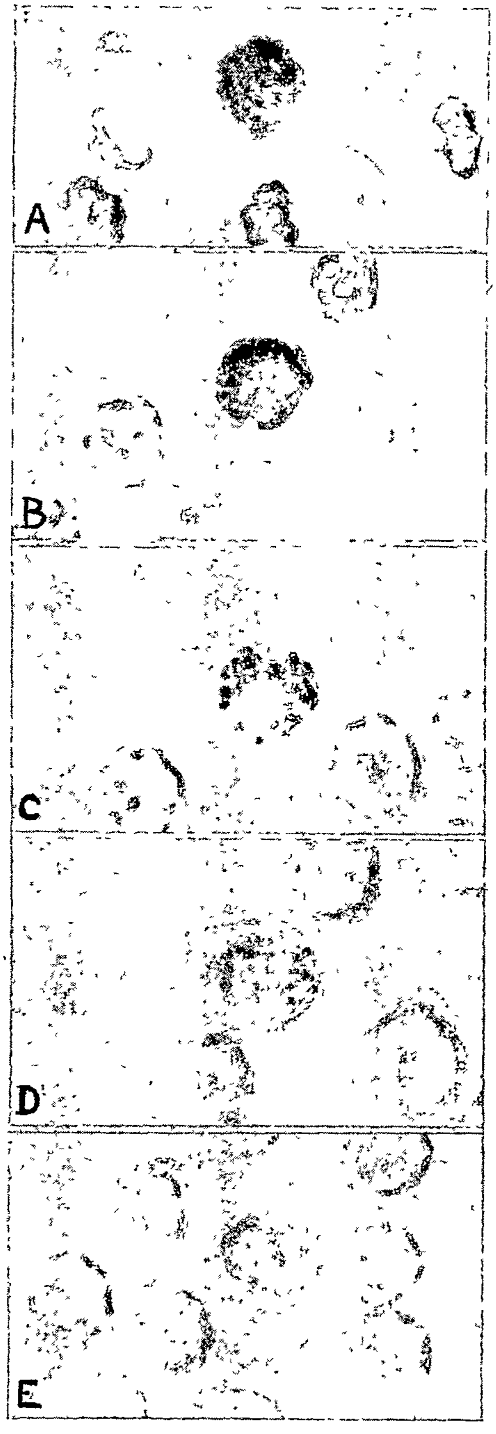

Fig 1 Views of progressive specific allergic degranulation of a single living donor basophil in presence of grass pollen extract (0 001 mgm N) and serum of hay fever patient Time *A*, 0, *B*, 5 min, *C*, 8 min, *D*, 10 min, *E*, 17 min Supra-vital stain Room temperature

itely in a dust-proof container Stain formula to 5 ml of absolute ethyl alcohol, add 0 6 ml of the neutral red stock solution (0 3 per cent in absolute ethyl alcohol)

Neutral red, as a *p*H indicator dye, rapidly points up the acidic nature of the basophil granules Toluidine blue staining of the heparin has also been used by us supra-vitally with success

Add 0 005 ml of patient's serum to stained slide using a micropipette or a micro-drop from 27 needle on tuberculin syringe Add entire buffy coat by blowing out of cut segment Add 0 005 ml of antigen solution Mix by gentle air agitation and cover with 'Vaseline'-edged cover-slip (No 1) Readings are made under oil by scanning the living field Within 5 min the basophil granules stain a distinctive brick red The cell can be sharply distinguished from all other leucocytes After 5 min read the first 20 basophils for changes in shape, stainability and degranulation Immediate readings can routinely be done within 10 min Slow reactions may be observed by later readings at 20–30 min Antigen controls consisting of test antigen and rabbit basophils as well as serum controls consisting of test serum and rabbit basophils must be done regularly

In the positive test the majority of the basophils show a loss of sphericity, cell distortion, swelling, granulolysis, granular swelling, less staining, rapid oscillation or dancing of granules, loss of granules. appearance of clear nucleus, cell wall bleb formation. and streaming of granules from the cells Explosive degranulation is seen in the more sensitive individuals We have found that the living cell permits more accurate assessment of its functional state than does the dead fixed cell, so that typing is not regularly necessary Observation of the behaviour of a single cell is not adequate since occasional cells in the control may undergo degranulation

Should the antigen control show degranulation, it is necessary to decrease the concentration In the event of the rare positive serum control, new serum should be secured Occasional patients may be sensitive to the traces of rabbit serum present in the buffy coat We have not seen anyone sensitive to the neutral red

This indirect technique has been successfully used in the study of many patients with immediate hypersensitivity states Among these were 22 men who had had penicillin reactions Fourteen of these gave a positive test Significantly, none of 10 control patients at present receiving penicillin treatment without incident gave any evidence of basophil degranulation The penicillin-sensitive patients regularly showed a positive test to aminopenicillanic acid as well as certain cross-sensitivity reactions to the new synthetic penicillins In six patients with atopic dermatitis, five showed specific degranulation reactions to chocolate, three to milk. three to tree pollens, and one each to grass pollens. wool and wheat In control tests with 30 different rabbits and 100 sera, but one positive reaction was seen and this in a patient with known serum hypersensitivity

By means of supra-vital staining, the allergic degranulation process has been observed and photographed for the first time in a single basophil Fig 1 shows such a progressive reaction It is apparent that without awareness of the sequence, few would identify Figs 1*D* and *E* as a basophil leucocyte This test is based on the rapid passive sensitization of donor basophils *in vitro* Hence, it may be successfully employed with other methods of observing degranulation such as the buffy coat smear technique[4]

Our experiences with this indirect basophil test in food, drug and insect bite reactors (both urticarial and anaphylactic) and atopic dermatitis patients, as well as those with serum sickness, asthma hay fever, contact dermatitis and ulcerative colitis have revealed an expanding vista of clinical usefulness for the basophil In our laboratory it has been a safe, reliable and sensitive indicator of serum antibodies not otherwise always evident It affords an assay of the allergic potential of new drugs, and other problems of cross-sensitization It aids in the diagnosis of obscure, as well as common, allergic states Furthermore, the therapist is given a method of objectively measuring changes in sensitivity during programmes of hyposensitization As noted above, potential anaphylactic reactors may be identified Nevertheless, the test remains an index only of the patient's status at the time the blood is drawn It cannot predict which patients will develop allergy, for example, to penicillin, or indeed which patients have temporarily depleted their antibody and thus are incapable of reacting at the time of blood sampling Finally, this technique has proved itself in work on sensitized animals as well as on man

This work was conducted under the sponsorship of the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board, and was sponsored by the Surgeon General, U S Department of the Army

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CHEMICAL EFFECTS OF MECHANICAL DISPERSION OF ALKALINE CHLORIDE

By Dr HENRY M. PAPÉE and Dr GIANNA L PETRICONI

Centre for Aerosol Nucleation, National Research Council of Italy

ELECTRICAL effects due to deformation of sodium chloride crystals are well known[1,2], and it has been shown that mechanically induced stress can lead to defects in alkali halides[3] It has been shown, on the other hand, that while thermal evaporation of sodium chloride results in chemically uncontaminated particles, considerable amounts of nitrate and colloidal sodium impurities appear on the surface of the salt when a dry sodium chloride smoke is electrostatically precipitated by means of excessively large voltages[4] It has to be emphasized that while formation of NO_3^- impurities readily occurs through the action of NO_2 gas on moist sodium chloride particles[5], the same appears to happen in a dry air carrier medium and in electrical discharges[4]

Alkaline chloride samples of reagent grade were ground using a mechanical, heated, agate mortar, and sieved to − 270 mesh by means of mechanical vibrator The product was then separated into monodisperse fractions by use of a Bahco centrifuge (Éts Neu Co , Lille) The rotor of the instrument was heated during centrifuging to about 100° C by means of an electrical tape which was wound round the shield of the centrifuge, and hot air was blown over the orifice of the instrument during its operation This was done to prevent caking and agglomeration of particles on exposure to air moisture at room temperature According to the stage of the preparation, samples were stored either in a stove at 150° C, or in a desiccator in an atmosphere of pure, dry nitrogen Since the size of the particles obtained was too large to warrant nitrogen Brunauer–Emmett–Teller surface determination, the dimensions of the granules were estimated microscopically by an optical micrometer attachment Fig 1 shows photomicrographs of the monodisperse samples obtained

If it is assumed that electric or triboelectric[6] phenomena induced chlorine vacancies on the surface of alkaline chlorides during grinding, then it follows that the relative increment of sodium per unit surface will be approximately proportional to the increase of surface area during the process Thus

$$\frac{d\,\Delta OH^-}{S} = K\,dS \qquad (1)$$

where ΔOH^-, which is measured after solution of the halide sample, is the total excess over stoichiometry of sodium (situated in the outer strata of the solid) in the product Integration of equation (1) between zero and S surface values, yields

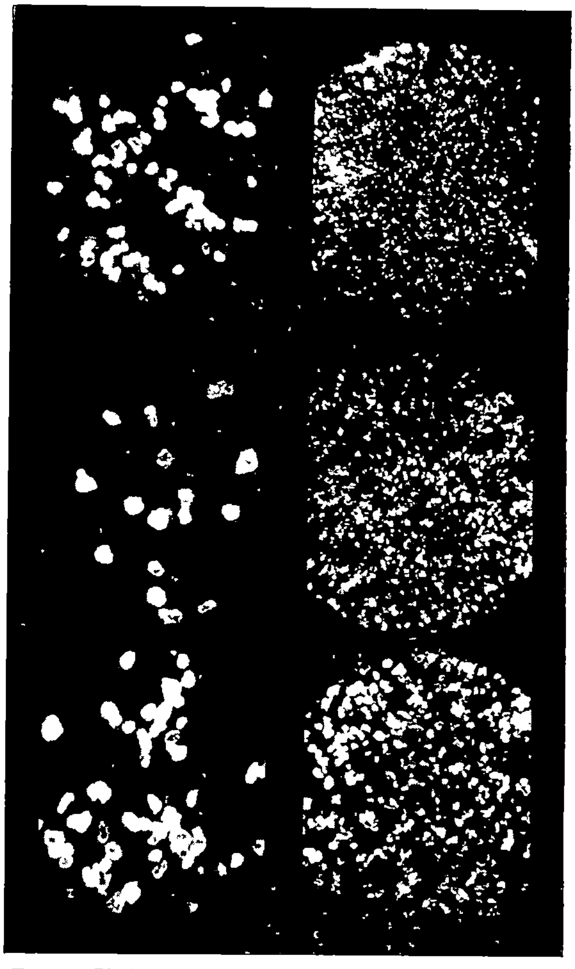

Fig 1 Photomicrographs of monodisperse sodium chloride particles used Visual field is 0 78 mm in diameter

$$\Delta OH^- = \tfrac{1}{2} K S^2 \qquad (1')$$

As expected, however, nitrate impurities were found in the samples after grinding, and although measurements of OH^- resulted in consistent trends according to equation (1), it was found that the behaviour of nitrates could not be fitted into a pattern such as given by equation (1). Surprisingly, however, vacuum outgassing of ground samples resulted in decreases of *p*H values of the product afterwards dissolved. This in turn led to the suspicion that triboelectrically generated nitrate, which is adsorbed on the surface, could be reduced to ammonia by the active centres on the surface in presence of very small amounts of moisture. Assuming this

$$\frac{dN}{dS} = K'S \qquad (2)$$

where N stands for the number of active centres created per unit surface.

Integrating

$$\int_0^N dN = K'\int_0^S S\,dS \qquad (2')$$

Therefore

$$N = \tfrac{1}{2}K' S^2 \qquad (2'')$$

and since it may be assumed that

$$NH_3 = K''\, N\, NO_3^- \qquad (3)$$

substitution of equation (2″) yields

$$NH_3 = K_3 S^2\, NO_3^- \qquad (4)$$

The alkalinity of the samples studied was determined using a sensitive *p*H meter, while nitrate and ammonia were measured colorimetrically by the brucin and Nessler reagent methods respectively. Triple-distilled water was used for the preparation of 100 ml. aliquots of solutions containing 300 mg reagent purity, ground, monodispersed salts, while 0.5-g aliquots were used for NH_4^+ determinations.

Fig. 2 gives plots of ΔOH^- against S^2, per unit weights of both sodium and potassium chlorides, while Fig. 3 gives corresponding plots according to equation (4).

The results indicate that mechanical fragmentation induces considerable changes in surfaces of alkali halide particles, and that therefore particles prepared in this way cannot be considered as pure halides from the point of view of their surface properties. It is possible that effects leading to a chemical change such as already described may be partly responsible for the power of ice nucleation shown by some freshly ground substances[7,8], it is furthermore possible that processes of repeated mechanical fragmentation, friction, and distortions induced by hydration and desiccation, which atmospheric aerosol undergoes, may to some extent contribute to the formation of atmospheric nitrate and ammonia largely contained by sodium chloride nuclei[9].

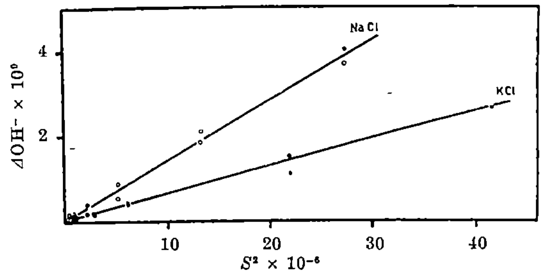

Fig. 2. Plot of the change of ΔOH^- (in g-equiv.) per gram of ground sodium and potassium chlorides, versus square of specific surface of monodispersed salt, separated by centrifuging (cm²)

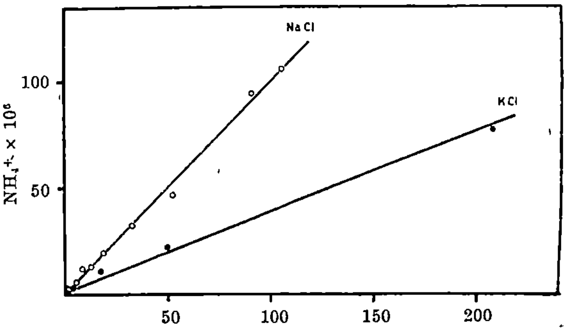

Fig. 3. Plot of the change of ammonium content (in g-equiv.) per unit weight of sodium and potassium chloride samples, against the square of specific surface (in square centimetres) of monodispersed salt obtained by grinding and centrifuging, multiplied by its content in nitrate (in g-equiv. per gram of salt)

We thank Prof. V. Caglioti for his interest and encouragement, and Mr. M. Armeni for the drawings and technical help.

This work was supported by Cassa per il Mezzogiorno and the Società Generale Elettrica della Sicilia.

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THORIUM AND URANIUM CONTENTS OF AUSTRALITES

By R. D. CHERRY

Department of Physics, University of Cape Town, Rondebosch

THE thorium and uranium contents of six australites have been measured in this laboratory. The technique used was that of 'thick-source' alpha counting[1-3] combined with the thorium-*A* pairs method of Mayneord *et al.*[4] A scintillation counter was used as a detector, and the experimental apparatus was similar to that described by Turner *et al.*[5] A total alpha count-rate, C, and a 'pairs' rate, P, are measured. If one assumes radioactive equilibrium and a known uranium-238 to uranium-235 ratio, then C and P are related to the uranium and thorium concentrations U and Th by equations of the type

$$C = k_1\text{Th} + k_2\text{U} \tag{1}$$

and

$$P = k_3\text{Th} \tag{2}$$

The constants k_1, k_2 and k_3 depend on the decay constants and atomic weights of uranium-238, uranium-235 and thorium-232, the area of the sample and, finally, the ranges of the alpha-particles of the thorium, uranium and actinium series. The evaluation of these constants has been discussed in detail by me[6]; here it will suffice to point out that in practice C is about thirty times larger than P, and is accordingly subject to a much smaller percentage error in the counting statistics.

The australites used included two from Victoria (Nos. 1 and 4), two from South Australia (Nos. 355 and 475) and two from Western Australia (Nos. 379 and 1176). The samples were supplied by Dr. S. R. Taylor and Mrs. M. Kaye. They had all been ground to 120 mesh and had previously been analysed for alkali metals[7,8], trace elements[9,10] and major elements[10]. No chemistry was necessary, the samples were merely packed and sealed on 'Perspex' rings in the manner described by Turner *et al.*[5], allowed to stand for a month or more and then counted. The total counting time amounted to about seven days per sample.

The results are summarized in Table 1, which includes also the potassium determinations of Taylor and Sachs[7,8]. The percentage standard deviations in C and P due to counting statistics alone average 1·8 and 11 per cent respectively for each sample. If it is assumed that counting statistics are the only appreciable source of error, then it follows from the appropriate numerical insertions into equations (1) and (2) that the analytical precision of the determinations of thorium and uranium are about 11 and 13 per cent respectively. The following features are worthy of note.

(*a*) The absolute values of uranium and thorium for the 'mean' tektite from our group are 2·0 and 9·4 p.p.m. respectively. Because of uncertainties in the ranges of alpha-particles in solids[6] (and consequent difficulty in determining absolutely the constants k_1, k_2 and k_3) it is difficult to estimate the errors in these values. It is thought unlikely, however, that these errors exceed 10 per cent. The agreement with the single high-quality value of Tilton[11] for a South Australian australite (namely, uranium at 1·74 p.p.m. and thorium at 9·19 p.p.m.) is reasonably satisfactory. The thorium/uranium ratio for our samples averages out at 4·9, and is quite comparable with that obtained for common terrestrial material[15].

(*b*) A high positive correlation between the count-rate C and the potassium content (as determined by Taylor and Sachs[7,8]) is immediately apparent. Because of the relative inaccuracy of the individual pairs rate values, this same trend is not obvious in the values of P, thorium and uranium. It seems fairly certain that a positive correlation does in fact exist between the potassium content and the uranium and/or thorium values, since it would be very difficult to account for the sympathetic variation of C with the potassium content on any other basis. Such a positive correlation should be taken into account by any theory which postulates tektite origin on the basis of a mixing hypothesis such as the recently suggested hypotheses of quartz–shale[12] and chondrite–soil[13] mixtures. On the quartz–shale hypothesis, if one follows Taylor[10] and assumes that quartz has a negligible uranium and thorium content, then, of course, the positive correlation between potassium and uranium and/or thorium is explained since the potassium content is itself negatively correlated with the silica content[10,12,14]. Similarly, the chondrite–soil mixture will also explain the positive correlation, since the levels of potassium, uranium and thorium in chondrites[15] are very much lower than in tektites and soil.

(*c*) Perhaps the most striking feature of the results in Table 1 is the small spread in the uranium and thorium values over the six samples counted. The percentage standard deviation of the six uranium values is 17 per cent, while that of the six thorium values is 15 per cent. Since we have estimated the analytical precision at 13 per cent and 11 per cent for uranium and thorium respectively, it would appear that the inherent spread in the results for the six samples is about 11 per cent for the uranium content and about 10 per cent for the thorium content. These figures are probably upper limits, and it is interesting to note that the relative standard deviation of the six count-rates, C, is as low as 5 per cent.

Table 1

Tektite No.	P (pairs/h)	C (counts/h)	K content (per cent)	U content (p.p.m.)	Th content (p.p.m.)
1,176	0·70	17·4	2·12	1·4	11·6
379	0·50	16·7	2·03	2·4	8·4
4	0·58	16·8	1·88	2·1	9·7
1	0·63	15·7	1·73	1·8	10·6
355	0·49	15·7	1·73	2·2	8·3
475	0·48	15·2	1·73	2·1	8·1

Relative spreads for other elements in australites have been quoted by Taylor[10]. Many of those (notably silicon, potassium, iron, lithium and barium) are as low or lower than the above results for thorium and uranium. The implications of this low variance in tektite composition relative to terrestrial rocks have been discussed in detail by Lowman[16], who concludes that it is a weighty factor against theories which suggest that tektites are produced by a mechanism which results in the fusion of terrestrial materials. Anders[17] has pointed out that tektites might possibly have been derived from smaller areas than the six million square miles assumed in Lowman's study, and has suggested that rock analyses should be selected from a few small areas for legitimate comparison with tektite variances. A desirable experiment at the present junction would appear to be the following. The variance of selected elements (for example, potassium, lithium, thorium) in possible parent materials (for example, shale[12], soil[13]) should be studied as a function of the surface area from which the samples of 'parent material' are collected. If it is found impossible to produce variances as low as those found in tektites even by limiting sampling to small areas, Lowman's conclusion will have been substantiated. If, on the other hand, variances approximating to those occurring in tektites are found at a particular size of sampling area, then some information will have been obtained as to the likely areal magnitude of any event leading to the formation of tektites from terrestrial materials.

I thank Dr S R Taylor and Mrs Maureen Kaye for the provision of the australite samples and Prof L H Ahrens for discussion

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STIMULATED ELECTRON SPIN-FLIP TRANSITION AS THE SOURCE OF 18-MEGACYCLE RADIATION ON JUPITER

By L LANDOVITZ
Yeshiva University

AND

LEONA MARSHALL
New York University

THE discovery of strong radio emission from the planet Jupiter was made in 1955 by Burke and Franklin[1] Since then a great deal has been learned about the characteristics of this radiation, but as yet no satisfactory explanation has been given[2–10,16] It is the purpose of this article to suggest a way in which the radiation may be produced which seems to account for most of the features peculiar to Jovian radio emission

First we summarize what is so far known about the radiations The average intensity of a burst of radiation is about 5×10^{-21} W m^{-2} $(cycle/sec)^{-1}$ The bursts are intermittent, and most of the energy is emitted in a narrow frequency-range extending from 10 to 35 Mc with a maximum intensity at 18 Mc and a half-width at half maximum of about 3 Mc In any given burst the energy is contained in an even narrower band width of about one-half Mc per sec This relatively monochromatic emission cannot be ascribed to black body radiation at any temperature

The rotational period of Jovian radio sources agrees with the accepted rotational period determined by observation of visible features on the surface of Jupiter The radio emission seems to come from a localized source having directional properties That is, the probability of emission of a burst is high during only about 1/6 of the Jovian day The probability of emission seems to show an inverse correlation with sunspot number It has been recognized that such a correlation would result from the existence of a Jovian ionosphere in that its increased ionization at sunspot maximum would make the escape of radiation from the lower levels of the ionosphere less probable

It is a peculiarity of the Jovian radio broadcasts that although they may be detected first at one frequency, they may be detected slightly later at either higher or lower frequencies The frequency of onset of these storms seems to drift to either higher or lower frequency with almost equal probability, and furthermore the drift in frequency toward the end of each of these broadcasts seems completely uncorrelated with the drift in frequency at the beginning The rate of frequency drift is a few Mc/sec The broadcasts last for about 3 sec on the average

The Jovian radio emission has been shown to be mainly right-hand circularly or elliptically polarized, an effect which suggests the existence of an intrinsic magnetic dipole field of Jupiter analogous to the Earth's magnetic field

Considerable evidence has accumulated which shows a correlation between solar coronal eruptions and Jovian radio emission 1–10 days later, indicating that the Jovian broadcasts are initiated by solar particles which travel from the Sun to Jupiter with travel times varying from 1 to 10 days Additional evidence for the correctness of this view is found in a correlation between solar flares and geomagnetic activity on one hand, and Jovian radio emission 8 days later, during a time when Jupiter and the Earth were aligned along the same radius from the Sun (in opposition) When Jupiter is no longer opposed to the Sun as seen from the Earth there is correlation between solar flares, geomagnetic storms, and Jovian noise storms delayed by 8 days plus the time required for the Sun to rotate through the heliocentric angle between the Earth and Jupiter, indicating that the same centre of activity on the Sun shoots out particles as it rotates, perturbing now the Earth, and now Jupiter

Various suggestions have been made about the origin of radio emission from Jupiter, none of them entirely satisfactory Lightning discharges in Jupiter's atmosphere would produce radiation at all wave-lengths, unpolarized, and unlikely to be strongly correlated to a fixed interval of longitude of the planet The mechanism of plasma oscillations should produce radiations over a broad band of frequencies, corresponding to a continuous decrease in ion density from high values deep in Jupiter's atmosphere to the low densities of interplanetary regions The mechanism of synchrotron radiation from solar particles spiralling in Jupiter's magnetic field would be expected to produce higher harmonics as well as the fundamental cyclotron frequency, because the solar particles must have velocities 0 01 c –0 1 c in order to reach Jupiter in 1–8 days All these mechanisms have the drawback that one expects from them radio emission over a much broader frequency range than 18 ± 3 Mc/sec

We suggest instead that this radio emission is a maser-like phenomenon, namely, is a decameter emission from electrons making spin-flip transitions in the perturbed magnetic field of Jupiter, and that furthermore the radio emission is stimulated as the perturbation travels through the ionosphere of Jupiter. We suggest that impinging clouds of solar particles stimulate perturbations which in turn cause very sudden changes (ΔH) in the local magnetic field of Jupiter, in the time of the Larmor precession, so that the local population of electrons initially having an equilibrium distribution of spin orientation with respect to the local field direction is suddenly stimulated to make spin-flip transitions. The incidence of a cloud of solar particles, therefore, subjects an electron population in a static magnetic field to an oscillating magnetic perturbation causing the electron population to emit radiation at the Larmor frequency characteristic of the static field[11].

It is reasonable to suppose that all atoms at low densities are ionized by sunlight. The atmosphere of Jupiter is thought to be largely hydrogen, consequently the exosphere contains mainly protons and electrons.

The characteristic frequency of the radio emission, 18 Mc, provides a determination of the local Jovian magnetic field of 6.4 gauss = H at the altitude where the emission occurs (according to $\nu_c = 2.8\ H$ Mc/gauss). The clouds of solar particles have average densities ~ 10^4 particles/cm^3, travel with velocities $v \sim 1 \times 10^8$ cm/sec, contain magnetic fields ~ 2×10^{-3} gauss, and have an average dimension $d \sim 2 \times 10^8$ cm (see, for example, ref. 12). The time of passage of such a cloud is given by $t \sim d/v = 2$ sec, in agreement with the duration time of radio emission.

Such high-velocity clouds of solar particles carrying magnetic fields are known to produce in the exosphere of the Earth sharp crested magnetic waves[13] of ~ ± 0.001 gauss amplitude, between 7 and 14 Earth radii. We assume that a similar coupling occurs when solar clouds impinge on the exosphere of Jupiter and that in fact magneto-acoustic waves[14] are produced in low-density plasma having high-frequency components (for example, ~ 10^7 c./sec) short wave-lengths, and magnetic amplitude ΔH ~ 2 milligauss. The local field in which the electrons find themselves is adiabatically changed from H to $H + \Delta H$ (where $\Delta H \sim 0.002$ gauss) in a time not large compared with the time of the Larmor precession, $t_L \sim 5 \times 10^{-8}$ sec. The rapid oscillations of magnetic field act as perturbations able to produce spin flip and radiation at the Larmor frequency.

The frequency at onset should drift to higher or lower frequencies depending on the path taken by the magneto-acoustic wave train in the exosphere. For example, if the wave-pocket travels toward the surface of Jupiter, the emitted Larmor frequency will drift to higher values corresponding to increased values of the local Jovian magnetic field.

An order of magnitude estimate of the spin-flip probability caused by a change of $\Delta H \sim 0.002$ gauss in $\Delta t \sim 2 \times 10^{-8}$ sec is given by[15]

$$|\alpha|^2 = \frac{\mu^2}{\hbar^2} [\Delta H]^2 [\Delta t]^2 = 16 \times 10^{-8}$$

where $\mu = eh/2\,m_e c$ is the electron magnetic moment. Suppose that an electron experiences q such changes per sec. Then the probability of spin flip per electron is $16q \times 10^{-8}$ per sec. Let N_e be the electron density, and assume that the radiation comes from a volume $V \sim R_J{}^3$ where R_J is the radius of Jupiter (which may be taken as an estimate of the thickness of a Van Allen belt on Jupiter). Then $V \simeq 3 \times 10^{29}$ cm^3. The number of electrons which contribute to the radio emission caused by the solar cloud is VN_e.

If the spin-flip radiation is produced isotropically, then the power P received on Earth is given, in units of energy per unit area per unit frequency interval, by

$$P = 16(h\nu_L)N_e V(q \times 10^{-8})/(D_J)^2(1/2 \times 10^6 \text{ c./sec})$$

where $D_J = 6 \times 10^{11}$ m is the distance between Earth and Jupiter, and where $1/2 \times 10^6$ c./sec represents the average band-width of a burst.

We find

$$P \simeq qN_e \times 10^{-33} \text{ W (m)}^{-2}(\text{c./sec})^{-1}$$

The average power is observed to be ~ 5×10^{-21} W (m)$^{-2}$ (c./sec)$^{-1}$. Then

$$qN_e \sim 1.5 \times 10^{12}$$

so that if $N_e \sim 10^7$ electrons/cm^3 (corresponding to the maximum density from which decametre radiation can escape), then q may be as small as 10^5 rapid changes in magnetic field per sec.

Actually one expects that the spin-flip radiation is not isotropic, but instead is emitted roughly parallel and anti-parallel to magnetic field lines on Jupiter. This feature is in agreement with the observed correlation of frequency of emission of 18 Mc/sec radiation with phase of the Jovian day, and may contribute an enhancement factor to the power of radiation received on Earth. (Note that the angular dependence of magnetic dipole radiation is $(1 + \cos^2\theta)d\Omega$ where θ is the angle between the propagation direction of the radiation and the magnetic field. Thus, the radiation tends to come out parallel and anti-parallel to the magnetic field lines of force. However, if there is a tipping of the magnetic axis with respect to the rotation axis, there will be a dependence of frequency of radio emission on phase of rotation, because of the displacement of geographic and magnetic poles, we are presented with portions of the field roughly parallel and perpendicular to the Earth's axis at different times of day.)

This work was supported in part by the U.S. National Science Foundation (L. L.) and by the U.S. Atomic Energy Commission Contract No. *AT* (30-1)-2625 (L. M.).

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LETTERS TO THE EDITOR

GEOPHYSICS

A Working Model illustrating Density of the Upper Air

In a previous communication[1] I proposed a preliminary model of the strong variability of the upper air density due to the extraterrestrial influence This model has now proved itself also for times of low solar activity at the beginning of the year 1962 At this time the solar radio noise at 10 7 cm had decreased to a value of 100 × 10^{-22} W /cm ^{2} cl Since the last sunspot maximum was extremely high, the solar activity was more strongly varied during 1958–62 than from a mean sunspot maximum to the minimum Four effects have been found predominant (1) the solar electromagnetic wave effect with its subdivision, (1*a*) solar short ultra-violet effect, (1*b*) day–night effect, (2) the corpuscle effect with, (2*a*) magnetic storm effect, and (2*b*) the plasma effect (solar wind) (annual and semi-annual effect)[2]

The plasma effect must cause an additional steady influx of heat from interplanetary matter besides the ultra-violet radiation as also other authors have recently concluded[3-5] The plasma effect was first found by me through its semi-annual and annual variation[6], which have now been independently confirmed[4]

With the expressions in ref 1, equations 1 and 2, the total completed formula for the air density $\rho(h)$ can be written now for low and mean latitudes as

$$\log \rho(h) = \log_{\text{stand}} \rho(h) - \Big[\iota(220, h)\Big(1 + \iota(\theta)\theta_t(h)\frac{220 - F}{F}\Big) + \theta_{\text{stand}}(h) f(\theta) + g(a, F)\, a(220, h) + K(200, h)\frac{A_P}{200}\Big] + \{\qquad\} \quad (1)$$

where F is intensity of the solar 10 7 cm. radio noise in units of 10^{-22} W /m ^{2} cl, θ is local time, a is month in the year, A_P is planetary magnetic index

The new functions $\iota(\theta)$ and $\theta_t(h)$ (Fig 1) give the dependence of the day–night effect on the solar activity The plasma-effect $g(a, F)\, a(220, h)$ markedly decreases with the solar activity according to equation (2), from which some conclusion can be gained about the dependence of the solar wind on the sunspot activity

$$g(a, F) = g(a) + (220-F)[0{\cdot}0060 - 0\ 0020 g(a)] \quad (2)$$

The dependence of the phase functions $g(a)$ and $f(\theta)$ on the month a in the year and local time θ has been already given in ref 1, Fig 1 The height functions $\iota(220, h)$ $a(220, h)$ and K (200, h) are given in Fig 2 up to an altitude of 1,500 km Table 1 represents the last values of the reference density profile $\rho_{\text{stand}}(h)$ In equation (1) terms of second order, which consider the dependence of the different terms on each other, have been neglected It has been found that these second-order terms are very small below an altitude of 1,000 km

Table 1 $\rho_{\text{stand}}(h)$

h (km)	gm /cm ^{3}	h (km)	gm /cm ^{3}
150	1 912 × 10^{-12}	600	1 623 × 10^{-15}
200	2 246 × 10^{-13}	700	7 418 × 10^{-16}
250	1 337	800	3 613
300	5 170 × 10^{-14}	900	1 782
350	2 445	1,000	8 765 × 10^{-17}
400	1 213	1,200	2 500
450	6 005 × 10^{-15}	1,400	8 600 × 10^{-18}
500	3 994	1,600	4 397

As a proof the theoretical accelerations were calculated from equations (1) and (2) The result is demonstrated for several satellites with different heights and day–night cycles for the perigee in Fig 3, where the value

$$\log\left[\frac{dP}{dt}_{\text{obs}} \Big/ \frac{dP}{dt}_{\text{calc}}\right]$$

is given for the monthly means

No systematic discrepancies can be seen The random scatter amounts to 10 per cent for *Explorer I* (1958 α), while the total amplitude of its acceleration goes up to the factor 10 For 1959 α the latter varies by the factor 50, while the single points in Fig 3 vary only by 20 per cent Since no more systematical residual variations exist in Fig 3, equation (1) must well represent all the major effects on the upper air density in low and mean latitude

Accepting this good agreement between calculation and observation, the density variations of the upper

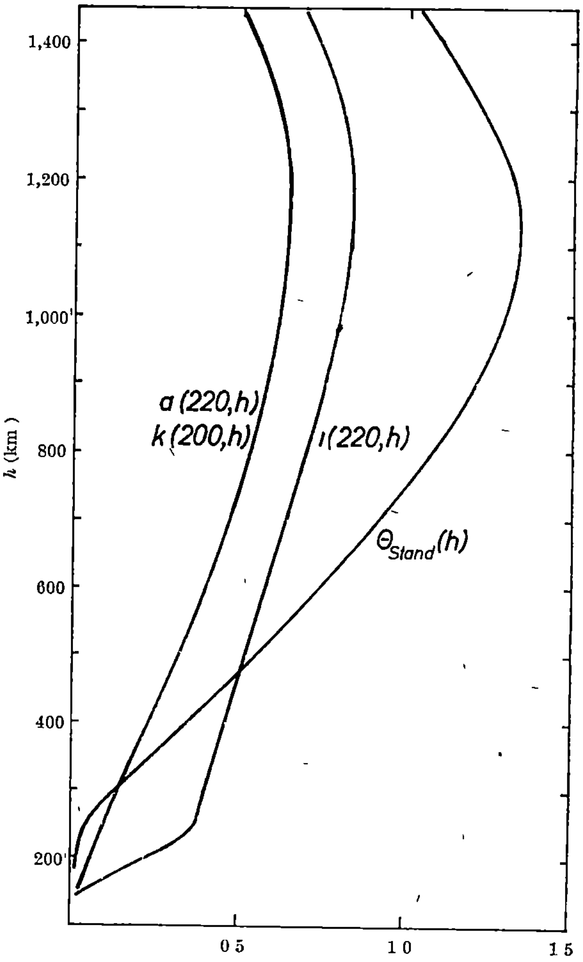

Fig 1 The functions $\iota(\theta)$ and $\theta_t(h)$

air can be described up to an altitude of about 1,000 km by a linear combination of several terms, which can be attributed to distinct physical processes The following conclusions can be drawn

(1) The upper air represents a much more reliable physical system than the lower Its physical state can be fairly predicted for the different parameters as solar and magnetic activity, the local time and the date in the year.

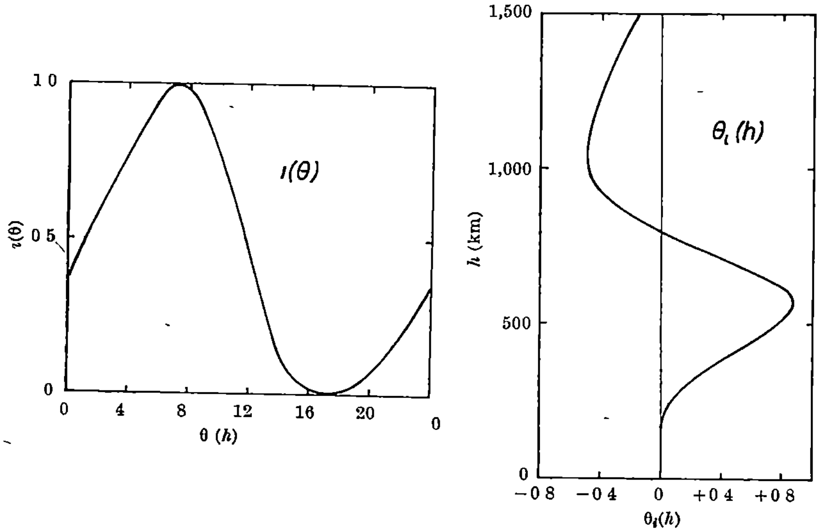

Fig 2 The height-functions $\iota(h)$, $a(h)$, $\theta_{\rm stand}(h)$ and $K(h)$

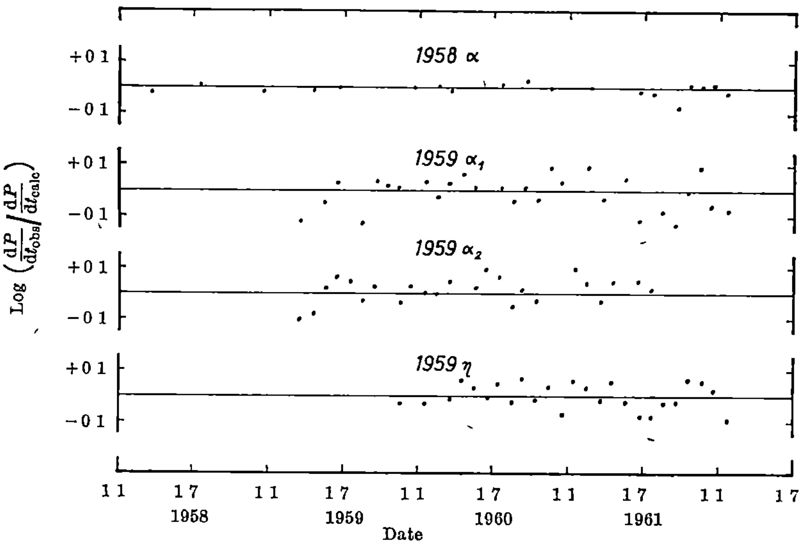

Fig 3 The comparison between calculated and observed satellite acceleration

(2) For geodetic satellites it seems possible to eliminate sufficiently second-order effects of atmospheric drag at least over longer periods

H K Paetzold

Institute of Geophysics and Meteorology,
University of Cologne

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PHYSICS

Radioactivation Analysis of Niobium in Tantalum by Irradiation with Protons in a Cyclotron

Considerable work has been done in the past decade on the separation and determination of relatively small amounts of tantalum and niobium However, no adequate method for the determination of these elements seems to have been devised Although neutron activation analysis has provided a good tool for the determination of many elements, no satisfactory results have been obtained for niobium in tantalum The reasons are as follows (1) small cross-section for the ^{93}Nb(n, γ)-^{94m}Nb reaction (1 1 ± 0 1 barn), (2) ^{94m}Nb has a short half-life (6 6 min) and emits a weak γ-ray (0 0415 MeV), (3) chemical similarity of these elements

We have undertaken to determine a small amount of niobium in tantalum foil of a tantalum capacitor, using the ^{93}Nb(p,n)^{93m}Mo reaction As shown in Fig 1, the target consists of five tantalum foils (29 mm × 23 mm , 18 2 mgm /cm ^{2}) and two niobium foils (29 mm × 23 mm , 7 5 mgm /cm ^{2}) The two tantalum foils outside were used for target covers, and the tantalum foil of the centre used for the determination of niobium in tantalum The two niobium foils were used for radioactivation standard The target was set in a water-cooled holder, and irradiated with 14 5 MeV protons in the internal beam of a cyclotron (10 μamp , 10 min) Proton energy was chosen from the excitation function for the reaction ^{93}Nb(p,n)^{93m}Mo determined by R A James[1] Loss of proton energy in the foils was calculated with the use of the rate of energy loss of protons in silver (for niobium) and gold (for tantalum), and it was found that in this case loss of energy was negligible The surfaces of the irradiated foils did not show any change

The following chemical treatment was used for the separation of ^{93m}Mo from the irradiated foils Small portions (3 ml) of molybdenum solution (1 mgm /ml) were added to a platinum crucible as carrier, and carefully dried up on a water bath The irradiated foil was then fused in this crucible with potassium bisulphate (5 gm), and the melt kept at dull redness for 10 min The resulting cake was dissolved in boiling 15 per cent tartaric acid (100 ml) After a clear solution was obtained, it was rapidly cooled with ice water A fairly rapid stream of hydrogen sulphide was passed into this cold solution for 5 min ,

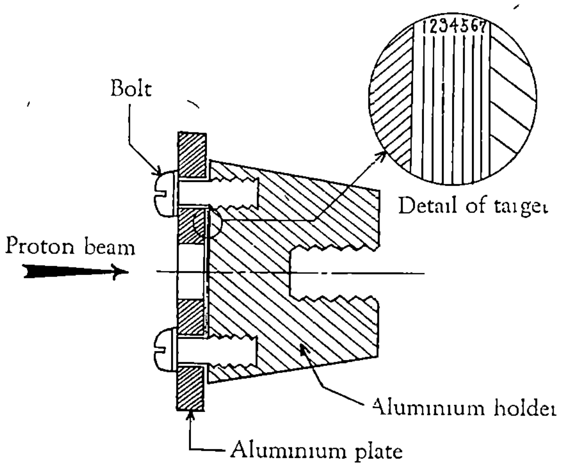

Fig. 1. Cross section of target and target holder. 1, 3, 4, 5, 7, tantalum foil; 2, 6, niobium foil

and then the solution was heated to boiling and kept near the boiling point for 5 min under continuous passage of hydrogen sulphide. After cooling the solution in ice water, the precipitation of molybdenum as sulphide was filtered off, washed, dried and radioactivity measured. This chemical process required less than 1.5 h, with a yield exceeding 80 per cent.

The total γ-ray activities of ^{93m}Mo were counted with a well-type sodium iodide (1.75 in. diam. × 2 in. high). Further, the ^{93m}Mo (0.263, 0.685 and 1.479 MeV) photopeaks were measured with an Argonne type 256 channel pulse height analyser with a large sodium iodide crystal (3 in. diam. × 3 in. high). The decay curve agreed with that of ^{93m}Mo. The niobium content in tantalum was estimated to be 0.2 per cent from the ^{93m}Mo activities of the two niobium foils. The contribution of the (Mo + p) reactions was negligible under our conditions.

This method appears to be a very sensitive one for the determination of niobium and is very effective for the analysis of niobium in tantalum. The determination of 10^{-1} p.p.m. niobium can be expected by this method.

We thank Mr. M. Furukawa for his valuable advice, and the crew of *Ins* (Tokyo) cyclotron for their assistance.

I. Tomita

Department of Chemistry,
University of Tokyo,
Hongo, Tokyo.

H. Saisho

Engineering and Research Laboratory,
Mitsubishi Atomic Power Industries, Inc.,
Ohmiya, Saitama, Japan.

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A Negative Ion Source using a Mercury Pool Cathode

Negative ion sources are now being used extensively in nuclear research. The demands from these sources are more rigorous than for their positive ion counterpart, and lives are around 100 h for hydrogen, much less for oxygen. Experiments in which the filament of the ion source has been replaced by a mercury cathode (Fig. 1) have given much larger currents (up to 700-amp pulse) and lives. With hydrogen beams, after 600 h operation the source showed only slight wear on the electrodes and indicated an indefinitely long life, while tests with oxygen and with sulphur beams, each for 300 h, indicated no difficulties. These tests were under conditions which gave magnetically analysed ion beams of H^-, 15 µamp, of O^-, 15 µamp, and of S^-, 8 µamp.

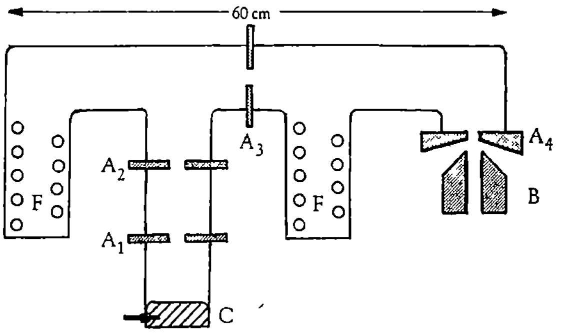

Fig. 1. A_1, A_2, A_3, auxiliary anodes; A_4, final anode; B, 40-kV ion extraction canal and electron donor target; C, mercury pool cathode; F, refrigerated coils

The mechanically refrigerated traps operate at − 50° C. A search was made for a mercury beam by running the ion source positive. None was found, which puts the upper limit in terms of the negative ion beam as less than 1 per cent.

Ralph H. V. M. Dawton

Electrostatic Generator Group,
National Institute for Research in Nuclear Science,
Harwell, Berks.

GEOLOGY

The Highland Boundary Fault: Reverse or Wrench Fault?

Detailed work which I have done in the Loch Lomond area suggests a definite answer to the above problem which is of some importance since it concerns one of the major geological features of the British Isles. The direction in which the principal stress acted in the production of the fault can be approximately determined from the pattern of structures in the Lower Old Red Sandstone conglomerates, and this supports the contention put forward in stratigraphical accounts (summarized by J. G. C. Anderson[1]) that the Highland Boundary Fault is a reverse fault of Caledonian age and not a Proto-Armorican wrench fault as envisaged by E. M. Anderson[2].

Immediately south of the fault, the chief rock type is a conglomerate composed of rounded to ellipsoidal boulders of quartzite with subordinate vein quartz, porphyry, and andesite. The inequidimensional boulders have their major and intermediate axes parallel or slightly oblique to the planes of bedding, and in the upturned to vertical attitude of the bedding close to the fault had their maximum surface areas normal to the direction of principal stress during most of the deformation.

The compressive stresses in the vicinity of the fault during deformation were sufficient to rupture most of the pebbles in the conglomerate for some distance south of the fault as noted by du Toit[3]. The cracking is megascopically conspicuous and is independent of shape and size of the boulders. The fracture planes may be regularly spaced or form highly shattered narrow zones concentrated chiefly in the central area of the pebbles, and similarly orientated directions of cracking can be traced for many yards in adjacent boulders although the matrix is not visibly affected Where the pebbles are subordinate to a sandy matrix no cracking occurs

The patterns developed in individual pebbles may be conjugate fractures with an average angle of 20°–30°, one set of parallel cracks agreeing in orientation with the mean bisectrix of the conjugate set or a combination of both These patterns compare with the laboratory experiments on the compression of rock in the brittle régime[4] Under such compression rocks are brittle, and rupture is expressed in extension fractures parallel to the major stress direction associated with acute-angled wedge fractures

Numerous small shear zones with slickensided surfaces and wrench displacement are prominent in both conglomerate and sandstone horizons, and these have an orientation parallel or oblique to conspicuous trends of cracking The orientation of the shear zones falls into two sets the bisectrix of which approximates to the direction of single cracking in pebbles mentioned above The structures described are the product of the main stresses responsible for the fault and are not secondary structures resulting from the movements on the fault

The direction which corresponds to the maximum of single cracks and bisectrices of conjugate cracks and shear zones is therefore taken, by analogy with laboratory behaviour of similar brittle materials, to be the direction in which the principal stress acted This direction is north-west–south-east, almost at right angles to the trend of the Highland Boundary Fault

The investigation so far reveals a definite pattern to the tectonic structures in the Lower Old Red Sandstone commensurate with horizontal compression of the rocks in a north-west–south-east direction, as in a vice, thus confirming the reverse fault theory of origin

D M RAMSAY

Department of Geology,
Queen's College,
University of St Andrews,
Dundee

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Post-Glacial Sea-Levels

FIG 1 compares eustatic data from New Zealand with a generalized eustatic curve calculated from raised strandlines in Sweden and Norway[1] The Fennoscandian region is undergoing isostatic rebound initiated by the retreat and melting of the Fennoscandian ice-cap, so that the Post-glacial raised strandlines owe their positions to the combination of two effects, changes in both land- and sea-level The basic assumption behind the calculated eustatic curve (Fig 1) is that the rates of isostatic rebound have been proportional from place to place—the raised strandlines used being from areas between Sauramo's Finniglacial and Gotiglacial hingelines[2,3]

Details of the calculations used by Dr H R Thompson, biometrician of the Applied Mathematics Laboratory, New Zealand Department of Scientific and Industrial Research, and of the data selected for the New Zealand curve, will be published shortly in the *N Z Journal of Geology and Geophysics* Note that some slight corrections have been made to that portion of the New Zealand curve younger than 4,000 years B P (compare with that given by Schofield[4,5])

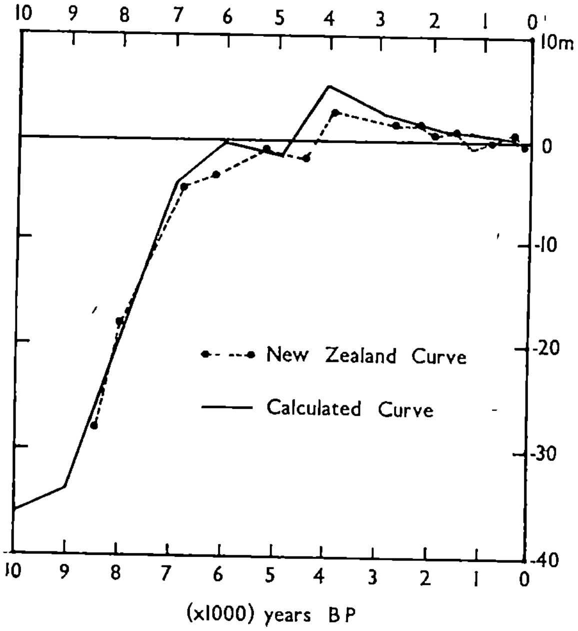

Fig 1

The calculated curve agrees with a Post-glacial high sea-level of about 4,000 years ago It is also similar to the curves of Godwin *et al* [6] and McFarlan[7] in that following the initial, rapid, Post-glacial rise, sea-level reached the present level 5–6,000 years ago Both the calculated and New Zealand curves parallel Fairbridge's[8] curve but differ in detail, the most important being the absence of his sea-level 'highs' of −15 m, 9,000 years ago and +3–4 m of 5–6,000 years B P

J C SCHOFIELD

New Zealand Geological Survey,
Otahuhu, Auckland

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Isotopic Ages from Some Spitsbergen Rocks

ISOTOPIC ages by potassium – argon and rubidium – strontium methods have been measured on some Spitsbergen rocks, in the hope of contributing to the Mid-Palæozoic time-scale and to the understanding of local geological history[1] Rubidium strontium and argon measurements were made by stable isotope dilution analysis, except for York's figure, which was by total volume (argon) measurement The potassium measurements were made by flame photometer

The following specimens were analysed, and the results are given in Tables 1 and 2

Table 1 POTASSIUM – ARGON ANALYTICAL RESULTS

Sample	Volume of argon (mm^3 N T P) Wt of sample (gm)	K_2O (per cent)	Atmospheric contamination (per cent)	Apparent age (m y)
Chydenius granite (biotite)				
H 626				385*
H 629	0.1087	7.35	3.5	388 ± 14
	0.1150	7.35	4.3	406 ± 15
Hecla Hoek mica schists (biotite)				
H 829	0.0899	6.07	3.6	389 ± 14
E 275	0.0935	5.65	14.8	384 ± 13
E 686	0.1576	9.42	5.6	425 ± 22

$\lambda_\beta = 4.72\ 10^{-10}\ yr^{-1}$ $\lambda_e = 0.584\ 10^{-10}\ yr^{-1}$
Analyst J Miller (Cambridge)
* Analyst D York (Oxford)

Table 2 RESULTS OF RUBIDIUM – STRONTIUM DATING

Sample	Rubidium (p p m)	Strontium (p p m)	Radiogenic strontium-87 (p p m)	Apparent age (m y)
Chydenius granite (biotite)				
H 626	1,240	15.4	2.08	401 ± 8
	1,270	16.3	2.14	402 ± 8
Hecla Hoek mica schists (biotite)				
H 828	515	25.8	0.82	382 ± 8
E 276B	541	7.1	0.86	379 ± 6
	542	7.3	0.86	380 ± 6
	541	8.8	0.85	375 ± 6
				378 ± 4

Analyst E Hamilton (Oxford)
(^{87}Rb $\lambda = 1.47 \times 10^{-11}\ yr^{-1}$)

Chydenius granite[2,3] *H* 686-9 This is a 'post-tectonic' porphyrite adamellite cutting and metamorphosing folded Hecla Hoek rocks The upper part of the succession contains Canadian (Ordovician) fossils This granite follows closely the main Caledonian (Ny Friesland) tectogenesis, but can only locally be shown to precede part of the Carboniferous where an apophysis of the batholith is truncated by rock, probably of Moscovian (Upper Carboniferous) age, and to the south-west probably of Tournaisian (Lower Carboniferous) age.

Hecla Hoek Schists[4,5] *H* 825-9, *E* 275-6, *E* 686 On the east shore of Raudfjorden, Red Bay conglomerates (L O R S) unconformably overlie Hecla Hoek schists 300 m above the base an Upper Downtonian (Upper Lower Gedinnian) fish fauna[6] is present The Red Bay conglomerates are unmetamorphosed and truncate mica schists, marbles and migmatites, which have been correlated with the Lower Hecla Hoek

The concordance of rubidium – strontium and potassium – argon ages would indicate an age of metamorphism of 383 ± 10 m y It was at this time that the Hecla Hoek mica schists were formed, and for the purpose of this communication it suffices to date this event Further work is being carried out using the isotope geochemistry of strontium to search for older ages So far, two older rubidium – strontium ages have been determined, and the possibility that older potassium – argon ages are preserved is being investigated

If 383 ± 10 m y is taken provisionally as the latest age of metamorphism of the schists, there would have to be considerable erosion and some deposition between this date and the base of the Upper Downtonian This suggests a date for the base of the Devonian (Downtonian) of 383 m y or less

The present status of the time-scale is summarized in Table 3 The generally accepted age for the base of the Devonian is 400 m y , but not all other ages dating this point in the time-scale are based on materials which are as well defined stratigraphically as the present ones Although it is not advisable to suggest a detailed revision of the time-scale based on one isolated area, the Spitsbergen ages would necessitate that the apparent length of the Devonian Period is reduced and that of the Silurian increased Leaving the other boundaries unchanged, this would give the Devonian and Silurian Periods durations of approximately 38 and 42 m y respectively, instead of 70 and 20 m y on Kulp's 1961 scale

Table 3 SUCCESSIVE MID-PALÆOZOIC TIME SCALES

To base of	Holmes (ref 7) 1960	Kulp (ref 8) 1960	Kulp (ref 9) 1961
Carboniferous	350 ± 10	350	345
Upper Devonian		365	(365)
Middle Devonian		380	390
Lower Devonian	400 ± 10	400	405
Silurian	425	430	425

The apparent ages given in this communication date a thermal event which may be regarded as marking the last phase of the 'Caledonian orogeny' in Spitsbergen The possibility that the absolute age may have been lowered by re-heating in Tertiary times is considered to be most unlikely The slightly older ages obtained for the Chydenius granite, and *E* 686 of the Hecla Hoek series, may indicate incomplete loss of argon or radiogenic strontium during the metamorphic event In addition, the rubidium – strontium ages have been calculated using an original ^{87}Sr/^{86}Sr of 0.712

In the time-scale compiled by Kulp[9], the Shap granite is given as Lower Devonian with an age of 395 ± 5 m y It would appear that this is an erroneous tabulation. The original data[10] from which this age was obtained gave

K-Ar — 391 ± 7 (weighted average of four determinations of 393 ± 12, 391 ± 20, 397 ± 12; 383 ± 12)

Rb–Sr — 381 ± 7 (weighted average of three determinations of 385 ± 11, 380 ± 19; 364 ± 24) The arithmetic mean of these two results indicates an apparent age of 386 m y which is in agreement with the Spitsbergen ages

We thank Prof L R Wager and Sir Edward Bullard for allowing us to investigate these rocks at Oxford and Cambridge, using the rubidium – strontium and potassium – argon methods, respectively

E HAMILTON

Geological Age Isotope Group,
University of Oxford

W B. HARLAND

Sedgwick Museum,
Cambridge

J A. MILLER

Department of Geodesy and Geophysics,
Cambridge

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ENGINEERING

Ultrasonic Wave Velocities in Stressed Nickel Steel

EXISTING methods for detection of residual stresses in metals are limited to surface or near-surface examination, and will not reveal stresses within the material. A possible method of detection is the use of ultrasonic waves in a manner analogous to the use of light waves in photoelasticity. An ultrasonic shear (transverse) wave entering a stressed solid body splits up into two components travelling at different velocities depending on the relevant elastic 'constants' which are slightly different along the two principal stress axes normal to the wave propagation direction. This action is analogous to photoelasticity, where the two components of a polarized light wave are due to electrical anisotropy (variation of permittivity with direction) caused by the stress within a transparent body. Internal stress, including residual stress in metallic bodies, might therefore be detected by observation of the phase shift between the two ultrasonic waves occurring in what has been called 'sonoelasticity'.

Preliminary to work on the practical application of 'sonoelasticity' to the detection of residual stresses, measurements have been made of the variations in velocity with applied stress of three types of ultrasonic wave in nickel steel. These measurements have yielded values of the second-order or Murnaghan elastic constants[1], very few of which have been found by other workers. They are difficult to evaluate directly with any accuracy, since this involves measurements of a small non-linearity in the relation between stress and strain, and the strain itself is small in metallic materials. Ultrasonic velocity variations with stress are also very small, but can be measured accurately by electronic techniques. In the present work, measurements have been made of the changes in velocity of a 2·5 Mc/s longitudinal and two 4·5 Mc/s shear waves generated by quartz crystals and propagated across the centre of a nickel steel bar under compressive stress acting along the axis of the bar. The two shear waves were polarized, one parallel to and the other normal to the stress axis. Changes in velocity were made by measuring both the changes in transit time of the waves and the changes in path-length related to the longitudinal strain by Poisson's ratio. The changes in transit time were measured by the 'sing-around' method[2], whereby a selected echo from the pulse transmitted into the bar was used to trigger the transmitter again, the pulse of energy thereafter 'singing-around' the system at a repetition rate (pulse repetition frequency) determined by the total effective ultrasonic transit time. The pulse repetition frequency was measured by a counter-type frequency meter.

Table 1. LAMÉ AND MURNAGHAN CONSTANTS (in BARS × 10^5)

Material	λ	μ	l	m	n	Source
Polystyrene	0·2889	0·1381	−1·89	−1·33	−1·00	H. and K
Armco iron	11·00	8·20	−34·8	−103·0	+110	H. and K.
Pyrex	1·353	2·75	+1·4	+9·2	+42	H. and K.
Nickel steel	9·09	7·80	−4·6	−59·0	−73·0	D.I.C.

H. and K., Hughes and Kelly; D.I.C., present work.

The measurements made to date are shown in Fig. 1, where it may be seen that, within the limits of measuring precision, there is a linear relationship between stress and 'sing-around' pulse repetition frequency for each of the three wave types. The relations between the three velocities and stress are given by the following equations derived by Hughes and Kelly[3]:

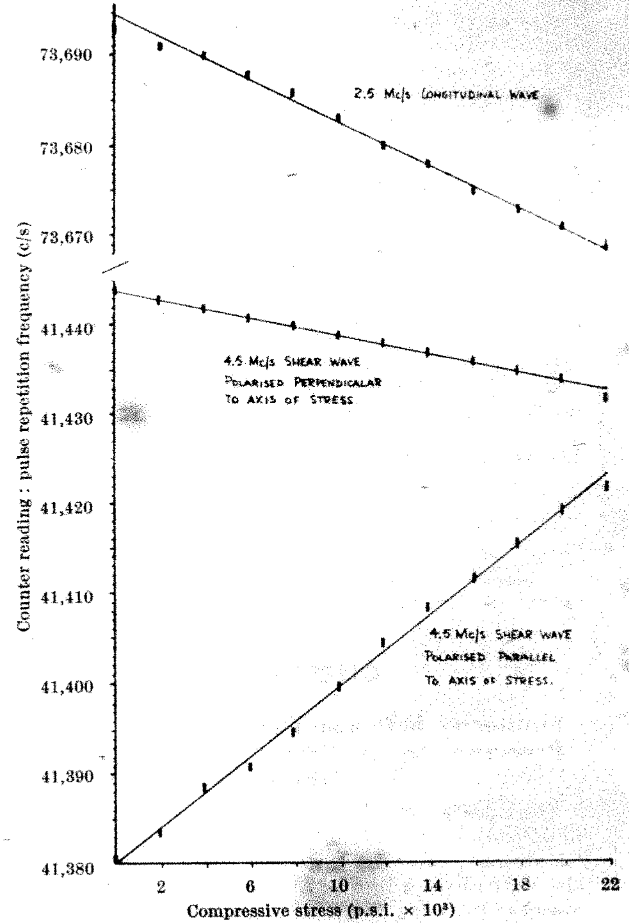

Fig. 1. Graphs of 'sing-around' pulse repetition frequency versus stress

$$\rho_0 c_{1_y}{}^2 = \lambda + 2\mu - \frac{T}{3K_0}\left[2l - \frac{2\lambda}{\mu}(m + \lambda + 2\mu)\right]$$

$$\rho_0 c_{2_y}{}^2 = \mu - \frac{T}{3K_0}\left[m + \frac{\lambda n}{4\mu} + \lambda + 2\mu\right] \qquad (1)$$

$$\rho_0 c_{2_z}{}^2 = \mu - \frac{T}{3K_0}\left[m - \frac{\lambda + \mu}{2\mu}\cdot n - 2\lambda\right]$$

where T is the compressive stress, acting along the y axis, c_{1_y} is the longitudinal wave velocity, c_{2_y} and c_{2_z} are the velocities of the shear waves, polarized parallel and normal respectively to the stress axis. The unstressed density is expressed as ρ_0, $K_0 = \lambda + 2/3\,\mu$ is the unstressed bulk modulus, λ and μ are the Lamé constants, and l, m, and n are the second-order or Murnaghan constants. Each velocity may be expressed in the form

$$c = c_0\left(l + \frac{\Delta c}{c_0}\right) \qquad (2)$$

where c_0 is the unstressed velocity and $\Delta c/c_0$ is the fractional change due to the stress. As this latter is very small, its square may be ignored, and thus for practical purposes:

$$c^2 = c_0{}^2\left(1 + 2\,\frac{\Delta c}{c_0}\right) \qquad (3)$$

If the last expression is substituted into equations (1), it may be seen that the linear relation between stress and velocity is to be expected on the basis of the second-order elastic theory.

The measured values of ρ_0 and c_0 were used to find λ and μ from the expressions:

$$c_{20}^2 = \frac{\mu}{\rho_0} \text{ and } c_{10}^2 = \frac{\lambda + 2\mu}{\rho_0} \qquad (4)$$

The above values and the measured values of T and fractional velocity changes were then used to find the values of l, m, and n from equations (1). The values are compared in Table 1 with those obtained by other workers[3].

The values for nickel steel will be checked in the near future by making measurements with further types of wave/stress combinations, and it is also intended to extend the work to other metallic materials.

D. I. CRECRAFT

Department of Electrical Engineering,
University of Birmingham.

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CHEMISTRY

Similarity between Theories of Rhythmic Precipitations of Crystalloids and Specific Proteins

HEDGES[1] pointed out that critical condition and mobilization of material are factors common to the Liesegang phenomenon. Mobilization occurs when the critical condition is being approached, with the material diffusing from the zones which become clear spaces to those which become rings. Spiers and Augustin[2], in their theory of the one-dimensional immunodiffusional problem, assumed a zone of reaction in which antigen and antibody combine to form non-diffusing complexes, causing diffusion of the antibody into the zone. Here, experimental evidence is given that inward diffusion actually occurs during precipitation with specific proteins as well as with crystalloids, and results in the occurrence of the translucent zone.

The simple diffusion in two dimensions[3] with two sources of diffusion was used. Fig. 1 shows such an experiment. Normal human serum as antigen and rabbit's antiserum were used. The translucent zone without precipitate at the plane of separation between two precipitation patterns is visible.

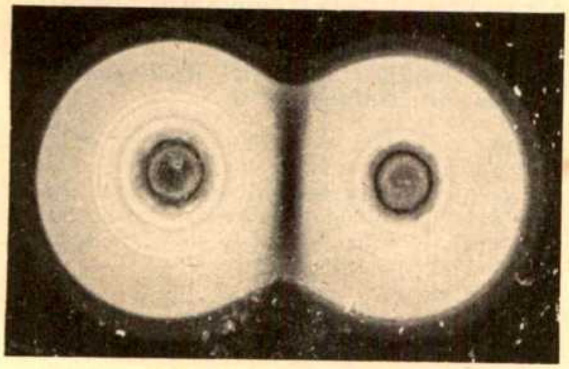

Fig. 1

There are two mechanisms which can possibly cause occurrence of the translucent zone: (1) dissolution of the precipitate or (2) decreased antibody concentration in this zone. Dissolution of the precipitate occurs in a case of excess of antigen[4]. In the system presented the diffusing reactants meet at the plane of separation, and in the absence of the concentration gradient across the plane the flow will be opposed. Therefore accumulation of the antigenic material will occur at this plane. Because the material always flows in the direction of a falling concentration gradient, the concentration of the antigenic material at the plane of separation cannot be higher than those at the points which are nearer to the sources of diffusion: and since intensity of the precipitate decreases in the direction of decreasing concentration, the mechanism of the dissolution of the precipitate cannot be responsible for the occurrence of the translucent zone. The remaining mechanism of decreased concentration of the antibody indicates inward diffusion.

Fig. 2 represents a diffusion experiment between equal concentrations of two sources of one salt. Gel of 0·5 per cent purified Difco-agar and 0·01 M solution of barium chloride was used as the indicator in the gel. A 1 M solution of ammonium sulphate was used. Here as in Fig. 1 the occurrence of the translucent zone also indicates inward diffusion.

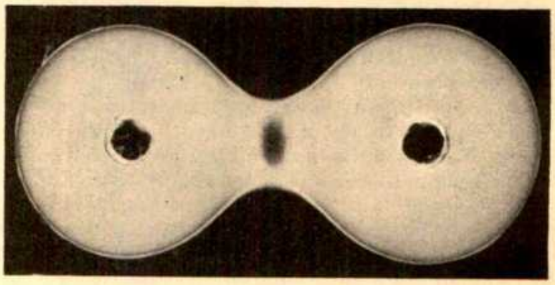

Fig. 2

Diffusion is defined as the mutual interdiffusion of at least two components. To explain the occurrence of the translucent zone this concept may be stated in the following way. In the system presented there is simultaneous movement, or inward diffusion, around two sources of diffusion. When at the given distance between sources of diffusion two zones of movement combine, the movement in this area is equal to the effects of both diffusing sources. Therefore, the concentration of the internal reactant is not distributed uniformly in the medium but is at the minimum concentration along a line of centres where combination first occurs, and gradually increases along other radii. This change in relative concentration of the internal reactant may also result in altered rate of diffusion of the reactants diffusing from adjacent wells[5].

ANTON BENAS

Pathophysiological Institute,
Faculty of Medicine,
University of Zagreb.

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Face-centred Cubic Molybdenum Monocarbide

THREE forms of molybdenum monocarbide (MoC) having a hexagonal unit cell have been reported in the literature[1]. In a recent note, Clougherty, Lottrop and Kafalas[2] have described a high-pressure synthesis of a new face-centred cubic molybdenum carbide, which they designate α-MoC. This material was prepared by heating an equiatomic mixture of molybdenum and carbon or dimolybdenum carbide and carbon at temperatures between 1,800° C and 2,500° C under pressures ranging from 40–70 kb. The reported lattice constant was 4·27 Å.

In recent work in these laboratories, the effect of heat-treating amorphous films of carbon *in vacuo* has been examined by electron diffraction methods. The carbon films were prepared in the usual way by evaporation *in vacuo* and deposition on a glass substrate. The films (about 1,000 Å thick) were detached and supported on grids or foils made from three different materials, namely, molybdenum, tantalum and carbon, so that they could be examined in the electron diffraction camera. The supported carbon films were heated in simple tube furnaces made from molybdenum or tantalum (Fig. 1) to temperatures up to 1,400° C, for periods of 15 sec in a residual air pressure of about 10^{-4} mm mercury.

The diffraction patterns obtained from the unsupported regions of the carbon films heated to 1,400° C on a molybdenum, tantalum or carbon support in the tantalum furnace showed only diffuse rings due to a highly disordered carbon, the rings becoming sharper as the temperature of heating was raised. Fig. 2 shows the diffraction pattern from one such carbon film supported on carbon and heated to 1,400° C. On heating the films on the three supporting materials in a molybdenum tube furnace at 1,000° C, the diffraction patterns were similar to those obtained in the tantalum furnace. However, a marked change was observed when the films were heated to a temperature greater than 1,200° C in the molybdenum furnace irrespective of the nature of the film support. The carbon reflexions were no longer visible and the diffraction pattern (Fig. 3) could now be indexed on the basis of a face-centred cubic unit cell having a lattice constant of 4·26 Å.

These observations can be interpreted by assuming that this cubic phase originates in the reaction between the carbon film and a material evaporating from the walls of the molybdenum tube. There are three possibilities to be examined. (1) Metallic molybdenum might be evaporating from the walls of the furnace. Considering that the vapour pressure of molybdenum is only about 5×10^{-13} mm of mercury at 1,200° C, this appears to be unlikely. (2) The molybdenum is transported from the furnace wall to the carbon specimen as a volatile oxide which then reacts to form molybdenum carbide. (It should be noted that the observed cubic structure is not consistent with any of the known oxides of molybdenum.)

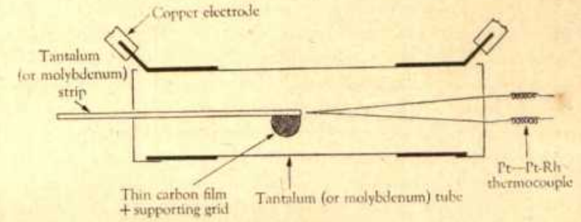
Copper electrode
Tantalum (or molybdenum) strip
Thin carbon film + supporting grid
Tantalum (or molybdenum) tube
Pt—Pt-Rh thermocouple

Fig. 1. Arrangement for heating thin films of amorphous carbon

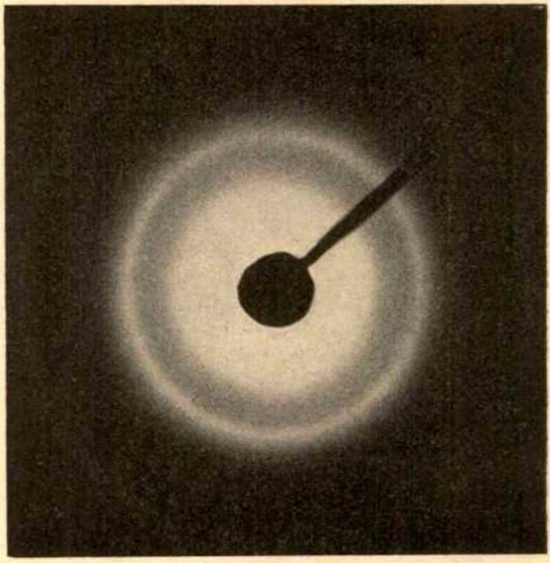

Fig. 2. The electron diffraction pattern from a thin film of carbon heated to 1,400° C for 15 sec in a tantalum tube furnace

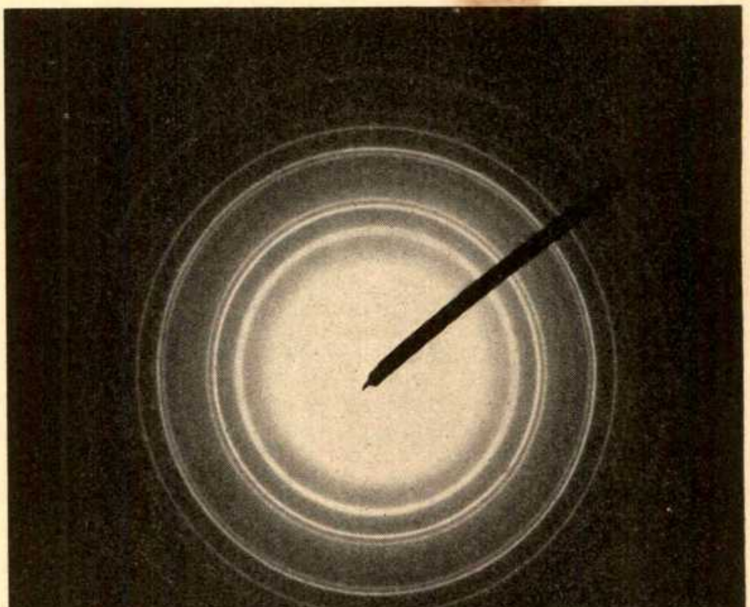

Fig. 3. The electron diffraction pattern from a thin film of carbon heated to 1,200° C for 15 sec in a molybdenum tube furnace

(3) There is a possibility that a volatile impurity in the molybdenum is responsible for the new diffraction pattern. The last suggestion seems to be excluded for the following reasons. The molybdenum sheet (from Murex Ltd.) contained 0·01 per cent iron as the only detectable impurity. Of the possible compounds which could be formed under the experimental conditions, the symmetry of the diffraction pattern is consistent only with cubic ferrous oxide. However, the lattice parameter of ferrous oxide is larger than that found for the phase. In any event the furnace was preheated at 1,500° C for 15 min to clean it up.

There is, therefore, reasonably good evidence that a face-centred cubic molybdenum carbide with a lattice constant of 4·26Å was formed at 1,200° C as a thin film phase in the experiments now reported. This is to be contrasted with the extreme condition of 40–70 kb and 2,000° C used by Clougherty, Lothrop and Kafalas[2] to produce the same structure in bulk.

I thank Mr. D. Clinton for carrying out some of the experimental work. I also thank Dr. G. L. Kington for useful discussions and my thanks are also due to Morganite Research and Development, Ltd., for permission to publish these results.

G. Kaye

Morganite Research and Development, Ltd.,
Northfields Laboratories,
Northfields, Wandsworth, London, S.W.18.

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Raman Spectra of Adsorbed Molecules : Quenching of Fluorescence by Adsorption

It is well known that the infra-red spectra of molecules go through changes in position and intensity by their adsorption on solid surfaces[1–8]. In earlier communications we have shown[9] that the infra-red spectra of organic molecules spread in molecular layers over the surfaces of inorganic salts show differences in position and intensity of the bands compared with the spectra of the bulk substances. The observed differences are greater the more the atomic distances of the vibrating groups coincide with the lattice distances of the salt surfaces. The distortion of molecules with a centre of symmetry by the local fields of the lattice surface causes the appearances of Raman active vibrations in the infra-red.

In this communication we wish to describe some results obtained on the Raman spectra of organic molecules in the adsorbed state. As adsorbing material, aerosil (spec. surface 175 m^2/g), porous glass or potassium bromide powder of great specific surface was used. The Raman spectra were obtained by Brandmüller's method[10] for crystal powders, which has been modified by introduction of an interference filter with great transparency for the Raman lines and a maximum of reflexion for the exciting frequency (4356 Å.). The scattered light was allowed to traverse this filter so that the exciting light could by reflexion pass again through the scattering sample increasing the intensity of Raman lines. Registration was made either photographically or with the aid of a photomultiplier and electronic amplification (*Steinheil*).

The thickness of the scattering powder is critical for the observed intensity of the Raman lines. Changes in that thickness by only 0·05 mm causes the maximum intensity to be passed over. The optimum thickness lies around 0·32 mm, varying from sample to sample. With the aid of a cuvette of variable length in intervals of 0·01 mm it was possible to work at the maximum intensity for the Raman lines.

Table 1

Substance	New Raman lines shown by adsorption on SiO_2 (cm^{-1})	Infra-red frequencies (cm^{-1})
Naphthaline	1,310	1,302
	1,360	1,364
	1,410	1,391 or 1,431
	1,500	1,506
Biphenyl	1,308	1,307
	1,380	1,386
	1,480	1,481
Trans-stilbene	1,220	1,255
	1,609	1,502
Terphenyl	1,112	1,121
Anthracene	960	957
	1,100	1,149

The spectra of biphenyl, naphthaline, anthracene, phenanthrene, terphenyl, trans-stilbene, *p*-nitrophenol, and *p*-nitrotoluol were registered in the state of monomolecular ($n = 1$) and polymolecular (up to $n = 10$) layers. Figs. 1 and 2 illustrate a comparison between the Raman spectra of adsorbed molecules and their spectra in the crystal state. It can be stated generally that the intensities of the lines are very much equalized by the adsorption and that new lines appear (indicated by arrows in the figures) in the case of molecules possessing a centre of symmetry. Table 1 shows that the frequencies of the new Raman lines almost coincide with well-known infra-red bands of the molecules.

It is then possible to register Raman lines of adsorbed molecules only if the adsorbing material has

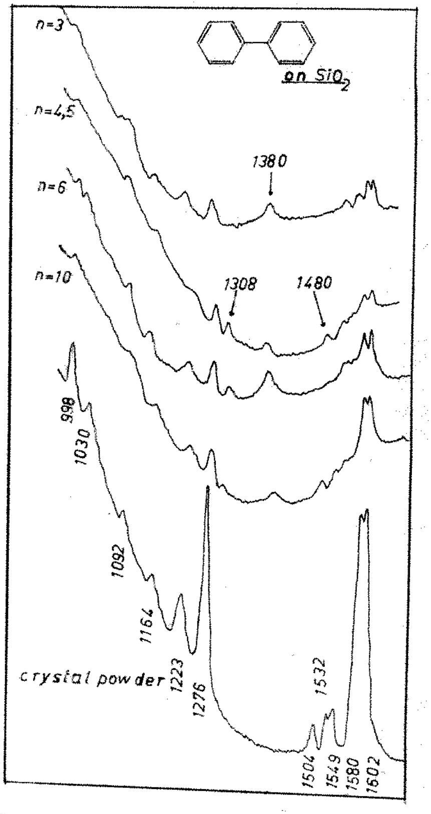

Fig. 1. Raman spectra of biphenyl as crystal powder and adsorbed on silica

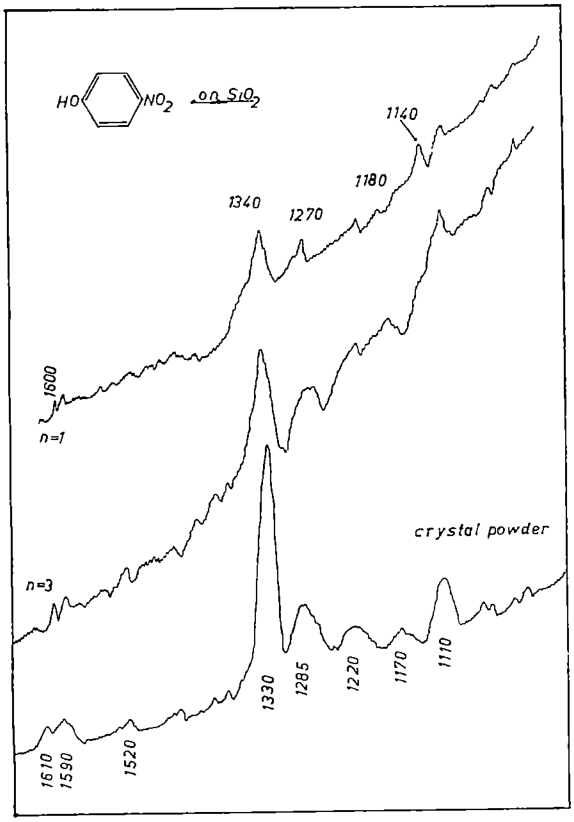

Fig 2 Raman spectra of *p*-nitrophenol as crystal powder and adsorbed on silica

a sufficiently great specific surface In the case of coloured substances detectable intensities of Raman lines are even observable below a monomolecular coverage of the surface, probably due to an increase of intensities by a resonance Raman effect

Another observation made on this occasion is that the fluorescence of molecules is completely quenched by adsorption at a monomolecular coverage The measurement of Raman spectra of fluorescent molecules is possible, using the substances in adsorbed state Fig 3 illustrates, for example, terphenyl, that the fluorescence disappears for $n = 1$, and the covered Raman lines appear

G KARAGOUNIS
R. ISSA

Department of Chemistry,
University of Freiburg i Br,
West Germany

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Water as a Chain-transferring Agent in Vinyl Polymerization

IN a previous communication[1] the chain-transfer constant (C_S) of a solvent in a mixed solvent system was determined This opens up the possibility of determining the chain-transfer constant of solvents which are immiscible with the monomer By a suitable choice of a common solvent the heterogeneous mixture of the monomer and the immiscible solvent can be kept in a homogeneous medium, and the C_S value of the latter can be determined An attempt is reported here to determine the chain-transferring capacity of water with the growing radical of poly-(methyl methacrylate) on the above principle

Tert-butyl alcohol was selected as the solvent to keep the mixture of water and methyl methacrylate in a homogeneous phase 1 1 ratio of *tert*-butyl alcohol and methyl methacrylate was taken and different weighed amount of water was added to the ampoule Polymerization was carried out at 60° ± 0 05° C in nitrogen-flushed vacuum-sealed ampoules[1,4] The polymer was precipitated by ethanol followed by reprecipitation from benzene solution The samples were dried *in vacuo* and the viscosity determined by Ostwald viscometer of flow-time 232 sec with benzene The results are shown in Table 1 and Fig 1. The arrow point in Fig 1 indicates the onset of precipitation.

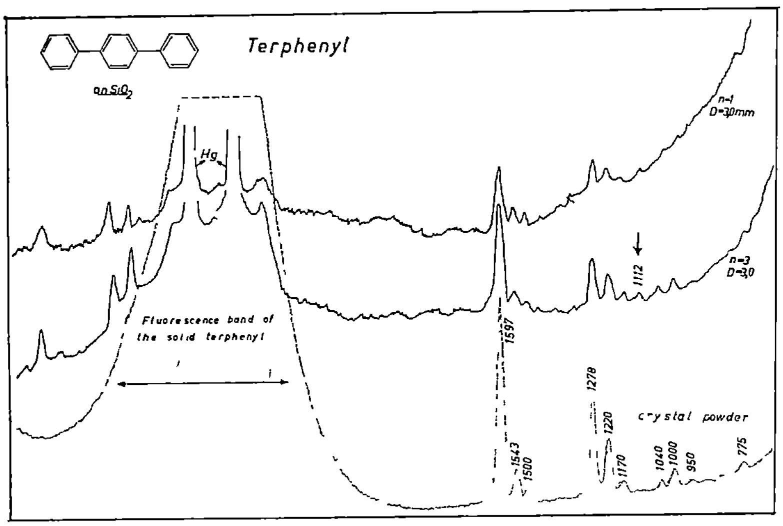

Fig 3 Quenching of fluorescence of terphenyl by adsorption

The most remarkable feature of the result is that addition of water even in small quantity brings about an almost monotonous increase in molecular weight instead of the expected decrease due to chain transfer and dilution A plot of the results, that is $1/\overline{P} - R_p\delta^2/M^2$ against $[H_2O]/[M]$, shows the peculiar nature of the curve Instead of the usual straight line with a positive slope, the curve takes a reverse course, finally becoming almost parallel with the H_2O/M axis This undoubtedly indicates that water has no chain-transferring capacity Jenkins and

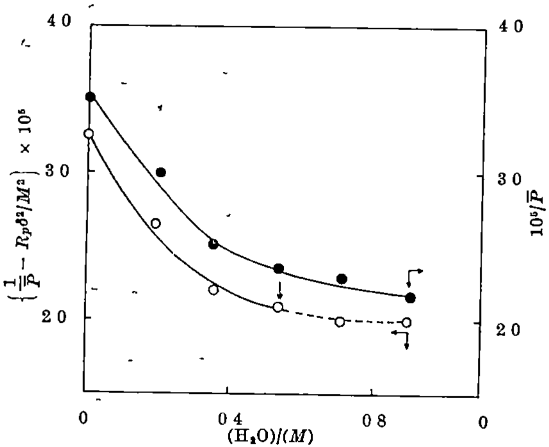

Fig 1 Effect of water on the polymerization of methyl methacrylate in *tert*-butyl alcohol at 60° C

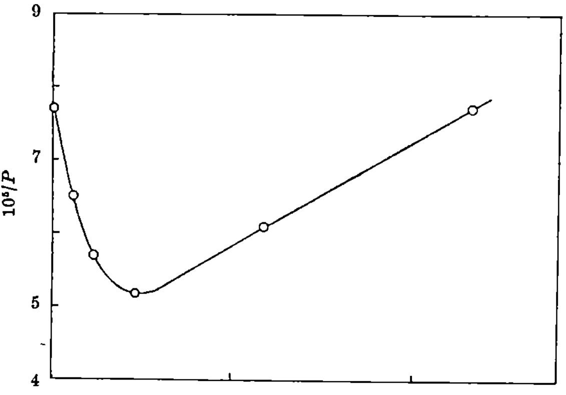

Fig 2 Effect of water on the polymerization of methyl methacrylate in acetic acid at 60° C

Johnston[5] also report that water reduces the reactivity of solvent as a transfer agent This is not surprising in view of the fact that alkyl radicals *X*—CH$_2$—CH*R*, do not react with water[2], and as shown by Merz and Waters[3] that even the highly reactive phenyl radical can persist in aqueous solution until it collides with a suitable solute radical or molecule The unexpected increase in degree of polymerization as also the negative slope of the plot of $1/\bar{P} - R_p\delta^2/M$ against [H$_2$O]/[*M*] can be attributed to a tighter coiling of the polymer chain leading to a reduced termination rate

Table 1 POLYMERIZATION OF METHYL METHACRYLATE IN *tert*-BUTYL ALCOHOL–WATER MIXTURE

t-BuOH/Monomer = 1 1 by volume in each experiment

[H$_2$O]/[*M*]	$10^5/\bar{P}$	$10^7 R_p$ moles l^{-1} sec^{-1}	$10^5\left\{\frac{1}{\bar{P}} - R_p\ \delta^2/M^2\right\}$
0 0000	3 45	9 24	3 25
0 1963	3 00	10 09	2 65
0 3529	2 53	8 93	2 02
0 5358	2 36	7 07	2 10
0 7089	2 29	6 40	2 00
0 8878*	2 14*	3 17*	2 00*

* Polymerization carried out in the heterogeneous phase The initial mixture was slightly heterogeneous

M, monomer concentration, $\bar{P}$, degree of polymerization, R_p, rate of polymerization, δ, the relation $k_t^{\frac{1}{2}}/k_p$ where k_t and k_p are rate constants for termination and propagation respectively

$\bar{P}$ has been calculated from intrinsic viscosity data after Baxendale *et al* (*J Polymer Sci*, 1, 237 (1946))

Table 2 POLYMERIZATION OF METHYL METHACRYLATE IN ACETIC ACID–WATER MIXTURE

Acetic acid/Monomer = 1 1 by volume in each experiment

[H$_2$O]/[*M*]	$10^5/\bar{P}$	Optical density at 515 mμ using rhodamine reagent (polymer solution 0 2%)	Average No of COOH per molecule
0 00	7 67	0 41	very low
0 29	6 56	0 46	0 922
0 58	5 72	0 50	1 75
1 16	5 19	0 50	1-93
2 90	6 11	0 50	1 65
5 80	7 67	0 55	1 79

A slightly different trend is noticeable in the polymerization of methyl methacrylate in acetic acid in the presence of water In this system the quantity of water was varied over a wider range (S/M = 0 29–5 8) The results are tabulated in Table 2 and represented in Fig 2 As in the previous case $1/\bar{P}$ decreases with addition of water and this might be presumed to be due to the same reasons as cited before

In this case also from the point marked with an arrow the system starts to show heterogeneity The new feature which appears here is that from about 1 1 (water/acetic acid) the curve takes an upward turn It is believed that this complication is caused by the increasing ionization of acetic acid introducing in the system a new species, the acetate ion CH$_3$COO$^-$, which probably is a much better chain-transferring agent for poly(methyl methacrylate) radical than acetic acid This also receives support from our results (Table 2, last col) of the number of carboxyl end-groups per polymer chain as determined by the dye interaction method[6] developed in this laboratory The number of carboxyl end groups increases with the addition of water and remains more or less steady on further addition of water

UMA SANKAR NANDI
PREMAMOY GHOSH
SANTI R PALIT

Indian Association for the Cultivation of Science,
Calcutta, 32

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Revision of the End Carbon Chain Concept in the Gas-Liquid Chromatography of Methyl Esters of Unsaturated Fatty Acids

THE 'end carbon chain', shown to be the key factor in relating the retention time of unsaturated fatty acids of differing chain-length but the same number of double bonds, was originally defined as the number of carbon atoms beyond the double bond farthest removed from the carboxyl group[1]

It would be advantageous to redefine this unit as the number of carbon atoms reckoned from the centre of the double bond farthest removed from the carboxyl group to, and including, the terminal methyl group The end carbon chain is then simply the fatty acid chain-length less the familiar position number usually employed to identify this particular double bond position Moreover, the revised 'end carbon chain' concept is immediately useful in assessing the biological activities of certain fatty acids as related through the terminal numbering system[2]

R G ACKMAN

Fisheries Research Board of Canada,
Technological Station,
Halifax, Nova Scotia

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Thermal Isomerization of Poly(triallyl cyanurate) using a Dynamic Mechanical Method

We wish to report a difference in thermo-mechanical behaviour between poly(triallyl cyanurate) and poly(triallyl isocyanurate). In this work a modified torsional pendulum was used in which the vertical specimen shaft consisted of a braid of glass fibres which was impregnated with the polymeric material under investigation. In principle any changes in the mechanical rigidity of this composite would be reflected by changes in frequency of the freely oscillating inertial mass of the pendulum. By expressing the calculated rigidity modulus, G, relative to some arbitrary value, G_0, in each experiment, a relative rigidity parameter, G/G_0, ensued. This parameter partially overcame the difficulties which were inherent in defining the geometry of the system. Experimentally, the relative rigidity reduces to P_0^2/P^2, where P_0 and P are the periods of oscillation at the reference and measured conditions, respectively. The applicability of the measurement of the dynamic mechanical properties of supported polymers to the study of chemical and physical transformations has been demonstrated previously[1-3].

Resins of poly(triallyl cyanurate) and poly(triallyl isocyanurate) were formed on braids of glass fibre from the monomer by polymerization with 1 per cent of benzoyl peroxide at 90° C in nitrogen. After 48 h the rigidity appeared to have levelled off; this was considered to represent the cured state. Fig. 1 represents the curing behaviour in terms of relative rigidity and time. The relative rigidity is based on the rigidity of the cured state, G_f.

The thermoset resins, cured as indicated in Fig. 1, were cooled to room temperature, after which the mechanical behaviour on heating was studied by heating to 500° C over an interval of 180 min. Fig. 2 summarizes these results. Here the relative rigidity is based relative to the rigidity of the composite at room temperature. The difference in thermal softening behaviour between the two resins is apparent. The drastic decrease in rigidity of poly(triallyl cyanurate) at 300° C which is succeeded by an increase at 340° C we believe to be associated with an internal rearrangement, possibly by an intra-molecular cyclic mechanism which is followed by subsequent reaction of allyl groups. The thermal isomerization of normal cyanurates to isocyanurates is well known. The isomerization reaction in poly(triallyl cyanurate) has been investigated by infra-red spectroscopy, differential thermal analysis and calorimetry[4-6]. Additional aspects of the relationship between dynamic mechanical changes and specific chemical reactions in this system will form the basis of a future communication.

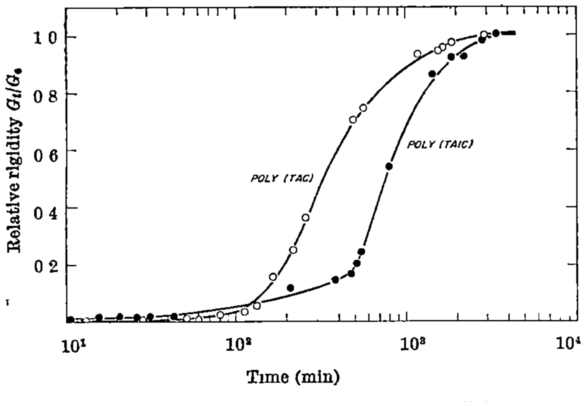

Fig. 1. Polymerization of triallyl cyanurate and triallyl isocyanurate

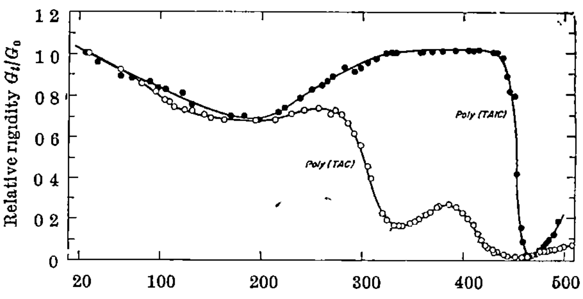

Fig. 2. Thermal softening behaviour of poly(triallyl cyanurate) and poly(triallyl isocyanurate)

It is evident that the torsional braid technique can be used in the examination of transformations which occur in polymeric systems. The method is particularly attractive because a relatively small amount of sample is required (less than 0·1 g) in each experiment and because the changes can be monitored non-destructively. The technique should prove valuable in the study of structural changes which occur in organic, inorganic, biochemical and physiological polymer chemistry.

J. K. Gillham
A. F. Lewis

Stamford Laboratories,
American Cyanamid Co.,
Stamford, Connecticut.

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Effect of Heat on the *p*H of Water and Aqueous Dye Solutions

It appears to be generally accepted that the spontaneous acidity of distilled water is due to dissolved carbon dioxide which can be expelled by boiling to restore the *p*H to about 7·0. Some of my recent work has involved a large number of experiments on the *p*H of water and staining solutions. This brought to light some interesting phenomena which may be of interest to research workers in the field of biology and medicine. In the first place it appears that freshly distilled water usually has a *p*H between 5·0 and 6·0, not 7·0. In the experiments referred to above, whatever the *p*H of the particular sample of distilled water at 20° C it rose gradually until at 100° C it had risen by *p*H 2·0. That is to say if at 20° C the *p*H of the water was 5·0, then at 100° C the *p*H became 7·0, and *p*H 5·5, for example, became 7·5 at 100° C. Graphs were perfectly linear in every case.

The instrument used was an *EIL* direct reading *p*H-meter model 23*A* (Electronic Instruments, Richmond, Surrey); the necessary adjustments were made to permit accurate readings over the temperature-ranges used It was observed that as the temperature of the samples of distilled water fell gradually from 100° C the same pH changes took place but in reverse, so that at 20° C the pH readings observed were exactly the same as those observed before the water was boiled The phenomena might be explained by the following equation

Instead of

$$n(\mathrm{H_2O}) \rightarrow n(\mathrm{H^+}) + n(^-\mathrm{OH})$$

the reaction might be

$$n(\mathrm{H_2O}) \underset{-\text{ heat}}{\overset{+\text{ heat}}{\rightleftarrows}} (n{-}m)\,(\mathrm{H^+}) + m(\mathrm{H}) + n)^-\mathrm{OH})$$

This equation implies that the effects of heat would be to increase the proportion of the hydroxyl ions by decreasing the proportion of hydrogen ions The changes in pH were checked by means of coloured organic indicators

Experiments were also carried out with London tap water, pH 8 2 at 20° C It was found as the temperature was increased up to 60° C so the water became less and less alkaline At 65° C the pH began to rise steadily so that at 100° C the pH of the samples tested was exactly the same as at 20° C

A number of aqueous solutions of acid dyes in distilled water were similarly tested over the temperature-range 0°–100° C As these solutions were heated gradually up to 60° C so their pH fell At 65° C the pH began to rise steadily so that at 100° C the pH observed were exactly the same as at 20° C On cooling the same changes in pH occurred but in reverse

Further investigations are being carried out on the effects of heat on the pH of a large number of acid and basic dyes and will be reported elsewhere Meanwhile it is felt the observations on heat-induced pH changes of distilled water reported here may help to explain, in part at least, the mechanism of some staining methods and also the alleged capriciousness of certain histological stains

EDWARD GURR

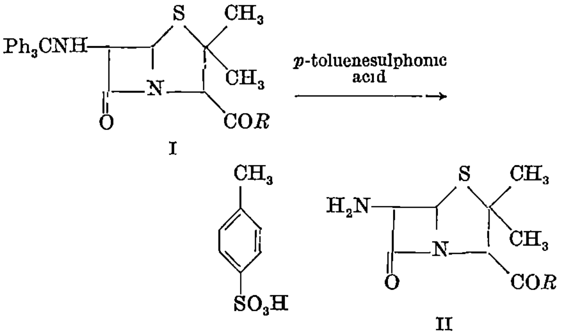
Department of Anatomy,
University College,
Cork, and
Michrome Laboratories,
East Sheen,
London, S W 14

BIOCHEMISTRY

6-Aminopenicillanamide and Methyl 6-Aminopenicillanate

SIMPLE derivatives of penicillins, such as esters[1] and amides[2], have been prepared from the penicillins themselves by conventional methods The availability of 6-aminopenicillanic acid (6-APA) led me to synthesize from it selected carboxylic derivatives which can be used conveniently to prepare corresponding series of penicillin derivatives by acylation of the appropriate 6-APA intermediate

6-Aminopenicillanamide and methyl 6-aminopenicillanate were synthesized *via* 6-tritylaminopenicillanic acid (I, R = OH) The latter compound and its methyl ester have been described by Sheehan and Henery-Logan[3] 6-Tritylaminopenicillanic acid was converted to the amide (I, R = NH_2) and methyl ester (I, R = OCH_3), which were then detritylated to the desired products Removal of the trityl group was readily effected by one equivalent of p-toluenesulphonic acid, which afforded 6-aminopenicillanamide (II, R = NH_2) and methyl 6-aminopenicillanate (II, R = OCH_3), respectively, directly as crystalline p-toluenesulphonate salts



The amide and methyl ester of 6-APA can be differentiated from 6-APA in several paper-chromatographic systems, for example, in one system, methyl ethyl ketone/acetone/water/formic acid, 80 6 12 2[4], the following R_F values are obtained 6-aminopenicillanamide, 0 4, 6-APA, 0 5, methyl 6-aminopenicillanate, 0 8 Detection can be carried out with chemical sprays or by the usual bioautographic method after first subjecting the developed paper-gram to phenylacetylation[5], which enables one to locate the three 6-APA compounds as bioactive spots

6-Aminopenicillanamide p-Toluenesulphonate. Crystalline 6-tritylaminopenicillanic acid diethylamine salt[3], $[\alpha]_D^{24}$ + 102° (C 2 in dioxan), λ_{max} 5 68 (vs) μ in KBr (obtained in 45–48 per cent yield from 6-APA), was transformed quantitatively to the free acid, non-crystalline, $[\alpha]_D^{25}$ + 121° (C 2 in $CHCl_3$), λ_{max} 5 64 (vs) μ in $CHCl_3$ The acid was treated with isobutyl chloroformate and triethylamine in dry acetone to form the mixed anhydride, which was converted *in situ* with aqueous 10 per cent $(NH_4)_2HPO_4$ to 6-tritylaminopenicillanamide in 83–92 per cent yield, non-crystalline, $[\alpha]_D^{26}$ + 175° (C 2 in $CHCl_3$), λ_{max} 5 64 (vs) μ in KBr A solution of 6-tritylaminopenicillanamide (160 g, 0 35 mol) in acetone (200 ml) was stirred, while a solution of p-toluenesulphonic acid (63 36 g, 0 334 mol) in acetone (137 ml) was added rapidly. Precipitation occurred in a few minutes on continued agitation After a total of 13 min , crystalline 6-aminopenicillanamide p-toluenesulphonate was collected by filtration, washed with acetone and ether, and dried (107 g, 83 per cent yield) For analysis a sample was recrystallized twice from aqueous acetone, m p 158–160° (dec), $[\alpha]_D^{24}$ + 197° (C 1 in pH 7 buffer), $[\alpha]_D^{23}$ + 175° (C 1 in ethanol), λ_{max} 5 62 (vs) μ in KBr Anal $C_{15}H_{21}O_5N_3S_2$ requires C, 46 49, H, 5 46, N, 10 85, S, 16 55 Found C, 46 59, H, 5 49, N, 10 54, S, 16 23

Methyl 6-Aminopenicillanate p-Toluenesulphonate Methyl 6-tritylaminopenicillanate[3] (3 64 g, 7 7 mmol) suspended in acetone (7 5 ml) was dissolved by the addition of p-toluenesulphonic acid (1 425 g, 7 5 mmol) Methyl 6-aminopenicillanate p-toluenesulphonate crystallized from solution after stirring for 25 min The product was recovered by filtration, washed with acetone and ether, and dried (1 97 g) The mother liquor furnished an additional 0 32 g, total yield = 76 per cent For analysis a sample was recrystallized twice from warm acetone, m p 133–136° (dec), $[\alpha]_D^{24}$ + 157° (C 1 in pH 7 buffer), $[\alpha]_D^{23}$ + 143° (C 1 in ethanol), λ_{max} 5 57 (vs) μ in KBr Anal

$C_{16}H_{22}O_6N_2S_2$ requires C, 47 74, H, 5 51, N, 6 96, S, 15 93 Found C, 47 85, H, 5 78, N, 6 52, S, 15 85

B K Koe

Medical Research Laboratories,
Chas Pfizer and Co , Inc ,
Groton, Conn

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Fermentation of Saccharose by Alpha-Glucosidase

I HAVE isolated two melibiose-fermenting yeasts, *Saccharomyces oleaginosus*[1] and *S hienipiensis*[2], which, by means of Wickerham's method[3], are capable of fermenting maltose, but which neither ferment nor assimilate saccharose With the Delft method (Kreger-van Rij, N J W , private communication), slow fermentation of the saccharose was obtained

Seven strains of *S oleaginosus* were inoculated in yeast nitrogen base Wickerham (YNB), with 0 5 per cent saccharose or 0 5 per cent galactose or 0 5 per cent galactose plus 0 5 per cent saccharose After 15 days, development was nil in the saccharose tubes but abundant in the rest, in all cases a higher degree of optical density was proved in the tubes containing the saccharose with galactose mixture By means of paper chromatography it was shown that in the tubes of galactose with saccharose only saccharose was present

Identical results were obtained by comparing saccharose, maltose, and saccharose with maltose

With 58 strains of *S oleaginosus*, fermentation of saccharose in 2 per cent concentration in yeast infusion (YI) was compared with fermentation in the same concentration in the medium used by the NRRL of Peoria After 24 days, only 2 strains in YI showed no gaseous fermentation· of the 56 which had fermented, the fastest began fermentation at 7 days, and the slowest at 29 days, in all the tubes, presence of saccharose only was proved by paper chromatography At 42 days, not one of the tubes in the Peoria medium had fermented

A yeast autolysate (YA) was compared with the YI, inoculating from 36 different slants of 2 strains of *S oleaginosus* after 21 days, one of the strains had fermented 2 tubes of the YI, and at 7 days, 24 tubes had fermented in the YA In the other strain, the figures were 5 and 18 respectively

Addition of bromthymol blue to the YI and to the YA had no influence on these results, neither was the result improved by omitting this stain from the Peoria medium

The same strains were inoculated from 10 different slants in the YNB with 0 5 per cent saccharose After 32 days there was no development in any tube, and at 44 days there was positive development in 6 tubes of one of the strains, and 3 in the other

One strain of *S oleaginosus* grown in tubes of YI with glucose, saccharose and maltose, and in YA with saccharose, all of which had fermented, were plated into YI containing agar, eosin, methylene blue[4] and glucose, or saccharose or maltose, also with these same sugars, substituting the YL for YA, in all the cases colonies appeared at 4 days inoculation of the plates, of a dark-red on the glucose and maltose plates, and rose-white on the saccharose plates, independently of the basal medium or whether the inoculation proceeded from tubes with saccharose, glucose or maltose

The former result seems to indicate that the maltose adaptation did not exert any influence on the capability to use saccharose This was confirmed by inoculating the YA and the YI with 2 per cent saccharose from cultures grown in glucose and maltose, because identical results were obtained Nevertheless, when the inoculation was carried out with a large quantity of yeast, there certainly was a more rapid fermentation of the cells which proceeded from maltose, that is to say, the adapted cell is capable of using saccharose directly, and when the number of such cells present in the medium is very high, they metabolize saccharose very fast, but if the inoculation is done with a small number of cells, which have to multiply before they begin fermentation, there is no difference between adapted or unadapted cells

Practically the same results are obtained with *S hienipiensis*

With *S italicus VB* 12–1 the results were as follows in YNB with 0 5 per cent saccharose, the development is positive from 8 to 10 days onwards in all the tubes, this result coincides with the fact that this species normally is considered capable of assimilating saccharose, by means of paper chromatography it was proved that there was no external hydrolysis of the saccharose, fermentation is as slow as or slower than with *S oleaginosus* in any of the media Agar with eosin, methylene blue and saccharose, either in YI or YA, yielded rose-white colonies

Therefore, it seems that it is possible to make the following deductions from these results

(*a*) *S italicus*, *S oleaginosus* and *S hienipiensis* contain no β-fructosidase However, they are capable of induced utilization of sucrose, which very likely involves the induced formation of a maltose transportase[5] that will transport saccharose across the yeast membrane, followed by intracellular hydrolysis of the saccharose by α-glucosidase

(*b*) The utilization (fermentation or assimilation) of saccharose by *S italicus*, *S oleaginosus* and *S hienipiensis* is a property which can be induced by saccharose

(*c*) In this synthesis, in the presence of saccharose, the composition of the medium has a decisive influence *S italicus* carries out synthesis quickly in comparatively simple media, while *S oleaginosus* and *S hienipiensis* demand a much more complex medium

(*d*) Though *S italicus* is quicker in its assimilation of saccharose than *S oleaginosus* and *S hienipiensis*, there is no notable difference among the three species as to their production of carbon dioxide from saccharose

(*e*) In small inoculations, the fact that the inoculations proceed from glucose, maltose or saccharose has no influence on the velocity of the utilization of the saccharose On the other hand, by using abundant cellular masses, the adaptation to maltose and saccharose determines a much more rapid fermentation

(*f*) In taxonomic studies of yeasts, it is impossible to omit specifying in detail all the media used, in order to avoid confusion (*g*) In doubtful cases, it would be convenient to determine whether the saccharose is utilized by the α-glucosidase or by β-fructosidase I consider the qualitative analysis of the culture medium by means of paper chromatography, when the phase of positive development has been reached, as sufficient proof

(*h*) It is necessary not to confuse the adaptive fermentation from saccharose by β-fructosidase (*S fragilis*) with the saccharose utilization by α-glucosidase (*S italicus*, *S oleagenosus* and *S hienipiensis*), though this reaction is also inducible

JUAN SANTA MARIA

Seccion de Bioquímica,
Instituto Nacional de Investigaciones Agronómicas,
Madrid, 3

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Adenosine Diphosphate Glucose and Glucoside Biosynthesis

URIDINE diphosphate glucose (UDPG) has been found to act as glucose donor in a number of enzymatic reactions, such as glucoside formation in plants[1] and in locust[2], sucrose and starch synthesis in plants[3,4] and glycogen formation in mammals[5]

Further work showed that adenosine diphosphate glucose (ADPG) is a better substrate for starch synthesis than UDPG, but half as effective as the latter for glycogen formation and nearly ineffective for sucrose synthesis[6]

Arbutin or very similar glucosides were reported to occur naturally in seeds and locusts[7,8] Since arbutin is formed with enzymes either from wheat germ or locust it was considered of interest to test ADPG with these systems, which are known to use UDPG as substrate

With the enzyme from locust[2] ADPG proved to be ineffective for glucoside synthesis On the other hand, using the enzyme from wheat germ[1], ADPG led to the formation of two glucosides which were indistinguishable from those formed when UDPG was used as substrate, that is, arbutin and the corresponding gentiobioside All experiments were carried out with enzyme extracts and methods described previously[1,2,6] For the characterization of the glucosides the reaction with bromine was also used[7,9]

The quantitative results obtained are shown in Tables 1 and 2 It may be observed that the synthesis of glucosides and gentiobiosides is faster when ADPG is substituted for UDPG, but the difference in rates of transfer is not so great as in starch synthesis[6]

Other nucleotides were also assayed as glucose donors in glucoside synthesis Deoxy-ADPG, UDP-maltose and an isomer of UDPG in which the glucose is joined to UDP through position 6 gave negative results Guanosine diphosphate glucose, and to a lesser extent cytidine diphosphate glucose, act slightly as glucose donors, as estimated by nucleoside diphosphate formation in the presence of hydroquinone

The reactions of ADPG and other nucleotides with different phenols and enzymes from several sources are now being examined

Table 1. FORMATION OF GLUCOSIDES AND GENTIOBIOSIDES

Substrates		µmoles formed	
Phenol	Nucleotide	Glucoside	Gentiobioside
Hydroquinone	UDPG	0 12	0 03
Hydroquinone	ADPG	0 15	0 05
Arbutin	UDPG	—	0 09
Arbutin	ADPG	—	0 15

The system contained 0 5 µmole, ADPG or UDPG, 0 8 µmole, hydroquinone or arbutin, 0 15 µmole, ethylenediamine tetraacetic acid, 0 15 µmole, cysteine, 30 µmoles *tris*(hydroxymethyl)aminomethane–maleate buffer (*p*H 6 8), 0 08 ml , wheat germ enzyme (fraction I), (ref 1) Total volume, 0 2 ml The reaction mixture was incubated 60 min at 37° and chromatographed The spots containing glucoside and gentiobioside were eluted and estimated as previously reported (ref 1)

Table 2 FORMATION OF UDP OR ADP

Substrates		µmoles of UDP or ADP formed		
Phenol	Nucleotide	10 min	25 min	40 min
Hydroquinone	UDPG	0 006	0 015	0 025
Hydroquinone	ADPG	0 011	0 023	0 045
Arbutin	UDPG	0 008	0 0125	0 020
Arbutin	ADPG	0 014	0 025	0 035

The system contained 0 4 µmole, hydroquinone or arbutin, 0 1 µmole, cysteine, 0 1 µmole, ethylenediamine tetraacetic acid, 20 µmoles *tris*(hydroxymethyl)aminomethane–malate buffer (*p*H 6 8), 0 04 ml , wheat germ enzyme (fraction I) Total volume, 0 1 ml The reaction mixture was incubated at 37° for the times indicated and the UDP or ADP formation was estimated (ref 6)

This investigation was supported in part by a research grant (No *G*–3442) from the National Institutes of Health, U S Public Health Service, by the Rockefeller Foundation and by the Consejo Nacional de Investigaciones Científicas y Técnicas

JUAN CARLOS TRIVELLONI
EDUARDO RECONDO
CARLOS E CARDINI

Instituto de Investigaciones Bioquímicas
'Fundación Campomar'
and Facultad de Ciencias Exactas y Naturales,
Obligado 2490,
Buenos Aires

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Phage Ghost-induced Spheroplasts of *E. coli* 'B' as a System for Phage Reproductions

THE investigations of various authors have shown that lysozyme protoplasts, as well as penicillin spheroplasts, preserve the biochemical activity of an intact bacterium Synthesis of proteins and nucleic acids is achieved in them[1,2]

The full metabolic value of protoplasts allowed them to be used as a system for the study of the reproduction of phages

In the work reported here, spheroplasts of *E coli B*, obtained by means of treatment with ghosts of phage

T4r, were examined The 'ghosts' were obtained by means of Herriott's method[3] somewhat modified. For the elimination of the residual infective phage, the preparations were subjected to an osmotic shock, and irradiated by ultra-violet light (3 lamps at 15 W) for 5–7 hr The spheroplasts were maintained in a hypertonic medium with sucrose and magnesium sulphate The number of 'ghosts' per bacterium was equal to 2,500–3,000 parts The spherical bacterial forms obtained were osmotically fragile but retained a modified cell wall and they had the capacity of absorbing partly the heterologous phages But in these ghost-induced spheroplasts, neither homologous nor heterologous phages were reproduced

During the preliminary infection of the cells by an intact phage and the subsequent transformation of them into spheroplasts, it was established that the suppression of phage reproduction results only from treatment of the infected cells by phage ghosts during first minutes after initial infection (Table 1)

Table 1. REPRODUCTION OF PHAGE *T4r*+ IN THE SPHEROPLASTS OF *E coli* INDUCED BY 'GHOSTS' OF THE PHAGE *T4r*+ FOLLOWING DIFFERENT INTERVALS OF TIME AFTER INFECTION

	Composition of the mixture	Time of treatment of bacteria after their infection by the phage (min) 1	2	4	5
		Coefficient of increase of the titre of phage during subsequent incubation of infected bacteria for 40 min at 37° C			
Exp	Bacteria infected by *T4r* and treated by the 'ghosts' of the same phage	2	1	1 4	18
Cont	Bacteria infected by phage *T4r*+ and treated by sucrose in medium *F*	22	29 4	15 6	16

It was concluded that the phage 'ghosts' suppress in the bacterial cells those reactions which are initiated immediately after the penetration of the phage DNA These reactions could be either the blockage of early protein synthesis[4] or of RNA[5]

In order to check this suggestion, biochemical experiments were carried out the aim of which was the examination of the influence of 'ghosts' on the synthesis of protein and nucleic acid of the non-infected cells

In the biochemical experiments a 4-hourly broth culture of *E coli B* was used, treated by the phage 'ghosts' in such a quantity that there were 100 phage particles per bacterium. A non-treated culture was used as control

The two bacterial preparations (experimental and control) were incubated at 37° C and during definite intervals of time samples were taken for the determination of the contents of protein, nucleic acids and acid-soluble compounds[6]

From Fig 1 it can be seen that in the bacterial culture treated with phage 'ghosts' a complete suppression of protein and DNA synthesis occurs and also a considerable diminution of RNA content Also, one can observe a corresponding reduction in the total quantity of nucleic acids and an increase of acid-soluble compounds absorbing ultra-violet light

The difference of our results relating to the velocity of accumulation of acid-soluble compounds from those obtained by Lehman and Herriott[7] possibly can be explained by the fact that in our experiments the number of 'ghosts' per bacterial cell was considerably greater

The mechanism of the diminution in RNA and acid-soluble compounds observed by us is not clear One may assume that the protein walls of the phage are activating RNase, but at the same time they can block the synthesis of RNA in the bacteria From these suppositions, one may assume that the accumulation of the acid-soluble compounds in the culture, subjected to the action of the phage 'ghosts', could occur at the expense of nucleotides produced by the action of RNase, or at the expense of the RNA precursors or at the expense of both The reduction of the synthesis of RNA can be produced by increase of the penetrability of the cell under the influence of 'ghosts', which is responsible for entry into the medium of the RNA precursors

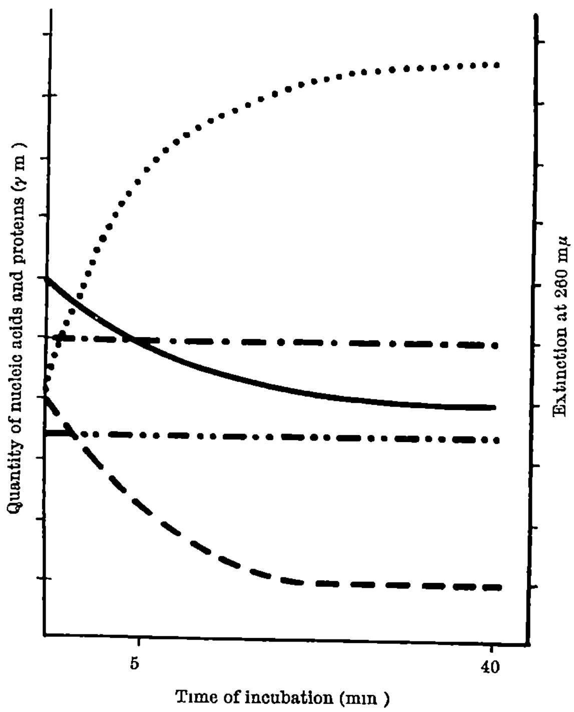

Fig 1 ——, Total quantity of nucleic acids, – – –, RNA, – –, DNA, , acid-soluble compounds, – –, protein

Comparison of the experimental results on phage reproduction with the results of the biochemical experiments suggests that the incomplete value of phage spheroplasts as a system for phage reproduction could be explained by the reduction of the synthesis and destruction of RNA, forming in the cell immediately after the infection of the phage, which as was shown in earlier work is necessary in the first stages of its reproduction[8–11]

D M GOLDFARB
A V AVDEEVA
N B BORISOVA

Institute of Epidemiology and Microbiology,
N F Gamaley Academy of Medical Science,
Moscow, U S S.R

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Isolation of Progesterone from Human Pregnant Myometrium

ZANDER[1] has reported that "with the methods presently available it has never been found possible to demonstrate non-labelled progesterone or either of the Δ4-3-ketopregnene-ol-one isomers in the target tissues, uterine muscle and mucosa" With the aid of gas-liquid phase chromatography we have isolated non-labelled progesterone from the human pregnant myometrium and this report deals with the method used and the concentrations of the steroid found

The technique for qualitative and quantitative isolation of progesterone, using gas-liquid phase chromatography, was first perfected in placental tissue The details of this procedure have been described elsewhere[2] Essentially it consists of 5 per cent sodium hydroxide digestion, extraction of crude lipids with ether, partial purification of cholesterol on silicic acid column and final separation of progesterone on gas chromatogram using 2 per cent '*SE* 30' (General Electric) as liquid phase

The qualitative identification of progesterone was confirmed by the following procedure

(1) The retention times on the gas column chromatogram for the other closely related steroids were obtained and none of the steroids tested had the same retention time as progesterone

(2) The peak from the gas column which displayed the same retention time as crystalline progesterone was collected and subjected to ultra-violet absorption analysis Both the tissue extract and the standard exhibited maxima at 240 mμ were suggestive of unsaturated λ-β ketonic di-substitution

(3) The 'progesterone' peak from analysis of the tissue on the gas column was also subjected to bioassay by a modification of the McGinty procedure[3] and gave a 4+ response for progesterone in the endometrium of rabbits (We thank Dr Arthur L Haskins, of the University of Maryland, Baltimore, for the bioassays and ultra-violet absorption spectra)

After establishing with placental tissue the technique for the isolation of progesterone by gas column chromatography, human myometrium was analysed using the same procedure In order to obtain a peak suitable for accurate measurement, however, the myometrial tissue samples had to be in the range of 40–45 g each, therefore confining the studies to women having cæsarean hysterectomy

Table 1 MYOMETRIAL PROGESTERONE

No	History	μg progesterone/g wet weight myometrial tissue *P*	*AP*
1	Pooled myometrial biopsies	0 63	0 31
2	Mrs A R —12 weeks	0 15	0 08
3	Mrs R C —38 weeks	0 16	0 10
4	Mrs V T —39 weeks	0 11	0 12

P, myometrium overlying the placental site, *AP*, myometrium directly opposite to placental attachment

With this procedure, 0 08–0 63 μg of progesterone per gram of wet weight of tissue was found (Table 1) Bioassay and ultra-violet absorption spectra were carried out as before The bioassay gave a 1 to 3+ progestin response, and the ultra-violet absorption was in the vicinity of 240 mμ due to very low concentrations In addition, the final extract was run in three different liquid phases, 2 per cent 949, 1 per cent 149–244, 2 per cent '*XF*–1125' (General Electric), and the 'progesterone' peak from the unknown sample had in each of these instances the same retention time as the standard progesterone

Finally, to eliminate the possibility that these small amounts represent measurements of plasma progesterone retained in the tissues despite the careful blotting of the cut pieces in order to remove blood, hæmoglobin determinations were carried out and the maximum plasma content estimated as 8 5 ml/100 g wet weight (We thank Mr Daniel Kooyman for the analysis of tissue plasma content) Even assuming 15 μg of progesterone per 100 ml of plasma, the determined tissue-levels exceed any reasonable plasma concentration by several-fold (0 012 μg calculated from plasma sources versus the 0 08–0 6 μg found)

D KUMAR
J A GOODNO
A C BARNES

Department of Gynecology and Obstetrics,
Johns Hopkins University
School of Medicine and Hospital,
Baltimore

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Development of H Activity by Human Blood-Group B Substance treated with Coffee Bean α-Galactosidase

THE appearance of H specificity in the non-diffusible products remaining after the enzymatic destruction of A and B blood-group specificities has been observed with enzyme preparations from several different micro-organisms Iseki and Masaki[1] first reported that an A-decomposing enzyme from the bacterium *Clostridium tertium* destroyed the A serological activity of human saliva and hog A preparations and gave products which had H serological activity not shown by the A preparations before enzyme treatment Enzyme preparations from the protozoan *Trichomonas foetus* similarly decompose A substance of human ovarian cyst and saliva origin with the disappearance of A specificity and the appearance of H specificity[2] Extracts from *T foetus* also contain a different enzyme which acts on human B substance with the destruction of B serological properties and the appearance of H specificity[2,3], and bacterial enzymes from *Bacillus cereus*[4] and *Clostridium maebashi*[5] induce the same specificity changes in B substance

The B-decomposing enzymes from these micro-organisms have not been obtained in a sufficiently pure state for their specificity to be determined unequivocally by their action on substrates of known structure On the basis of enzymatic hydrolysis and inhibition experiments, however, a D-galactosidase specificity was inferred Loss of B activity of human ovarian cyst B substance by the action of the partially purified enzyme from *T foetus* was accompanied by the release of reducing sugars (equivalent to 5 per cent of glucose) which consisted of a major galactose component with only traces of fucose and *N*-acetylhexosamine[3], and the enzyme from *Cl maebashi*, when allowed to act on B substance from human stomach linings, liberated about 8 per cent of the original galactose and no other sugar[6] The action of the *T foetus* enzyme on B substance was inhibited by D-galactose and not by any of the other sugars in the blood-group substances[7], and when this inhibition occurred there was no development of H specific activity[2,3] These results therefore suggested that the

Table 1. HÆMAGGLUTINATION INHIBITION OF ANTI-B, ANTI-H AND ANTI-Le^a SERA BY B AND BP1 SUBSTANCES BEFORE AND AFTER TREATMENT WITH COFFEE BEAN α-GALACTOSIDASE*

Substance	Treatment	Minimum amount of substance (μgm./ml.) giving complete inhibition with Human anti-B serum	Rabbit anti-H serum	Human anti-Le^a serum
B substance (Beach)	Untreated	0.1	100	50
	Enzyme treated Beach E.T.	200	1.5	50
	Enzyme treated Beach E.T. (phenol insol.)	200	1.5	50
	Enzyme treated Beach E.T. (20 per cent ppt.)	200	0.75	25
BP1 substance (Beach P1)	Untreated	3	>200	>200
	Enzyme treated Beach P1 E.T.	>200	50	>200
	Enzyme treated Beach P1 E.T. (10 per cent ppt.)	>200	25	>200
	Enzyme treated Beach P1 E.T. (20 per cent ppt.)	>200	50	>200
H substance	Untreated	—	0.75	—
Le^a	Untreated	—	—	0.37

* Conditions for hæmagglutination inhibition tests: 0.1 ml. serum, diluted to contain 2–3 completely hæmagglutinating doses, was added to 0.1 ml. of serial dilutions of blood-group substances. The mixtures were left 15 min. at room temperature and 0.1 ml. of a 1 per cent suspension of erythrocytes of the appropriate group were then added. The degree of agglutination was determined by examination under the microscope after 2 hr. at room temperature.

enzymes destroying B specificity were removing terminal D-galactosyl residues and thereby exposing structures closely related to, or identical with, those present in H substance.

The anomeric form of the galactosyl residues in B substance hydrolysed by the enzymes, and consequently the α- or β-specificity of the galactosidases which destroy B serological activity, could not be deduced from the enzymic degradation or inhibition experiments. Insight into the serologically active grouping in B substance had been obtained independently, however, from immunochemical inhibition experiments. Kabat and Leskowitz[8] found that galactose inhibited precipitation in the B-anti-B system and that α-methyl-D-galactoside and other α-D-galactosyl compounds were better inhibitors than galactose, whereas β-methyl-D-galactoside was a poorer inhibitor. These results indicated that a terminal non-reducing galactosyl unit in the α-configuration is involved in B specificity, and the recent isolation by Painter, Watkins and Morgan[9] of a B serologically active disaccharide, *O*-α-D-galactosyl-(1 → 3)-D-galactose, from human B substance confirms this deduction. The enzymes which destroy B specificity are, therefore, by inference, α-galactosidases. Nevertheless, enzymes which split α-D-galactosyl linkages do not invariably act on B substance with a loss of B serological activity. Enzyme preparations from brewers' yeast[10], almond emulsion[11] and *Patella vulgata*[12], which actively hydrolysed melibiose and *p*-nitrophenyl-α-D-galactoside, were without action on B substance[13]. Zarnitz and Kabat[14], however, found that a purified α-galactosidase from coffee beans[15] splits galactose from B substance with a reduction in the power of the substance to precipitate a human anti-B serum and a complete destruction of the capacity of the substance to inhibit hæmagglutination of B red-cells by an anti-B serum. About 5 per cent of reducing sugar was liberated from the B substance and, on fractionation on a charcoal: celite column, this sugar was shown to consist almost entirely of galactose with only a trace of methyl pentose and no detectable hexosamine or acetylhexosamine. The enzyme preparation was without action on lactose, showing the absence of a β-galactosidase, and it was therefore concluded that loss of B activity resulted from the action of the α-galactosidase in the coffee bean preparation. In this communication we wish to report that a development of H activity accompanies the loss of B activity when B substance is treated with the coffee bean α-galactosidase.

H activity was measured in hæmagglutination inhibition tests with a rabbit anti-H serum[16]. The materials tested have been described previously[14] and were (1) the untreated B substance (Beach), (2) the whole lyophilized residue obtained after enzyme treatment (Beach E.T.), (3) the insoluble material obtained on extraction of the whole lyophilized residue with 90 per cent phenol (Beach E.T., phenol insoluble), (4) the material recovered from phenol by the addition of 20 per cent (v/v) ethanol (Beach E.T., 20 per cent p.p.t.). The low H activity of the untreated B preparation is considerably increased as the result of the action of the coffee bean α-galactosidase (Table 1), and this H activity is associated with both the fractions obtained after extraction of the enzyme-treated non-dialysable residue with phenol.

The H activity developed by B substance after treatment with the B-decomposing enzyme from *T. foetus* was first reported to be equivalent to about 10 per cent of the activity of a purified H substance[3]. In later experiments, when a more highly purified enzyme was used, H inhibition titres equivalent to 25–50 per cent of the activity of H substance were obtained[2]. Crude extracts from *T. foetus* contain an enzyme which destroys H serological activity, however, and it is difficult to free the enzyme acting on B substance from all traces of this contaminant. The materials which remain after treatment with the coffee bean α-galactosidase have H activities equal to 50–100 per cent of the activity of a purified human H substance (Table 1), in the absence of an enzyme which destroys the newly exposed H reactive structures, therefore, the removal of galactose from B substance gives rise to a material which has virtually the same activity in hæmagglutination inhibition tests as an untreated H substance. In double diffusion tests in agar gel, the α-galactosidase-treated B substance gave a precipitin line with rabbit anti-H serum which fused with that given by human H substance, this line was not given by the untreated B substance.

Mild acid hydrolysis of group B substance liberates fucose and various oligosaccharides and exposes other terminal non-reducing α-galactosyl units which confer a new, BP1, specificity on the molecule[17]. Galactose is also split from BP1 substance by the coffee bean α-galactosidase with a resultant loss of both the residual B specificity and the newly formed BP1 specificity[14]. The enzyme-treated BP1 substance (Beach P1 *E.T.*) was divided into two fractions, Beach P1 *E.T.* 10 per cent, and Beach P1 *E.T.* 20 per cent, by precipitation from phenol solution with ethanol[14], and when the BP1 materials were tested for development of H specificity only a small increase in activity was observed (Table 1), it can be assumed that this increase results from removal of galactose from the remaining B specific groupings in the molecules and not from the BP1 structures.

Stepwise enzymic degradation of group B substance, first with a purified B decomposing enzyme from *T foetus*, and, secondly, with an H decomposing enzyme from the same organism, results in a material which has blood-group Le^a activity considerably in excess of that detectable in the original preparation or in the H-active material formed as the result of the first enzyme treatment[2,18] Since treatment with the coffee bean preparation corresponded to the first step in this degradation procedure, an increase in Le^a activity was not to be expected When the B and BP1 fractions were tested for hæmagglutination inhibition in the Le^a system, no significant change in the Le^a titres was observed between the control and the enzyme-treated substance (Table 1)

The demonstration that H activity is developed by B-substance on treatment with the purified α-galactosidase from coffee beans provides confirmatory evidence that the H active groupings are exposed as the result of the removal of terminal α-linked galactosyl units from the B specific structures

This work was aided in part by a grant from the National Science Foundation (*G*–18727)

Winifred M Watkins
Lister Institute of Preventive Medicine,
London, S W 1

Marie Luise Zarnitz*
Elvin A Kabat
Departments of Microbiology and Neurology,
College of Physicians and Surgeons,
Columbia University
and the Neurological Institute,
Presbyterian Hospital,
New York

* Present address Max Planck Institut für Biologie (Abt. Weidel), Tübingen, Germany

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Activation of Endogenous Plasma Proteolytic Enzymes with the Consequent Production of Pharmacologically Active Polypeptides

The transfusion of human antihæmophilic globulin concentrate (EHF) routinely prepared in this laboratory by the method of Kekwick and Wolf[1] was found to give clinical reactions[2] resembling the effects of a plasma kinin[3]. As EHF contains plasminogen and fibrinogen as well as other clotting factors, and as human plasmin is known to produce a kinin from pseudoglobulins[4], the possibility that the reactions were due to a kinin produced as a result of plasminogen activation in EHF was explored

Kinin was assayed on the isolated guinea pig ileum against an alcoholic extract of a tryptic digest of human α-globulin, the potency of which had been determined in terms of synthetic bradykinin (Sandoz '*BRS* 640') Results are expressed as ng synthetic bradykinin/ml The activation of plasminogen was determined by estimating the amount of fibrinogen in the EHF hydrolysed after incubation at 37° as indicated by the increase in the optical density at 280 mμ of a trichloroacetic acid filtrate, expressed as μg tyrosine/ml When EHF was incubated with streptokinase (Lederle) plasminogen activation was followed by kinin production In 10 batches of EHF the increase in kinin, which was maximal after about 60 min, although fibrinogenolysis continued, varied from 19–83 ng bradykinin/ml The kinin was isolated by distillation at reduced pressure from the alcoholic filtrate obtained after precipitating the protein of activated EHF with 3 volumes of boiling ethanol , or by chromatography on 'Sephadex *G* 75', when 92 per cent of the active material was found in association with a small molecular fraction equivalent to 1 2 per cent of the initial protein A kininogenetic system was thus demonstrated in EHF, and the clinical reactions observed could be due to the activation of EHF plasminogen by a plasma or tissue activator.

The initiation of blood clotting by the activation of Hageman factor by glass[5] is followed by the production of a plasma kinin[6], and, it has been claimed[7,8], by the activation of plasminogen It seemed possible, therefore, that an intrinsic plasminogen activator which could be isolated from plasma by adsorption on a silica surface could cause the *in vivo* production of kinin from EHF As Hageman factor[9] and kininogen activator[10] have been eluted from silica by alkaline saline, the effects of such a fraction were tested on the plasminogen and kininogen in EHF, and in systems where the proenzyme was purified human plasminogen, and the substrate plasminogen-poor fibrinogen or casein

Plasma, derived from blood taken into ACD anticoagulant in plastic bags, centrifuged at 0°, and held at −20° in siliconed bottles until used, was allowed to flow through a column of 'Celite Hyflo' (100 mg/ml), which was then washed with 0 15 M sodium chloride until the effluent was protein free The column was then eluted with 0 15 M sodium chloride brought to *p*H 11 0 by adding sodium hydroxide, until the effluent was again free from protein In 10 different preparations 'Celite' eluate (CE) containing 0 24–0 44 mg N/ml and 0 7–1 4 per cent of the initial plasma protein was obtained Plasminogen was prepared by the method of Sgouris *et al* [11] and plasminogen-poor fibrinogen (SF) by that of Kekwick *et al* [12] A comparison of the effects of incubating EHF and SF with saline, CE or streptokinase for 60 min at 37° showed that from EHF, CE produced 13 μg acid-soluble tyrosine, and 42 ng bradykinin, while streptokinase produced 115 μg acid-soluble tyrosine and 53 ng bradykinin Because of the almost complete lack of plasminogen only traces of both substances were produced from SF When EHF and SF were incubated with glycerol-activated plasmin, EHF produced 98 μg tyrosine and 72 ng bradykinin, and SF 69 μg tyrosine, but no bradykinin. This shows that the kinin is not produced by the hydrolysis of fibrinogen

Table 1 Effect of Incubating CE and Streptokinase with Plasminogen at 37° before adding Substrate

Activator	Plasminogen batch	Substrate	Tyrosine μg/ml. 2 h	4 h
CE_4	291 P_3	Fibrinogen SF_4	27	36
None	291 P_3	,,	9	22
CE_7	295 P_3	,, SF_4	19	32
None	295 P_3		7	22
CE_9	307 S_3	,, SF_6	4	20
None	307 S_3		3	7
CE_9	None	,, SF_6	0	4
Streptokinase 200 units	307 S_3	,, SF_6	81	112
Streptokinase 200 units	None	,, SF_6	2	2
None	None	,, SF_6	3	4
CE_4	298 S_3	Casein	14	34
CE_8	298 S_3	,,	7	33
CE_9	298 S_3	,,	9	27
Streptokinase 200 units	298 S_3	,,	135	162
None	298 S_3	,,	0	8

CE was also incubated with purified plasminogen for 30 min at 37° before adding the mixture to substrate In each experiment activation by CE was compared with that by streptokinase, with the effect of plasminogen alone and with the effect of activators without added plasminogen Table 1 shows that plasminogen was activated by CE, in excess of the autoactivation taking place, and CE itself contains no plasmin, since the small amount of plasminogen in SF was equally activated by CE and streptokinase

A preparation of CE, concentrated by pressure dialysis against pH 8 6 veronal buffer I 0 1, was examined by electrophoresis on acetyl cellulose in the same buffer[13], the protein being stained with naphthalein black By comparison with normal plasma examined at the same time, CE was a mixture of α_1-, α_2- and β_2-globulins The relative proportions were calculated by estimating, in a Hilger spectrophotometer[14], the optical density at 630 mμ of dyed protein on a strip cleared with Whitemor oil 120, and found to be 12 per cent α_1-, 78 per cent α_2- and 10 per cent β_2-globulin

The observations described confirm those of previous workers[7-10] In addition we have found that CE activates plasminogen as well as kininogen, and in EHF could produce conditions which might give rise to a clinical reaction on transfusion The kininogen substrate is not known, but differences between EHF and SF as substrate for plasmin digestion indicate that it is not fibrinogen

Margaret Mackay

Medical Research Council
External Scientific Staff

W d'A Maycock
B S Combridge

Lister Institute, Elstree, Herts

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Gastric-stimulating Factor in Meat Extract

It was first shown by Pavlov[1] that meat extract was a powerful stimulant of gastric secretion when given by mouth Later workers confirmed this but failed to identify the compounds responsible for the effect (for a review, see ref 2) Following a full investigation of the constituents of meat extract[3,4], a sustained effort was made to identify the gastric stimulating factor, with the results presented here

Gastric secretion was measured with Davies's technique[5] by following the uptake of carbon dioxide of a bag of isolated toad mucosa contained in a double side-armed Warburg flask A plot of manometer readings against time gave a line of constant slope changing abruptly to a line of greater slope (increased secretion) when a stimulating agent was added from the side-arm At the end of each experiment in which no stimulation of gastric secretion was observed, histamine (to a concentration of 5 6 μg/ml) was added as a control to determine whether the failure to stimulate was due to the use of the occasional non-secreting mucosa A measure of the activity of a given sample was obtained by dilution with saline to a point where a response was no longer obtained Using this technique, commercial meat extract and an extract of fresh muscle were active, while a mixture of the compounds identified in meat extract[6] was inactive, both before and after heating Riboflavin, 5-hydroxytryptamine, synthetic carnosine and synthetic carnitine were all inactive

The histamine in the sample was concentrated by saturating a solution of meat extract at pH 10 with sodium sulphate and extracting with n-butanol The amount of histamine was estimated, by comparison with known amounts of standard, on paper chromatograms and found to lie between 0 004 and 0 008 per cent of the extract This is equivalent to 0 012–0 024 μg/ml in the 300 μg/ml of extract, which is the minimum amount that gives a response in the assay

Since the minimum amount of pure histamine giving a response was in the region of 0 05 μg/ml and the extract still stimulated after the free histamine had been extracted, it was concluded, at this stage of the investigation, that histamine was not the active principle

A ten-fold purification of the activity of meat extract was achieved as follows Meat extract was blended with 5 vol of ethanol in a Waring blender, the alcohol extract concentrated to half-volume and an equal volume of acetone added The amorphous cream-coloured precipitate was centrifuged off and redissolved in water Paper chromatography showed the presence of creatine, creatinine, hypoxanthine, carnosine, carnitine, and traces of amino-acids. Physical and chemical tests combined with bioassay revealed that the active factor was dialysable, soluble in polar organic solvents, basic and probably contained an amino-group since the activity was destroyed by acetylation, deamination and oxidation with hydrogen peroxide, and was unaffected by hydrolysis in 6 N acid or alkali

Active material was chromatographed on paper in a number of solvents and the activity located by cutting up the chromatograms and assaying the zones After chromatography of meat extract in butanol–acetic acid the active principle was found to coincide with a narrow band of yellow material having a blue fluorescence in ultra-violet light of wave-length 247 mμ, but in isopropanol–ammonia it was separated from the fluorescent material which remained near the origin. As evident from Table 1, the active factor had quite different R_F values from histamine in two of the solvents tested.

Table 1

	R_F factor Active factor	 Histamine
n-Butanol–acetic acid–water (ref. 7)	0·45	0·22
Isopropanol–concentration ammonium–water (ref. 7)	0·52	0·52
n-Butanol–pyridine–water (ref. 7)	0·37	0·47

When the concentrated material was run in butanol-acetic acid, activity was found at two positions on the chromatogram corresponding to $R_F = 0·22$ and 0·45. However, after acid- or alkaline-hydrolysis only one active spot $R_F = 0·22$ was found. Examination of the action of the two materials R_F's 0·22 and 0·45 on the isolated guinea pig intestine indicated that both these compounds had a histamine-like effect which was inhibited by promethazine.

The concentrated material was treated to chromatography in ethanol solution on a column of cellulose powder. The effluent was fractionated arbitrarily and each fraction tested for activity. The active fractions were combined to give a further purification of approximately five-fold. Paper chromatography of this material showed the presence of a faint diazo-reacting spot at R_F 0·45 in butanol–acetic acid. When histamine hydrochloride was added and the mixture chromatographed, two strong diazo reactors now appeared at R_F 0·22 and 0·45. After hydrolysis only the material at 0·22 was found.

It is suggested that the added histamine reacted with one of the substances present in the partially purified sample from meat extract to form a complex at R_F 0·45 and that a similar material is the active principle naturally present in the extract. West and Riley[8] have observed a similar change in the chromatographic behaviour of histamine present in tissue extracts, and Beckett, Beaven and Robinson[9] have shown that double spot formation may take place with a number of naturally occurring amines, and is repressed by basic solvents.

The activity of meat extract in stimulating acid gastric secretion when administered by mouth has not been explained, but it seems likely that the postulated histamine complex is active by this route at low concentrations whereas free histamine causes secretion only in very large doses.

T. Wood*
E. P. Adams†
A. E. Bender‡

Research Department,
Bovril, Ltd.,
148/166 Old Street,
London, E.C.1.

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* Present address: Biochemistry Department, Institute of Psychiatry, The Maudsley Hospital, London, S.E.5.

† Present address: Department of Experimental Pathology, Australian National University, Canberra, Australia.

‡ Present address: Research and Development Department, Farley's Infant Food, Ltd., Colnbrook, Bucks.

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PHYSIOLOGY

Tolerance to Severe Heat and the Behaviour of Respiratory Minute Volume in Cattle

In an attempt to find whether various degrees of tolerance to severe heat in cattle are associated with significant changes in respiratory minute volume, the following experiment was conducted. Each of 8 Ayrshire bull calves, aged 7–11 months, was exposed 3 times in a climatic room[1] to an atmosphere of 40° C dry bulb and 38° C wet bulb temperature, until its rectal temperature reached 42·0° C. Every 10 min., throughout exposure, the volume and frequency of respiration were determined with a pneumotachometer[2] and the rectal temperatures measured with thermocouples[3]. Heat tolerance was expressed in terms of rate of rise of rectal temperature (°C/hr.).

The most heat-tolerant animal (No. 3) could tolerate the hot atmosphere for 175 min., corresponding to a rate of rise of rectal temperature of 1·03° C/hr. The corresponding figures for the least heat-tolerant animal (No. 7) were 97 min. and 1·85° C/hr.

The relation between rectal temperature response and respiratory response of the 8 animals is shown in Fig. 1. The regression coefficient given in the equation is statistically significant. Under the conditions of this experiment, where rectal temperature rose virtually linearly with time of exposure, a mean increase in the rate of rise of rectal temperature of 1° C/hr. corresponded to a mean increase in respiratory minute volume of 90 litres.

The animals tested from July until September (Nos. 1, 2, 3 and 4 in Fig. 1) were more heat tolerant than those tested from November until January (Nos. 5, 6, 7 and 8). The averages of these two groups for rate of rise of rectal temperature were 1·14 ± 0·053° C/hr. and 1·64 ± 0·075° C/hr., the difference of 0·50° C/hr. being statistically significant. This might suggest seasonal acclimatization of the animals tested in the summer.

The finding of a positive correlation between rectal temperature and respiratory minute volume is in agreement with the results of McDowell, Lee, Fohr-

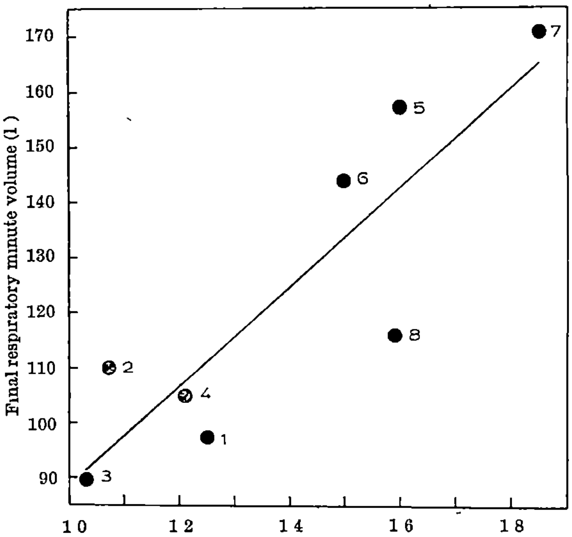

Fig. 1. Regression of respiratory minute volume in litres on rate of rise of rectal temperature. Regression equation $y = 90x - 1·4$. The figures in the diagram refer to the serial numbers of the calves.

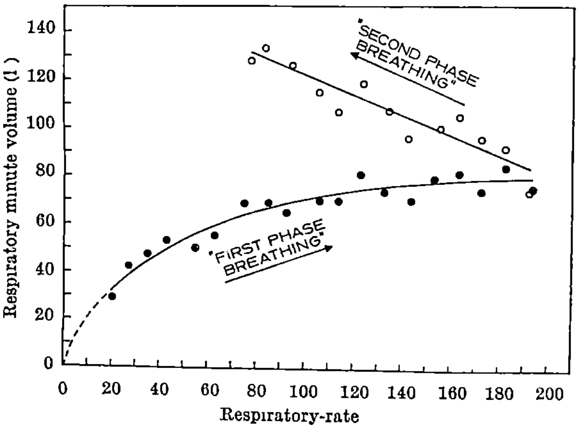

Fig 2 Relation between respiratory-rate and respiratory minute volume of calves exposed to an atmosphere of 40° C dry-bulb and 38° C wet-bulb temperature (Mean results for 8 animals)

man and Andersen[4] obtained under less stressful environmental conditions in cows, and it confirms their contention that a high respiratory activity is an attempted compensation for a poor heat balance brought about by some other cause

The inverse relationship between heat tolerance and respiratory minute volume gave rise to the question whether it would be possible to use respiratory-rate (which is easily determined) as an indicator of respiratory minute volume (which is less easily determined) The relationship of these two variables in severely heat-stressed calves is shown in Fig 2 Owing to the bi-phasic behaviour of respiratory-rate[5,6], in the higher range, a given respiratory-rate is associated with two different values of minute volume a lower minute volume during the rising phase ('first phase breathing'), and a higher one during the declining phase ('second phase breathing') Respiratory-rate cannot, therefore, be used to predict respiratory minute volume unless the 'phase' of respiration is known

W BIANCA

Physiology Department,
Hannah Dairy Research Institute,
Ayr, Scotland

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Failure of Amino-nucleoside to produce Abnormalities in the Fœtus of the Rat

IN an experimental study of urinary tract malformations, an attempt is being made in our laboratory to influence with chemical agents the embryonic development of the kidney One substance that has been used is the amino-nucleoside derivative of 'Puromycin(R)', which is known to produce increased albuminuria and the nephrotic syndrome in rats Hallman *et al*[1] have used this substance in attempts to induce a congenital nephrotic syndrome in experimental animals, and they have described renal tubular dilatation in some offspring of rats treated during the last days of pregnancy

The test animals used were pregnant albino rats of the Sprague–Dawley strain, weighing 150–200 g Fifty-one animals were given amino-nucleoside for periods of 7 days, beginning on the fifth to fourteenth day of gestation Each course of treatment consisted of daily subcutaneous injections of 0 3 ml per 100 g of body-weight of one of the following solutions of amino-nucleoside in sterile distilled water 0 375 mg per 100 ml , 0 50 mg per 100 ml , 0 675 mg per 100 ml , and 0 750 mg per 100 ml (Table 1) Control animals were injected with sterile distilled water Offspring were killed at intervals up to 2 months after birth and their kidneys were examined histologically Available sera from offspring were pooled according to litters to make 17 batches, which were examined electrophoretically by the method of Smithies[2] Parent animals were also killed and examined in a similar fashion

Table 1 INJECTION OF PREGNANT RATS WITH AMINO-NUCLEOSIDE

Solutions	Day of gestation on which injections were started									
(mg/100 ml)	5	6	7	8	9	10	11	12	13	14
0 750	1*	1	1	1	1	1	1	0	0	0
	0†	0	0	0	0	0	3	0	0	0
	9‡	11	13	13	12	12	3	0	0	0
0 675	1	1	1	1	1	1	1	1	1	1
	0	6	10	6	7	6	5	6	11	8
	8	0	0	0	0	0	0	0	0	0
0 500	3	3	3	1	1	3	2	1	1	1
	8	6	9	0	0	0	6	0	0	0
	26	21	12	11	11	35	13	13	12	10
0 375	3	1	3	1	1	2	0	3	1	0
	18	10	17	11	13	20	0	25	9	0
	0	0	0	0	0	0	0	0	0	0
Control	3	3	3	3	3	3	3	3	3	3
	32	33	33	27	26	33	33	32	30	34
	0	0	0	0	0	0	0	0	0	0

* No of mothers
† No of offspring examined
‡ No of abortion sites

The highest dosage of amino-nucleoside caused abortion, as determined by the examination of the uterine implantation sites, in most animals Examination of the 170 living offspring in the experiment disclosed no gross malformations or microscopic abnormalities of the kidneys, and the electrophoretic patterns of serum proteins were normal No other anomalies were observed Eight parent animals which were treated with amino-nucleoside solution in a concentration of 0 5 mg per 100 ml did, however, develop microscopic renal abnormalities consisting of variable tubular dilatation, with proteinaceous precipitates and cast formation Five of these eight also had electrophoretic patterns of serum protein consistent with the nephrotic syndrome

These investigations have not produced congenital or developmental abnormalities in the kidneys of rats, and we have not been able to reproduce the findings of Hallman *et al* That the amino-nucleoside either produced abortion or left the fœtus unaffected, according to the dosage used, suggests that its toxic effect may have been mediated through interference with placental function

This work was supported by funds from the Michigan Kidney Disease Foundation and by research grant *A*-4418 from the National Institute of Arthritis and Metabolic Diseases, U S Public Health Service We also thank the Lederle Laboratories, American Cyanamid Co , Pearl River, New York, for providing samples of amino-nucleoside

JAY BERNSTEIN
RUBEN MEYER
DONALD S PICKMAN

Child Research Center,
Detroit, Michigan

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Luteotrophic Activity of Human Growth Hormone (Raben)

I HAVE developed an assay for the measurement of luteotrophin in hypophysectomized adult mice which depends on the increase in the weight of a damaged horn of the uterus (formation of deciduomata) Mice were hypophysectomized on the first day of diœstrus and 48 h later the endometrium of the right horn of the uterus was injured The animals were injected daily for 5 days with a preparation of luteotrophin commencing immediately after hypophysectomy and killed on the sixth day The response was recorded as positive if the weight of the damaged horn was at least 11 mg greater than the weight of the control horn The results were expressed as the percentage of animals with a positive response and a probit transformation was used for parallel line assays Details of this method will be published shortly

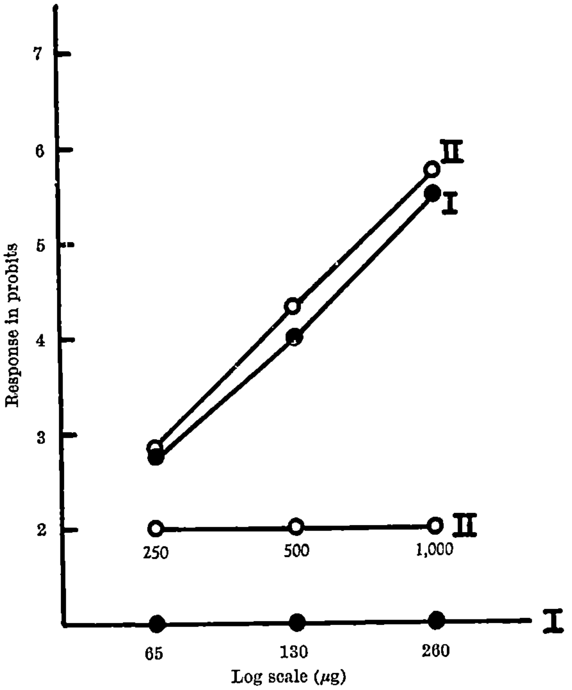

Fig 1 Dose-response curves of human pituitary growth hormone and of bovine prolactin I, bovine prolactin, Armour Laboratories, Lot No *D* 14083-2*B*, II, human pituitary growth hormone (Raben)

Human growth hormone prepared by the Raben method gave the same effect in hypophysectomized mice as the bovine prolactin, Armour Laboratories, Lot No *D* 14083-2*B* (assumed potency, 20 I U /mg) In positive responses the difference in weight between the two horns varied from 14 5 mg to 37 4 mg with human growth hormone and from 14 7 mg to 70 mg with bovine prolactin The doses for human growth hormone were 250, 500 and 1,000 μg per animal and for the bovine prolactin 65, 130 and 260 μg The potency of human growth hormone was 29 per cent (95 per cent fiducial limits 17–51) of the reference substance The results are given in Table 1 and Fig 1 These figures are in keeping with those previously obtained by the diœstrus method[1] and are of the same order as the results published by Ferguson and Wallace[2]

Table 1. RESULTS OF AN ASSAY OF HUMAN PITUITARY GROWTH HORMONE USING THE ARMOUR PROLACTIN LOT NO *D* 14083–2*B* (BOVINE) AS A STANDARD

Preparation	Design of assay	No of animals	Potency of unknown per cent of standard	95 per cent fiducial limits	Index of precision	Regression	Validity tests					
							Parallelism		Linearity			
									Standard		Growth hormone	
					λ	g	t	P	t	P	t	P
Human growth hormone	3 3	37	29	17–51	0 2	0 182	0 23	0 9–0 8	0 17	0 9–0 8	– 0 17	0 9–0 8

I am grateful for the supply of human growth hormone, which was prepared by Dr Anne S Hartree in the Department of Biochemistry, University of Cambridge, with the aid of a grant from the Medical Research Council to Prof F G Young, and to Armour Laboratories, U S A, for the supply of bovine prolactin

NADA KOVAČIĆ*
United Birmingham Hospitals,
Department of Clinical Endocrinology,
Birmingham and Midland Hospital for Women
Showell Green Lane,
Birmingham 11

* Fellow of the World Health Organization

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A Physiological Basis for the 'Optimum' Level of Energy Expenditure

THE level of oxygen consumption, compared with the maximum intake, at which an individual can work safely for 6–8 h, from month to month, has been variously defined The Max Planck Arbeitsphysiologie Institute group in Dortmund support the idea that the rate of oxygen intake equivalent to the "Pulsdauerleistungsgrenze" (a heart-rate of about 120 beats/min) is the safe limit The sole basis of this criterion is that, in their experience, the heart-rate continues to rise and does not remain in a steady state when the level of oxygen consumption has caused the heart-rate to exceed 120 beats/min The "Pulsdauerleistungsgrenze" is said to occur at about 30 per cent of the individual's maximum level of oxygen intake[1] Christensen *et al* [2] have a sounder, even if empirical, basis for their safe limit They found that men engaged on hard manual work, such as lumberjacking, on high financial incentives, do not, in general, work at a level which exceeds 50 per cent of the maximum level of oxygen intake

Neither of these criteria has a real physiological basis An objective and sound physiological basis for the optimum level of work would be the level of oxygen consumption, compared with the maximum oxygen intake, at which the rate of supply of oxygen by cardio-respiratory mechanisms to working muscles no longer balances the rate of expenditure of energy, and anaerobic metabolism commences in working muscles Increase in blood lactate, alone, is not a reliable index of anaerobic metabolism, Huckabee has demonstrated that 'excess' lactate, derived from the ratio of total-body lactate to pyruvate, is the only reliable measure of anaerobic metabolism[3] This communication deals with the level of oxygen intake, compared with the maximum, at which 'excess' lactate appears in 6 young Bantu males highly trained on a bicycle ergometer

These subjects were tested at work-levels from 2,000 ft lb /min to 11,000 ft lb /min on a bicycle

Table 1

Subject No	Estimated parameters					Break off		Max	% Max
	$\hat{\alpha}$	$\hat{\beta}$	$\hat{\gamma}$	$\hat{\delta}$	$\hat{\lambda}$	WR	O_2	O_2	O_2
319	0 555	0 229	−6 16	1 17	0 74	5,900	1 9	3 1	61 3
1209	0 578	0 216	−2 58	0 86	1 32	4,600	1 6	3 0	53 3
3557	0 507	0 228	−2 66	0 53	0 54	6,000	1 9	3 3	57 6
358	0 334	0 270	−1 11	0 27	0 60	6,300	2 0	3 5	57 1
3529	0 486	0 239	−2 08	0 64	1 10	5,000	1 7	3 8	44 7
3630	0 542	0 227	−4 71	0 96	1 10	6,100	1 9	3 3	57 6
Mean							1 83	3 33	55 3

ergometer, working for 7 min at lower levels of work and for 5 min above 9,000 ft lb /min Measurements of oxygen intake and blood pyruvate and lactate were repeated on 4 separate occasions at each work-level Blood samples were analysed for lactate by the method of Barker and Summerson[4] as modified by Hullin and Noble[5] and for pyruvate by the method of Friedemann and Haugen[6] Huckabee's equation was used for calculating 'excess' lactate Maximum oxygen intakes were determined by working the men up to their individual maximal efforts, which in all cases were above 11,000 ft lb /min

Representative values of oxygen intake (means of 4 measurements) are plotted against work-rates (WR) for one subject in Fig 1, and those for 'excess' lactate plotted against work-rate in Fig 2 Appropriate straight lines are fitted by least squares A derived curve of excess lactate against oxygen intake is given in Fig 3 and illustrates the point at which excess lactate increases sharply A similar procedure was used for the other 5 subjects to determine for each individual the level of oxygen intake at which 'excess' lactate increases Confidence limits have not been determined for the present estimated curves because the scatter of values about the mean is relatively wide, and insufficient values have been obtained at the turning point to justify the procedure of fitting confidence limits A further experiment is planned for this purpose The parameters of the equation for the various curves is given in Table 1 for the 6 subjects together with the level of oxygen intake at which excess lactate began to increase, the maximum oxygen intake of the subjects and the percentage of the maximum at which 'excess' lactate occurs The average of this last item for the six subjects is 55 3 per cent

It appears then that men who are highly trained, at both aerobic (or endurance work) and anaerobic oxygen intakes, can work to a level of about 55 per cent of their maximum oxygen intakes without anaerobic metabolism occurring in the body This percentage is in excess of the levels based on the previous criteria, which have no sound physiological basis, and may be due to the fact that these men were so highly trained Also it may well be that even under high financial incentives, men do not 'will' themselves in ordinary industrial situations to work at the optimum level of which they are capable The application of Huckabee's 'excess' lactate concept to the level of oxygen intake at which anaerobic metabolism occurs, compared with the maximum level of oxygen intake, opens the way to study objectively these two problems and many others which bear both

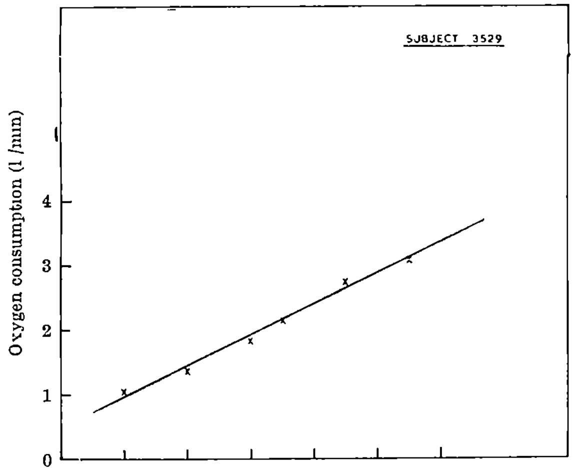

Fig 1

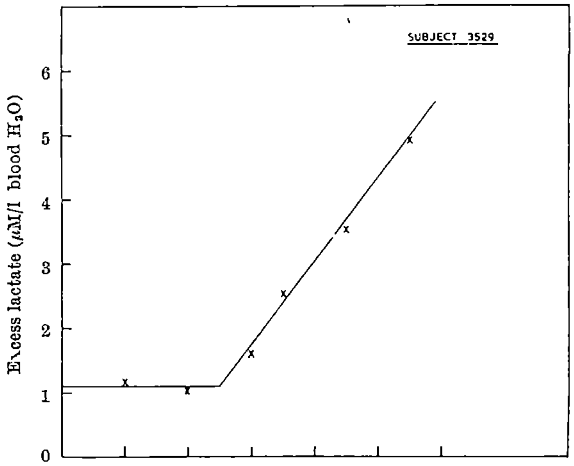

Fig 2

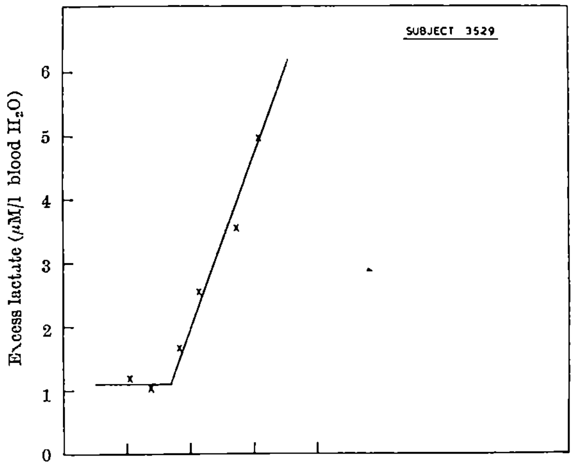

Fig 3

on athletic performance and, possibly of more importance, the question of human performance in industries where manual work is carried out

C H WYNDHAM
N B STRYDOM
C G WILLIAMS
M VON RAHDEN

Applied Physiology Laboratory,
Transvaal and Orange Free State Chamber of Mines,
Johannesburg

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PHARMACOLOGY

Antagonism of Bretylium by Sympathomimetic Amines

BRETYLIUM, an antihypertensive agent, inhibits the function of the adrenergic nerves without antagonizing the actions of epinephrine and *nor*epinephrine[1] It appears that bretylium blocks the terminal portion of the post-ganglionic sympathetic fibre by preventing the release of the neurohumoral agent Bretylium should then block the effects of certain agents which cause the release of the transmitter substance from the terminals of the sympathetic post-ganglionic fibres According to Burn and Rand[2], such sympathomimetic amines as amphetamine, ephedrine, tyramine and phenylethylamine elicit their action by releasing the neuro-humoral agent from the terminals of the nerve fibres However, Hukovic[3] and Azarnoff and Burn[4] have reported that the actions of tyramine are potentiated in isolated atrial preparations after pretreatment with bretylium

Cocaine is known to antagonize the actions of *TM*10 (ref 5) and bretylium[1] According to Wilson and Lang[6], the hypotensive effects of bretylium are not seen in hypertensive patients receiving amphetamine Although cocaine and amphetamine are dissimilar chemically, they do possess certain pharmacological properties in common One property exhibited by both compounds is the ability to potentiate the actions of the catecholamines, presumably by preventing their uptake by tissue[7] In this communication we record preliminary experiments on the interactions of bretylium and some agents that are known to possess this property

Cats of either sex, weighing 2–3 kg, were used in this work Pentobarbital, 30 mg/kg intraperitoneally, was used for anæsthesia and supplemental amounts were given intravenously as required The blood pressure was measured from the femoral artery using a Statham pressure transducer and a Gilson macropolygraph Contractions of the nictitating membrane were measured with a Grass strain gauge (Model *FT* 0 03) and a Gilson macropolygraph A tension of 5 gm was placed on the membrane All drugs were prepared in saline and injected into the femoral vein

The following drugs were used *dl*-amphetamine sulphate (350 μg/kg), tyramine hydrochloride (350 μg/kg), phenylethylamine (350 μg/kg), ephedrine (350 μg/kg), deoxyephedrine (300 μg/kg), bretylium (15 mg/kg in three divided doses) and imipramine (20 mg/kg intraperitoneal)

The general pattern of the experiments was as follows Control responses to nerve stimulation were obtained The stimulus used was a square wave of 7 V, 2 msec duration, 25/sec for 5 sec This was followed by the administration of the respective drug under study After an appropriate period of time following the drug, usually 5 min, bretylium was given

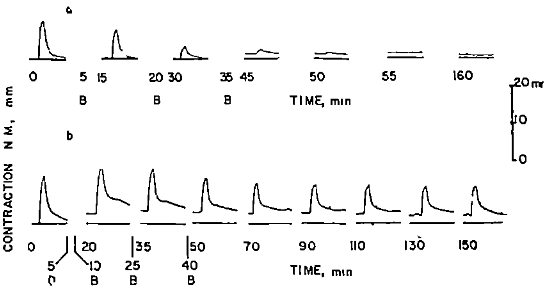

Fig 1 *a*, Response of nictitating membrane to nerve stimulation At times indicated by *B*, bretylium (5 mg/kg) was given Black dot indicates initial point of stimulus *b*, Responses of nictitating membrane to nerve stimulation At *D*, deoxyephedrine (350 μg/kg) was given, at *B*, bretylium (5 mg/kg) was given

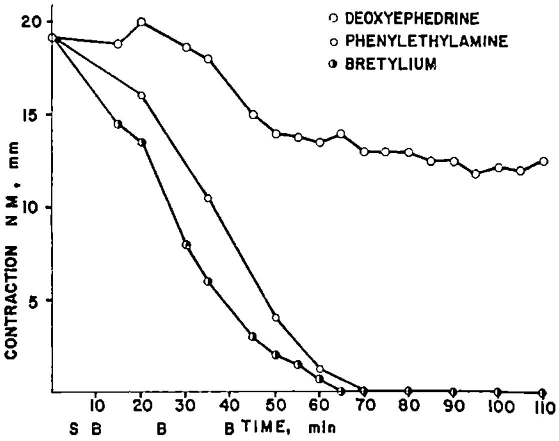

Fig 2 Response of nictitating membrane to nerve stimulation At *S*, sympathomimetic amine (350 μg/kg either deoxyephedrine or phenylethylamine) was given, at *B*, bretylium (5 mg/kg) was given Three animals were used with each drug

After bretylium, the nictitating membrane failed to respond to the stimulation of the sympathetic cervical nerve (Fig 1*a*) The effects of bretylium were apparent after the first administration, however, complete blockade did not occur until approximately 20 min after the last administration of bretylium This block was long lasting, in one experiment the block continued for 4 h, at which time the experiment was terminated In cases where bretylium was preceded by deoxyephedrine, the nictitating membrane did not show failure to nerve stimulation (Fig 1*b*) Although the response was diminished slightly after bretylium, it never demonstrated complete failure of response during the period of experimentation (up to 4 h) The other long-lasting sympathomimetic amines, amphetamine and ephedrine, also prevented the action of bretylium on the cervical nerve Imipramine, an antidepressant drug, also antagonized the actions of bretylium in a manner qualitatively similar to deoxyephedrine The short acting sympathomimetic drug, phenylethylamine, did not antagonize the action of bretylium on the cervical nerve (Fig 2) The response to nerve stimulation showed failure approximately 20 min after the last administration of bretylium This was similar to those experiments which received only bretylium Although it is not shown here, tyramine possessed properties that were qualitatively similar to phenylethylamine.

The antagonism of bretylium displayed by the long-acting sympathomimetic amines and imipramine may be explained by their common property, that is, the potentiation of catecholamines by the inhibition of their uptake by tissues. It is possible that during blockade of cervical nerve stimulation, bretylium allows only sub-threshold amounts of norepinephrine to be released. The agents that do antagonize bretylium may do so by preventing the tissue uptake of the subliminal amounts of norepinephrine released during stimulation. This would allow the concentration of norepinephrine to reach threshold levels, resulting in the contraction of the effector organ.

Hertting *et al.*[7] have shown that tyramine will also inhibit the tissue uptake of norepinephrine. The inability of tyramine to antagonize bretylium in our experiments may be explained on the basis of the differences in dosage. It is possible that, with higher doses, tyramine will also antagonize bretylium.

An alternative explanation for the antagonism of bretylium may be the competition for receptor sites on the terminal of the nerve. It is possible that the sympathomimetic amines and bretylium may occupy identical receptors or those in close juxtaposition. The long lasting amine may have a greater affinity for the receptor than bretylium. On the other hand, the short lasting amines occupy the receptor for a short time, allowing bretylium to act upon the terminals and resulting in blockade. However, the actions of imipramine cannot be explained on this basis. Since it potentiates the actions of catecholamines, the first explanation appears more tenable.

Further experiments are being carried out in this laboratory on the role of the inhibition of tissue uptake of norepinephrine as the possible mode of action of the antagonism of bretylium.

This work was supported in part by the U.S. Public Health Service pharmacology training grant (2*G*-109 (*C*2)). We thank Dr. S. T. Bloomfield of Burroughs Wellcome and Co., Inc., Tuckahoe, New York, for supplying the bretylium tosylate.

C. Matsumoto
A. Horita
Department of Pharmacology, School of Medicine,
University of Washington, Seattle 5.

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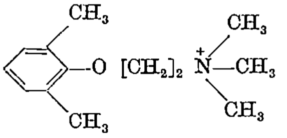
Amidines as Adrenergic Neurone Blocking Agents

2,6-Xylylether bromide (I), bretylium (II) and guanethidine (III) cause several similar adrenergic neurone blocking effects. The finding of similar properties in the benzyl- and phenoxy-ethylguanidines (IV), (V) and (VI) supported the view that quaternary ammonium and guanidine groups might have similar influences on pharmacological action[1]. These compounds are strongly basic and almost completely ionized at physiological *p*H values. It was therefore of interest to examine the compounds (VII) wherein high basicity was conferred by an amidine group. Several of these compounds showed powerful adrenergic neurone blocking properties, one of the most potent being *N*-[2-(hexahydro-1-azepinyl)ethyl]acetamidine (VII: $n=6$, $m=2$, $R=CH_3$) (*BW* 1113*C*60), which was prepared and tested as a stable non-deliquescent dihydrochloride[2].



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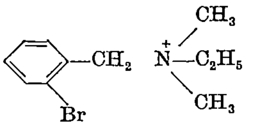

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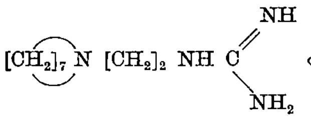

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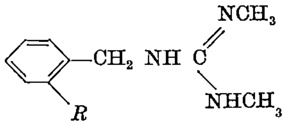
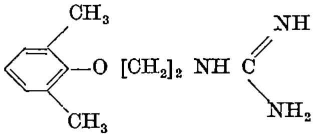
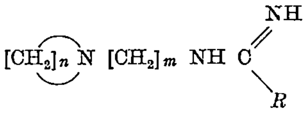


IV $R = H$
V $R = Cl$



VI



VII

In unanæsthetized cats, the dose of *BW* 1113*C*60 dihydrochloride required to relax the nictitating membranes was similar to that of guanethidine (3–10 mg/kg subcutaneously), but threshold doses of the compound administered daily for a week showed less cumulative action. In anæsthetized cats, intravenous doses of 1–3 mg/kg blocked responses of the nictitating membrane to postganglionic sympathetic nerve stimulation and showed a variety of other adrenergic neurone blocking effects. Experiments with nerves stimulated at different frequencies showed that the responses to low rates of stimulation were preferentially suppressed and that the curve relating the log frequency of stimulation and the nictitating membrane contraction was shifted to the right without depression of slope. This change is similar to that seen after guanethidine[3] and is distinct from that with bretylium, which depresses the slope of the response curve without first suppressing the effects of low stimulation frequencies[4]. Guanethidine, in contrast to bretylium, lowers the concentration of catechol amines in tissues with a rich adrenergic nerve supply, this depletion occurring after the blockade of the adrenergic nerves[5]. That the effect of *BW* 1113*C*60 on catechol amine stores may resemble that of guanethidine is suggested by the finding that the pressor amine content of the iris of cats was found to be reduced 24 h after subcutaneous injection of 10 mg/kg of either of these compounds. *BW* 1113*C*60 also showed prominent sympathomimetic effects resembling those of guanethidine and, similarly, large intravenous doses caused brief blockade of transmission in autonomic ganglia and neuromuscular junctions of voluntary muscle.

Comparison with related compounds in unanæsthetized cats showed that, as in the guanethidine series[6], the presence of a large heterocyclic ring was essential to high adrenergic neurone blocking activity, but in the present series of compounds the seven-membered ring produced the highest potency. Among compounds containing this ring, *BW* 1113*C*60 was more active than its homologue *N*-[3-(hexahydro-1-azepinyl) propyl]acetamidine (VII, $n=6$, $m=3$, $R=CH_3$), which in turn was more active than the propionamidine (VII, $n=6$, $m=2$, $R=C_2H_5$), and the formamidine (VII, $n=6$, $m=2$, $R=H$) (Repeated preparations of the formamidine gave poor elementary analyses, but its structure was supported by electrometric titration data provided by Dr A J Everett) The acyclic compound, *N*-(2-dipropylaminoethyl) acetamidine, was also relatively inactive

A L A Boura
F C Copp
A F Green

Wellcome Research Laboratories,
Beckenham, Kent

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HÆMATOLOGY

Effects of Corticosteroids on Coagulation of the Blood

Hypercoagulability of the blood in individuals who were receiving large doses of corticosteroids or adrenocorticotrophic hormone (ACTH) has been observed[1–3] This effect has also been observed in patients receiving ACTH for cirrhosis of the liver[4], a condition that usually depresses certain clotting factors For investigation of this problem heparin and antithrombic activity were evaluated, fibrinogen level and platelet counts were determined[1,4] Shortening of the clotting time was the most consistent coagulation finding in persons who were on corticosteroid or ACTH[1,4] The effect has been observed, particularly in siliconized tubes, in as little as 2–4 h after administration of 10 mg of prednisone[5,6] This effect was somewhat less marked after administration of 25–50 units of ACTH[5,6]

In the present investigations the following coagulation tests have been used Quick time[7], partial thromboplastin time[8], assays of prothrombin[9], factor V[9], and factor VII–X[9], thromboplastin generation test[10], assay of antihæmophilic factor[11], and fibrinogen[12]

We had the opportunity to perform coagulation investigations in a patient with Cushing's syndrome due to bilateral adrenal hyperplasia The Quick time of 9 2 sec (normal range 12–15 sec), partial thromboplastin time of 65 sec (normal range 60–100 sec), and minimum substrate clotting time in thromboplastin generation test of 9 0 sec (normal 9–11 sec) were unusually short The antihæmophilic factor-level of 420 per cent was quite high (normal range 50–200 per cent) The other coagulation tests were in the normal range Coagulation studies repeated 14 days after bilateral adrenalectomy, while the patient was maintained on 'Decadron' 2 mg and fluorohydrocortisone 0 1 mg daily, revealed a reversion to normal values (Quick time 13 2 sec, partial thromboplastin time 71 sec, minimum substrate clotting time in thromboplastin generation test 10 sec, antihæmophilic factor 110 per cent)

In vitro studies were carried out by adding prednisone in various concentrations to the patient's preoperative plasma and normal plasma and performing the Quick time and partial thromboplastin time on these mixtures No differences, compared to the normal saline controls, were observed

Coagulation studies were performed on four patients who had been on prednisone 60 mg a day for at least 10 days Three of these patients had rheumatic fever with carditis and one patient had idiopathic thrombocytopænic purpura Their ages ranged from 9 to 13 years Shortened Quick times ranging from 10 4 to 12 2 sec, short partial thromboplastin times ranging from 57 to 65 sec and short minimum substrate clotting times in the thromboplastin generation test ranging from 8 to 9 sec were noted Antihæmophilic factor-levels measured in the three patients with rheumatic heart disease ranged from 200 to 260 per cent In one of these, factors V, and VII–X were also slightly elevated (170 and 190 per cent respectively with normal range of 60–120 per cent) and in the other two, fibrinogen-levels were 460 and 504 mg per cent respectively (normal 200–400 mg per cent)

A 13-year-old patient with idiopathic thrombocytopænic purpura and a 7-year-old patient with rheumatic heart disease were examined before as well as 3 days after institution of oral prednisone, 60 mg daily Shortening of Quick time and partial thromboplastin time, as well as a rise of antihæmophilic factor (from 125 to 250 per cent and from 160 to 200 per cent respectively), were noted

The effect of corticosteroids on three patients with classic hæmophilia was then examined (Table 1) They were placed on prednisone 60 mg a day in 4 divided doses for 7 days Coagulation data were obtained before the medication was started, after 7 days of administration, and one week after the drug was discontinued

Table 1 Prednisone Effect on Classic Hæmophilia (severe, moderately severe and mild)

		Severe hæmophiliac aged 13 years			Moderate hæmophiliac aged 10 years			Mild hæmophiliac aged 12 years		
	Normal	Pre-steroid	1 week steroid treatment	1 week off steroid	Pre-steroid	1 week steroid treatment	1 week off steroid	Pre-steroid	1 week steroid treatment	1 week off steroid
Quick time	12–15 sec	13 6	13 0	13 2	13 5	12 5	13 5	13 0	11 2	11 2
Partial thromboplastin time	60–100 sec	237	89	300	174	106	218	94	95	95
Prothrombin	60–120%	48	100	180	76	110	82	74	72	80
Factor V	60–120%	63	83	60	68	76	90	58	40	38
Factor VII–X	60–120%	55	100	78	68	95	64	97	94	94
Antihæmophilic factor	50–200%	<1	<1	<1	2 5	5 2	1 6	7	13	7
Fibrinogen	200–400 mg %	254	257	270	284	282	294	—	230	200

In the severely affected patient, although the antihæmophilic factor level did not rise above 1 per cent of normal, the partial thromboplastin time decreased to normal range after one week of treatment There was a moderate rise of prothrombin, factor V, and factors VII–X One week after cessation of treatment, the partial thromboplastin time was back to the original value

In the moderately severe classic hæmophiliac under the same treatment, the antihæmophilic factor-level gradually increased over a week's period from 2 5 to 5 per cent with a disproportionate shortening of the partial thromboplastin time and some shortening of Quick time A week after cessation of treatment, these findings returned to original values

In the mild case of classic hæmophilia under corticosteroid treatment the antihæmophilic factor-level almost doubled from 7 to 13 per cent

These preliminary results seem to indicate that the antihæmophilic factor-level increases considerably in individuals under treatment with high doses of corticosteroids This rise occurs both in non-hæmophiliacs and in moderately or mildly affected classic hæmophiliacs Shortening of the Quick time in some of the steroid-treated individuals without concomitant rises in the coagulation factors of the second stage and in particular the disproportionate shortening of the partial thromboplastin time in the treated hæmophiliacs suggests that corticosteroids have some clot-promoting effect apart from raising the level of antihæmophilic factor The nature of this other clot-promoting principle is not elucidated

This investigation was supported by grants from the National Institutes of Health, *HTS* 5255, the John A Hartford Foundation, and the Wimpfheimer Foundation

SINASI OZSOYLU *
HERBERT S STRAUSS †
LOUIS K DIAMOND

Department of Pediatrics,
Harvard Medical School, and
Children's Hospital Medical Center,
Boston,
Massachusetts

* Trainee in Pediatric Hæmatology, Children's Hospital Medical Center, Boston, Mass

† Research Fellow in Pediatric Hæmatology, Children's Hospital Medical Center, Boston, Mass

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Absence of Hæmoglobin A_2 in an Adult

HÆMOGLOBIN A_2, the minor component of normal adult hæmoglobin, amounts to about 2 5 per cent of the total hæmoglobin, it can be distinguished from the major adult component, hæmoglobin A_1, by its electrophoretic and chromatographic properties

Recent investigations have shown that hæmoglobin A_2 consists of two types of polypeptide chains the α-chain, identical with the α-chain of hæmoglobins A_1 and F, and the δ-chain, which differs in constitution from the corresponding chains of hæmoglobin A_1 (the β-chain) and F (the γ-chain)[1,2] A two-fold increase of the normal percentage of hæmoglobin A_2 occurs in the usual type of β-chain thalassæmia trait, prevalent in Greece[3], while a decrease is observed in hæmoglobin-H disease A decrease of the A_2 fraction down to 1 per cent has been observed as a reversible phenomenon in iron-deficiency anæmia[4] Traces of hæmoglobin A_2 can be detected in cord blood hæmoglobin by the application of special techniques

Qualitative alterations of hæmoglobin A_2 have been reported in a few cases[5,6], these, similarly to the variants of the hæmoglobins A_1 and F, are considered variants of hæmoglobin A_2

We wish to report the complete absence of hæmoglobin A_2 in a 60-year-old man, he belongs to a family several members of which carry a β-chain thalassæmia trait unassociated with an elevated hæmoglobin A_2 He had been subjected to gastrectomy three months previously, but there was no history of loss of blood The laboratory findings (hæmoglobin 11 2 gm /100 ml, red cells 6,500,000/mm ^{3}, volume of packed red cells 37 5 per cent, pronounced microcytosis and poikilocytosis, moderate hypochromia, serum iron 90 μgm /100 ml) were typical of thalassæmia trait Hæmoglobin electrophoresis disclosed the presence of hæmoglobin A_1 only, hæmoglobin F amounted to 2 5 per cent

The complete absence of hæmoglobin A_2 was repeatedly demonstrated in this case on samples taken at intervals prior to, and also after, intravenous treatment with iron Starch-gel electrophoresis and agar electrophoresis at *p*H 8 4, in conjunction with intense benzidine staining, were used to demonstrate the absence of A_2 (Fig 1) Hæmoglobin solutions from a normal adult containing a low percentage of hæmoglobin A_2 (1 5 per cent) were diluted serially and run simultaneously as controls The hæmoglobin A_2 fraction was well demonstrated in the 100-fold dilution of the control sample, which corresponds to a percentage of 0 015 A_2 in an undiluted sample Absence of a band corresponding either to normal or abnormal A_2 fraction was also noted by chromatography on 'Amberlite *IRC*–50' and by starch block electrophoresis

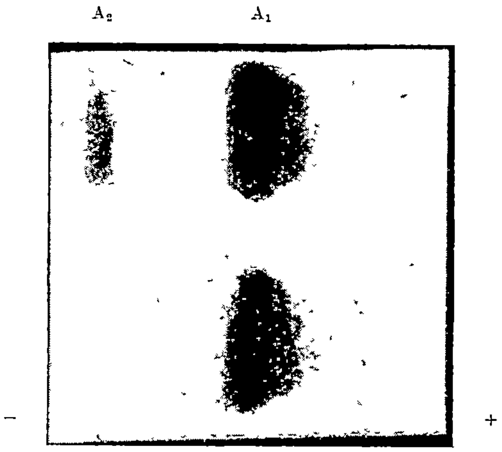

Fig 1 Starch-gel electrophoresis, discontinuous buffer system at *p*H 8 4, benzidine stain Above, normal adult control, below, sample lacking hæmoglobin A_2

Among this individual's offspring from an entirely normal wife, there were both normal persons and persons with thalassæmia trait, all having a percentage of hæmoglobin A_2 within normal limits, none displayed complete absence of hæmoglobin A_2

Present-day evidence indicates that the synthesis of the δ-chain is under separate genetic control[2], the genes for β-chains and δ-chains being probably linked[5] Absence of normal hæmoglobin A_2 is expected to occur in individuals homozygous for an electrophoretically detectable α-chain or δ-chain variant, in these cases, the variant hæmoglobin replaces normal A_2[6,7] Complete absence of A_2 has been further observed in a case of homozygosity for hæmoglobin 'Pylos'[8] In the case under discussion, no evidence was obtained that another component had replaced hæmoglobin A_2

So far as we are aware, this is the first observation of an adult having almost exclusively hæmoglobin A_1 and entirely lacking hæmoglobin A_2 In the light of the known disturbances in hæmoglobin synthesis, we assume that the complete inability of this individual to synthesize δ-chains is genetically determined On one hand, this person carries a type of thalassæmia affecting apparently the synthesis of both β- and δ-chains, such a type of abnormality, transmitted as a single gene defect, has been assumed to exist in certain cases of thalassæmia trait presenting normal percentage of A_2, observed elsewhere[9] as well as here[3,11] On the other hand, the complete absence of the A_2 fraction, while the offspring with thalassæmia trait presents normal percentage of A_2, make it necessary to assume that, in this particular case, an additional defect concerning the other δ-chain allele is also present

The possibility of the existence of a gene suppressing the synthesis of δ-chains has already been considered[8], the action of such a gene may be visualized as similar to the action of the known thalassæmia genes on either α- or β-genes[10] It is known that β-chain thalassæmia genes at least may lead to complete absence of β-chains[11,12] In the case described here, we assume that the genetic lesion causing suppression of both β- and δ-chains and leading to the hæmatological picture of thalassæmia trait is associated to a gene affecting solely δ-chain production, this results in failure to synthesize hæmoglobin A_2 Both healthy and thalassæmic offspring are expected to present hæmoglobin A_2, having of necessity inherited a normal δ-chain allele from the mother

In view of the fairly wide range of the normal A_2 values, persons heterozygous for such an isolated δ-chain anomaly (a δ-chain thalassæmia) may not easily be detected by quantitation of hæmoglobin A_2 Furthermore, the reduction or the complete absence of the small quota of the A_2 fraction may not be expected to give rise, *per se*, to alterations of the red cell morphology However, the simultaneous occurrence of such a gene and of the gene for typical (high A_2) β-chain thalassæmia in one person may prevent hæmoglobin A_2 from rising over normal values Should these two genes be in the coupling phase, their association is likely to manifest itself as a thalassæmia trait, without increased hæmoglobin A_2, transmitted as a single gene defect The possibility has to be considered that such may be the situation in some of the thalassæmia traits which are known to be β-chain thalassæmias but which do not present an elevated A_2 fraction

The complete absence of hæmoglobin A_2 in an individual carrying exclusively hæmoglobin A_1 provides further proof that hæmoglobin A_2 is not a by-product of the synthesis or catabolism of A_1

This investigation was supported by Research grant No *H*–4541 of the National Institutes of Health, U S Public Health Service

PHAEDON FESSAS
G STAMATOYANNOPOULOS

Hæmatology Section and Laboratory,
Department of Clinical Therapeutics,
Alexandra Hospital,
Athens

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RADIOBIOLOGY

A Correlation between Radiation Tolerance and Nuclear Surface Area

FROM results of morphological investigations it was concluded[1] that the Feulgen-stained material in somatic cell nuclei is distributed peripherally and it was suggested that this peripheral zone consists of two concentric zones in close proximity Sparrow and Miksche's data[2] on a correlation of nuclear volume and DNA content with higher plant tolerance to chronic radiation seem to offer evidence that such distribution of nuclear DNA is also found in the living somatic plant cell

These investigators determined the dose (r /day) required to produce severe growth inhibition in 23 species of plants and found a linear relationship between the logarithm of the nuclear volume, the volume being taken as a measure of the DNA-content and determined by micrometry in 10μ thick sections, and the logarithm of the dose

They give data in their Table 1 for six species from which the following equations can be calculated

$$\log(\text{nuclear volume}) = 4\ 42 - 0\ 82 \log \text{dose} \qquad (1)$$

and

$$\log(\text{nuclear volume}) = 1\ 66 + 0\ 66 \log \text{DNA} \qquad (2)$$

where log DNA denotes the average nuclear DNA-content, determined by chemical extraction The photometrically determined DNA-content values given in Table 1 have been disregarded, as it has been demonstrated[3] that the product of absorbance and nuclear area is not a direct measure of mass

If all the nuclear DNA is distributed in two peripheral zones of such close proximity that the difference in surface area between the two zones is negligible and therefore each zonal surface area $\simeq$ nuclear surface area, we have from (2)

$$2(\log \text{nuclear surface area}) = 1\ 33(\log \text{nuclear volume}) = 2\ 21 + 0\ 88 \log \text{DNA} \qquad (3)$$

The numerical value of the slope is thus only 12 per cent lower than the expected value of 1·0. From eqv. (1)

$$2\,(\text{log nuclear surface area}) = 1{\cdot}33\,(\text{log nuclear volume}) = 5{\cdot}88 - 1{\cdot}09 \text{ log dose} \quad (4)$$

The value of the slope in (4) is also lower—by 9 per cent—than the expected value of 1·0. When considering the very small numbers of experimental observations and considering that the deviations are both in the same direction and of the same magnitude, the agreement is remarkably good.

For the 23 species, Sparrow and Miksche found

$$\text{log nuclear volume} = 4{\cdot}07 - 0{\cdot}73 \text{ log dose} \quad (5)$$

from which we obtain

$$2\,(\text{log nuclear surface area}) = 5{\cdot}41 - 0{\cdot}97 \text{ log dose} \quad (6)$$

where the value of the slope is as near the theoretical value of 1·0 as can be expected. This demonstrates that the product of log surface area and log dose is constant, and indicates that each zone is equally sensitive towards radiation and implies that each (diploid) interphase nucleus for the greater part of this period carries a DNA-charge of $2 \times 2n$.

SIMON IVERSEN

Cancer Research Department,
Royal Beatson Memorial Hospital,
Glasgow, C.3.

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Effect of Radiation on the Electrophoretic Mobility of Ehrlich Ascites Tumour Cells

STEIN *et al.*[1] have shown that low doses of radiation cause a small increase in the negative electrophoretic mobility of Ehrlich ascites tumour cells.

In the course of our investigations of the effect of X-irradiation on Ehrlich ascites tumour cells we have measured the electrophoretic mobility of tumour cells withdrawn from *C3H* mice (for source, see ref. 2). Nine days after implantation of the tumour the animals were given a whole-body dose of 1,400 rads at a rate of 350 rads/min and killed 24 h later. After withdrawal from the mouse, the tumour cells were washed three times in 0·145 N sodium chloride, prior to a determination of the electrophoretic mobility (for method, see ref. 3).

An experiment involved determining the mobility of tumour cells removed from one irradiated mouse and one control mouse (that is, not irradiated). The electrophoretic mobility of cells withdrawn from the same mouse was reproducible but considerable variation of mobility was shown with cells from different mice. Bangham *et al.*[4] have commented upon the range of mobility values observed for tumour cells from non-irradiated mice.

The negative electrophoretic mobility of tumour cells from irradiated mice and control mice is 1·39 ± 0·03 (*S.E.*) μ/sec/V/cm and 1·41 ± 0·04 (*S.E.*) μ/sec/V/cm respectively, the quoted mobilities are mean values obtained from the results of 19 experiments. Applying Student's *t* test to the distribution of results indicates that there is no significant difference between the two mean values. Hence, we must conclude that the radiation has no measurable effect on the electrophoretic mobility of the tumour cells.

A different source of tumour material giving rise to a different electrophoretic pattern may be the cause of the disparity between the results of our experiments and the observations previously reported[1].

We thank Dr. G. M. Bell and Dr. L. R. Shenton for discussion, Mr. D. Porteous for preparation of the irradiated mice, and the British Empire Cancer Campaign for a supporting grant to one of us (S. H.).

SHEILA HOLLINGSHEAD

Department of Chemistry,
Manchester College of Science and
Technology,
Manchester 1.

DOREEN THOMASON

Christie Hospital and Holt Radium Institute,
Manchester 20.

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Dependence of Radiation Diarrhœa on the Presence of Bile in the Intestine

SYMPTOMS after exposure of the abdominal region of rats to X-radiation doses of 1,000 r., or greater, almost invariably include diarrhœa beginning at 3 days after X-ray irrespective of the dose. If death does not occur within the next 3 or 4 days there is a rapid improvement and the diarrhœa stops. Both the early onset and rapid improvement are usually attributed to the short turnover-time of the epithelial cells of the intestine. However, when the biliary flow was interrupted by ligation or cannulation of the common bile duct the incidental observation was made that diarrhœa did not occur[1,2].

Albino male rats weighing about 300 g were prepared by placing polythene cannulæ in the proximal duodenum and when necessary in the common bile duct. Then 2 days later the abdomina of these rats were exposed to 1,000 r. of 250 kV X-rays. When the bile was diverted from the lumen of the intestine by cannulation of the bile duct, diarrhœa did not develop even when a volume of isotonic saline equal to the volume of bile (15 ml./day) was administered intraduodenally (Table 1). However, in irradiated cannulated rats diarrhœa occurred as in unoperated controls, when bile was injected daily into the duodenum in quantities comparable to the normal daily output of bile. This occurred whether the bile had been collected from irradiated or non-irradiated rats. Diarrhœa also followed daily intraduodenal injections of bile salts (50 mg taurocholate or tauroglycocholate or 6 mg deoxycholate, each in 15 ml. of 0·9 per cent sodium chloride).

Table 1. INFLUENCE OF BILE ON DIARRHŒA INDUCED BY EXPOSURE OF THE ABDOMEN TO 1,000 R. OF X-RADIATION

Bile duct cannula	Injection into duodenum	Initial	No. of rats Diarrhœa at 3 and 4 days	Deaths at 7 days
No	Saline	10	10	8
Yes	Saline	19	1	16
Yes	Rat bile	8	8	7
Yes	Saline + bile salts	14	14	12

It is apparent from these results that the bile and, more specifically, the bile salts, are intimately involved in the development of radiation-induced diarrhœa, and that the diarrhœa cannot be due merely to a radiation-induced defect in the cellular turnover of the intestinal epithelium. Full details of these and other experiments to elucidate the mechanism by which bile salts act on the irradiated intestine will be reported later

This work was supported in part by a post-doctoral fellowship grant No *AF*-12,198 from the U S National Institute of Arthritis and Metabolic Diseases

MAURICE F SULLIVAN

Medical Research Council,
Radiobiological Research Unit,
Harwell, Didcot

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Comparative X-ray Sensitivity of *Tribolium confusum* and *T. castaneum* (Coleoptera : Tenebrionidae) at Different Developmental Stages during their Life-cycle

THE X-ray sensitivities of two ecologically similar species of flour beetles, *Tribolium confusum* (Chicago 'Standard' strain) and *Tribolium castaneum* (Brazil *C*I strain), were compared at eight different ages which span the life-cycle

The X-ray was delivered at 1 kr /min by a General Electric Maxitron generator operated at 30 m amp, 250 kV, 0 25 mm copper + 1 0 mm aluminium filtration and a 2 5 in anode-subject distance Below 10 kr , doses were given in 0 5 kr increments, while higher doses were given in 10 kr increments Groups of 50–100 individuals per life-cycle stage were exposed at each dose Culture conditions were 29 ± 2 C and 70 ± 10 per cent relative humidity

A dose was assumed to induce sterility if no progeny developed, and lethality if exposed individuals failed to mature into adults or if they died within 3–4 weeks after eclosion

The radiosensitivities for both species are compared in Fig 1 The egg (1–3 h after oviposition) was the most radiosensitive stage At this highly mitotic period, 1–2 kr was lethal In subsequent life-cycle stages, lethal doses were higher than sterilizing doses *T confusum* was consistently more X-ray sensitive than *T castaneum* Radiation sensitivity remained more or less constant during the larval stages except the tolerance of *T castaneum* decreased at the 15-day old larval stage The marked radioresistance in terms of lethality of *T castaneum* pupæ persisted through adulthood Young pupæ of *T confusum* were less radioresistant than old pupæ or adults

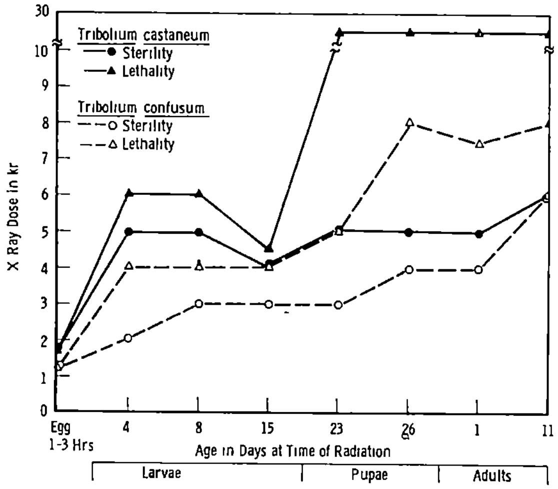

Fig 1 Comparative sterilizing and lethal X-ray doses at various development stages in the life-cycles of *Tribolium confusum* and *T castaneum*

The differential radiosensitivity of gametes in adults (more sensitive in 1 than in 11-day old adults) corroborates Dick's histological observation[1] that *T confusum* females were not sexually mature until the fourth or fifth day of adult life A differential radiation response of reproductive tissues was also observed in *Habrobracon*[2–4] and *Drosophila*[5]

Experiments are planned to explain the species differences in response to radiation Perhaps the one more pair of autosomal chromosomes in *T castaneum*[6], nuclear volumes, DNA contents, oxygen consumptions, or specific chromosomal (genic) sensitivities, contribute to the differential radiotolerances between these species of flour beetles

This work was carried out under contract *AT*–(45–1)–1350 between the U S Atomic Energy Commission and the General Electric Co

H E ERDMAN

Biology Laboratory,
Hanford Laboratories General Electric Co ,
Richland,
Washington

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BIOLOGY

Relations between Day-length and Annual Patterns of Plant Development

THE relatively unchanging annual sequence of anthesis of plant species which occurs naturally each year could be explained in terms of a primary effect of light duration with which secondary factors such as temperature and rainfall interact, provided all species were sensitive to the effects of light duration with respect to anthesis, that is, if there were no completely day-neutral plants

The tomato (*Lycopersicum esculentum*) has long been regarded as a typical day-neutral plant with respect to flowering Results from work at this Institute suggest that there is a hyperbolic relation between day-length and the age of the tomato at first anthesis of the form shown in Fig 1 by the continuous line, the longer the day-length the younger the plant at anthesis This would suggest that the tomato is basically a weak long-day plant, and that it is not day-neutral The interaction of a hyperbolic response curve of this type with the parabolic curve of annual variation in day-length at the latitude of the Institute would mathematically produce an annual pattern of age at anthesis of the skew form shown in Fig 2 by the continuous line The mathematically derived curve was found to correspond with the experimentally determined annual pattern of anthesis I thank my colleague, Mr D Cooke, for the calculation of the curves in Fig 1 and 2

By recalculation of the data on the anthesis of kenaf (*Hibiscus cannabinus*) collected by Walker, Sierra, Bedia and Hopp[1] it can be shown that there is a hyperbolic relation between day-length and the age of kenaf at anthesis of the form shown in Fig 1 by the broken line, the shorter the day-length the

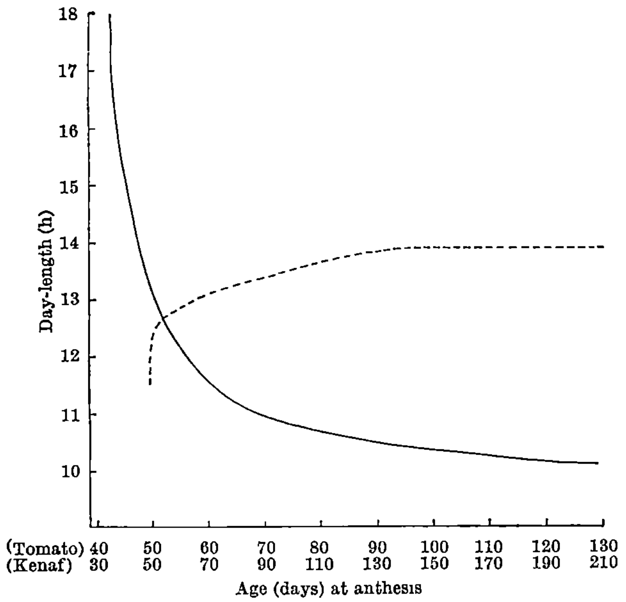

Fig 1 Relation between age at anthesis and day-length

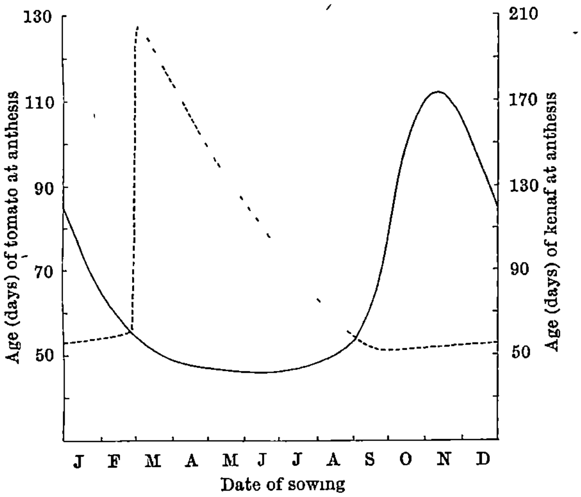

Fig 2 Seasonal patterns of the age at anthesis for tomato and kenaf

younger the plant at anthesis This would suggest that kenaf is basically a short-day plant with respect to anthesis The interaction of a hyperbolic response curve of this type with the parabolic curve of annual variation in day-length at the latitude of Santiago de las Vegas, Cuba, produces an annual pattern of age at anthesis of the skew form shown in Fig 2 by the broken line Each of the curves in Figs 1 and 2 are defined by 52 points and relate to weekly sowings in natural daylight at the two places concerned

These data suggest that there may be no completely day-neutral plants, but that apparently day-neutral plants may be included in two main groups of (*a*) long-day plants with hyperbolic response curves of the form shown by the continuous line in Fig 1; (*b*) short-day plants with hyperbolic response curves of the form shown by the broken line in Fig 1 The degree of sensitivity to day-length would be determined by the equation of the response curve For the long-day group the higher the value of the day-length asymptote the more sharply defined would be the long-day response, the lower the value the less sharply defined the long-day response For the short-day group the lower the value of the day-length asymptote the more sharply defined would be the short-day response, the higher the value the less sharply defined the short-day response Thus apparently day-neutral plants would be basically either long-day plants with low day-length asymptotes or short-day plants with high day-length asymptotes, and there would be two continuous series of species ranging from strongly short-day (or long-day) to weakly short-day (or long-day)

The interaction of these two continuous series of hyperbolic day-length responses with the parabolic annual variation in day-length at a given latitude would primarily determine the unchanging annual sequence of anthesis of plant species at that latitude, although other factors such as temperature and rainfall would interact with the hyperbolic response curves

A J Cooper

Glasshouse Crops Research Institute,
Worthing Road,
Littlehampton,
Sussex

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Effects of Coconut Milk and Kinetin on the Development of the Protonema and Bud Formation in *Pohlia nutans*

In previous experiments[1] on the effects of kinetin on the development of the protonema and the formation of shoot-buds in *Pohlia nutans* (Hedw) Lindb , it was found that buds were formed earlier in cultures containing kinetin and it seemed that in this respect its action was similar to red light[2] Buds were not produced on the protonema growing on a basal medium under blue light Since certain responses of higher plants to coconut milk are analogous to those induced by kinetin, for example, the induction of cell-division in mature tissue and the differentiation of a complete plant from a mass of such dividing cells, it seemed that it might be of interest to study the effects of coconut milk alone and in combination with kinetin on the development of the protonema and the initiation of shoot-buds in *Pohlia nutans*

Aseptic cultures were prepared from small protonema inocula on modified Voth's medium supplemented with various concentrations of coconut milk, coconut milk plus 2,4-dichlorophenoxy-acetic acid, kinetin, kinetin plus coconut milk, kinetin plus indoleacetic acid and indoleacetic acid alone The cultures were grown under fluorescent light ('Natural') giving 3,500 lux at the level of the cultures for 17 hr in 24-hr cycle The temperature was regulated at 24–26° C Some cultures with or without kinetin (0 1 mgm /l) were kept at the same temperature under low intensity of fluorescent light (700 lux) and blue light (400–570μ wave-length) giving 292 4 ergs/cm ^{2}/sec and 620 ergs/cm ^{2}/sec respectively at the level of the cultures for the same light hours Some of the results obtained are summarized in Table 1

It will be seen from Table 1 that coconut milk when supplied at 15 per cent concentration to the basal medium resulted in complete inhibition of bud formation in *Pohlia nutans* and in the decrease of radial growth of the protonema, but when supplied at

Table 1 Effects of Growth Substances on the Growth of the Protonema on Cell-Size and on Bud Formation in *Pohlia nutans*

Growth substances	Protonemal diam (mm)*	No of buds†		Cell-width (W) and cell-length (L) (μ)‡
Coconut milk, 15 per cent	16	nil	W	32— 36— 41
			L	135—166—193
Coconut milk, 1 per cent	48	6	W	32— 35— 39
			L	89—123—168
Kinetin, 0 1 mgm /l	39	98	W	27— 29— 30
			L	75—122—149
Kinetin, 0 01 mgm /l	38	32	W	28— 33— 36
			L	75—109—139
Kinetin (+ 15 per cent coconut milk), 0 1 mgm /l	14	nil	W	32— 35— 39
			L	143—198—246
Kinetin (+ 15 per cent coconut milk), 1 0 mgm /l	30	15	W	21— 26— 33
			L	111—182—239
Kinetin (+ 15 per cent coconut milk), 2 0 mgm /l	28	51	W	18— 24— 32
			L	96—168—218
Indoleacetic acid, 10 0 mgm /l	13	nil	W	25 — 27—32
			L	43— 89—150
Kinetin (+ 10 0 mgm /l IAA), 1 mgm /l	13	4	W	24 —29— 34
			L	61—121—178
Kinetin (+ 10 0 mgm./l IAA), 2 mgm /l	24	30	W	26— 29— 32
			L	71—114—185
Control	41	14	W	32— 34— 36
			L	72—114—145

* Mean measurements of three similar agar cultures grown for 27 days

† Mean of three similar agar cultures of 27 days old

‡ Mean measurements of 20 cells from 20 different prostrate protonemal filaments taken at random from 47 days old agar cultures, figures in the following sequence minimum—mean—maximum

1 per cent concentration produced lesser number of buds than the control The inhibitory effect of coconut milk was overcome by the inclusion of 1 or 2 mgm /l kinetin into the basal medium containing 15 per cent coconut milk Table 1 also shows that almost similar effects were observed in cultures containing 10 mgm /l indoleacetic acid with or without kinetin Measurements of cell-size of protonemal filaments growing in different cultures containing the growth substances revealed that cells of maximum size were produced in a basal medium supplemented with 15 per cent coconut milk and of minimum size in a basal medium supplied with 10 mgm /l indoleacetic acid Addition of kinetin at 1 mgm /l concentration to the medium containing 15 per cent coconut milk resulted in the decrease of cell-width but produced a synergistic effect on cell-length of protonemal filaments, whereas the addition of the same to the medium containing 10 mgm /l indoleacetic acid could not affect cell-width but could increase cell-length

Experiments on the effects of low intensity of fluorescent light and blue light showed that buds were formed in cultures containing kinetin but not in the control

The findings with coconut milk are of interest in that not only the cell-size and cell-division are affected but also the organized development of the protonema remains least disturbed Coconut milk even in combination with 6 mgm /l 2,4-dichlorophenoxyacetic acid failed to produce abnormal protonemal growth, and in cultures containing 6 mgm /l 2,4-dichlorophenoxyacetic acid alone *Pohlia nutans* not only grew but also was characterized by bud formation Lal[3] observed the formation of callus-like protonema in *Physcomitrium coorgense* growing in a basal medium supplemented with 10 per cent coconut milk That 15 per cent coconut milk induces cell-enlargement but limits rate of cell-division to a certain extent will be seen when we correlate the extent of radial growth of the protonema with the cell-size of protonemal filaments of each treatment Otherwise we can expect the greatest radial growth in cultures containing 15 per cent coconut milk Furthermore, in the presence of kinetin, coconut milk and indoleacetic acid affected cell-width and cell-length of protonemal filaments in different manners

It may be inferred from the results reported here that the formative effect of kinetin on bud formation in *Pohlia nutans* has been further emphasized in the presence of low intensity of white light, of blue light and of coconut milk, which are found to interfere with bud formation The effects of 'active' and 'neutral'[4] fractions isolated from coconut milk and their effects in combination with kinetin may further illustrate the action of coconut milk on the development of the protonema and the initiation of shoot-buds in *Pohlia nutans*

G C Mitra
L P Misra
K N Kaul

Tissue Culture Laboratory,
National Botanic Gardens,
Lucknow,
India

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Biosynthetic Pathways in Nutritionally Deficient Mutants of *Marchantia polymorpha* L.

Nutritionally deficient mutants of micro-organisms have provided valuable tools for investigating biosynthetic pathways Relatively few examples of such mutants are known in 'higher' plants[1,2] Methods have been devised to obtain nutritionally deficient mutants of a liverwort species, *Marchantia polymorpha*

Gemmæ are irradiated with X-rays and grown on a solid (agar) defined medium[3] at 17°–22° C A thallus arises presumably from a single apical cell at each of the two notches, at opposite ends of a gemma Thalli are observed at the end of the second week and scored either as normal (35 units long, measured by means of an ocular micrometer and a dissecting microscope) or as impaired (15 units or less) The latter thalli are excised and transferred to solid medium supplemented with 0 3 per cent yeast extract After three weeks on this medium, each thallus is diced, and fragments are added to defined yeast extract-medium to determine differences in growth This process is repeated twice Presumptive, nutritionally deficient mutants are then tested for growth on defined medium supplemented with vitamins, growth factors, purine, pyrimidines, or amino-acids, using techniques used for micro-organisms[4]

A population of 1,588 gemmæ was given 15,000 r X-rays A sample of 279 impaired thalli were screened to find nutritionally deficient mutants Of the 12 mutants which were found, 4 required either arginine or methionine or both Two mutants required arginine and one, methionine, a fourth mutant required either arginine or methionine Two arginine-deficient mutants were also able to use citrulline or ornithine and the third mutant, only citrulline Of the two methionine-requiring mutants, one was able and the other unable to use cysteine The poor growth of the latter mutant was noted visually The fresh and dry weights of the 4 mutants on defined medium and on defined medium containing specific supplements are summarized in Table 1 These results suggest that the ornithine cycle as well as the biosynthetic pathway

Table 1 GROWTH OF FRAGMENTS OF NUTRITIONALLY DEFICIENT MUTANTS OF *Marchantia polymorpha* REQUIRING EITHER ARGININE OR METHIONINE OR BOTH ON DEFINED MEDIUM WITH OR WITHOUT PRECURSORS OF THESE AMINO-ACIDS

Mutant No	Supplement	Fresh weight (mgm)			Dry weight (mgm)		
		Defined medium	+Supplement	Difference	Defined medium	+Supplement	Difference
44	arginine	71 05	167 69	96 64	1 84	4 95	3 11
44	citrulline	71 05	165 28	94 23	1 84	3 58	1 74
44	ornithine	71 05	192 12	121 07	1 84	5 09	3 25
245	arginine	76 32	144 40	68 08	1 14	3 65	2 51
245	citrulline	76 32	136 08	59 76	1 14	2 36	1 22
245	ornithine	76 32	70 22	−6 10	1 14	1 01	−0 13
55	arginine	35 29	93 96	58 67	0 74	2 30	1 56
55	citrulline	35 29	118 49	83 20	0 74	3 83	3 09
55	ornithine	35 29	96 44	61 15	0 74	2 54	1 80
55	methionine	35 29	56 62	21 33	0 74	1 49	0 75
55	cysteine	35 29	204 61	169 32	0 74	5 52	4 78

Five fragments of each nutritionally deficient mutant were added to a plate containing 35 ml defined medium and 1 ml of stock of supplement arginine, 0 2 mgm /ml , citrulline, 0 2 mgm /ml , ornithine, 0 2 mgm /ml , methionine, 0 2 mgm /ml , and cysteine, 0 4 mgm /ml

leading to methionine in *M polymorpha* are similar to those reported in micro-organisms

M W MILLER
E D GARBER
P D VOTH

Department of Botany,
University of Chicago

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Reduction of Plant Transpiration by Cetyl Alcohol

SURFACE monomolecular films of cetyl alcohol ($CH_3(CH_2)_{14}CH_2OH$) have been shown to reduce losses by evaporation from open water surfaces[1], and this application has had some successful use in Australia[2] There recently appeared a report[3] that the mixing of cetyl alcohol with the soil in which corn (*Zea mays*) plants were growing reduced their loss of water by transpiration by 40 per cent without reducing yield If it was confirmed that some substance could be taken up by plants which reduced their water needs without impairing growth, such a finding would have great potential agricultural importance. It was therefore decided to repeat Roberts's experiment on a pot scale, measuring the effects of cetyl alcohol on growth, transpiration rate and photosynthetic capacity

Wheat seedlings (var Sherpa) were raised under growth room conditions in pots containing 75 g of 'Vermiculite' Cetyl alcohol (obtained from British Drug Houses) was supplied at two levels, namely, 1 and 5 per cent of the dry weight for 'Vermiculite' in each pot There were twelve plants for each treatment plus eight controls Over the 20-day growing period pots were watered on two occasions with 100 ml of half-strength Hoagland solution, and daily with tap water The growth room was illuminated from a combination of warm white fluorescent tubes and incandescent strip lamps, and gave a light intensity of 1,200 ft candles at the base of the plants The carbon dioxide concentration was 400 p p m , air temperature 21° C Transpiration was measured gravimetrically in an air stream of a velocity of 7 ft /sec in the growth room The relative humidity was 80 per cent Net photosynthesis was determined at a carbon dioxide concentration of 300 p p m by measuring, with an infra-red gas analyser, the carbon dioxide consumed by the plant from a recirculating gas stream Light intensity was saturating (13 6 × 10^4 ergs/sec/cm^2 from high-pressure mercury-vapour lamps), leaf temperature was 25° C. After determination of leaf area, all plant material was dried for three days at 80° C in an oven with forced draught

The addition of 1 and 5 per cent cetyl alcohol induced foliar chlorosis and significantly reduced (P = 0 05 and P = 0 01) the growth of the wheat plants (Fig 1) However, the transpiration-rate on a leaf area basis was also reduced, there being a significant reduction (P = 0 01) with an application of 5 per cent cetyl alcohol, but the depression with 1 per cent was not significant (P = 0 05) (This effect has been reproduced in a second experiment) The rate of carbon dioxide fixation, on a leaf-area basis, was significantly reduced (P = 0 01) by both rates of

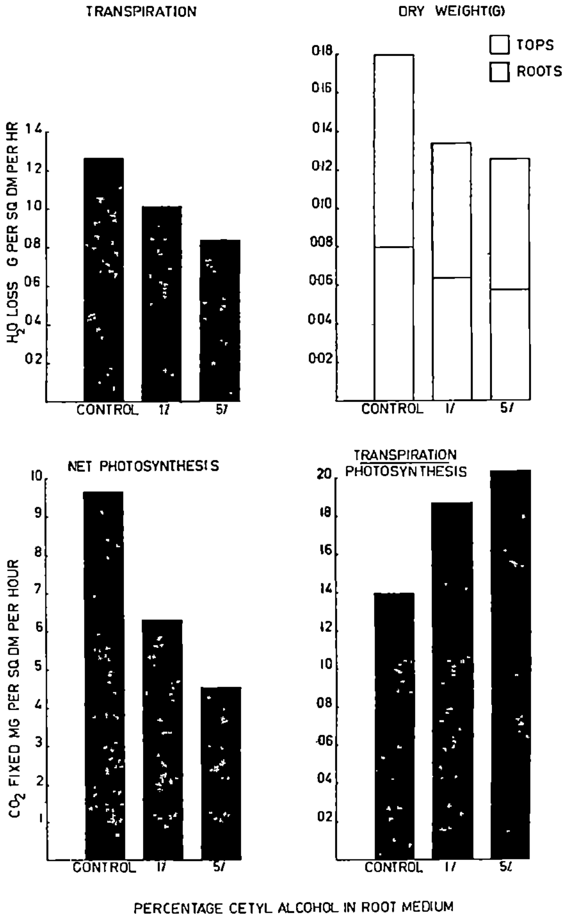

Fig 1

application of cetyl alcohol The resultant of these two effects on rates of transpiration and photosynthesis can be seen in the ratio of grams of water lost per milligrams of carbon dioxide This ratio increases with the application of cetyl alcohol

Thus, contrary to Roberts's[3] report, we find that cetyl alcohol when placed in the root medium of wheat significantly reduces growth, probably via reduction in photosynthetic rate However, it is also apparent that the rate of transpiration, on a leaf-area basis, is reduced The mechanism of this reduction in rate of foliar water vapour exchange is uncertain, however, it is possible that it is due to the translocation of cetyl alcohol into the vicinity of the spongy mesophyll of the leaf This is suggested, but not proved, by Roberts's observation of the movement of radioactivity into the leaves of plants to the roots of which cetyl alcohol labelled with carbon-14 has been applied

We thank Prof J S Turner, at whose suggestion this project was started, and also the Wheat Industry Research Council of Australia for support

T F NEALES
P. E KRIEDEMAN

Botany School,
University of Melbourne.

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Lack of Effect of Plasma Erythropoietin on Formate Incorporation into Nucleic Acids *in vitro*

RECENTLY, Perretta and Thomson[1] published a report on the marked stimulatory action exerted by a preparation of erythropoietin on the incorporation of formate labelled with carbon-14 into the bases of spleen and liver-slice nucleic acids This same preparation, they reported, had no such action on marrow suspensions incubated under the same conditions Since this finding appeared to offer a useful approach to the biochemical examination of the mechanism of action of erythropoietin and might also furnish a rapid sensitive *in vitro* assay for the hormone we undertook a confirmatory investigation Instead of the extract of alcohol-precipitated urine from anæmic rabbits we used a partially purified sheep plasma fraction, with the results summarized here These results do not confirm the findings of Perretta and Thomson

Experimental details were essentially as reported by Perretta and Thomson with the following exceptions as a source of erythropoietin, we used what we have termed the step III fraction[2], which had an activity of about 3 units per mg, the concentration used being 15 units/ml , and the incubation time 130 min The isolation and determination of specific activities of the purines and thymine were as described by Smellie *et al*[3] with some slight modifications All samples were run in duplicate and the averages are reported

It is clear from the results in Table 1 that we observed no marked stimulation of formate incorporation by any of the tissues In the case of spleen and marrow RNA and DNA, in fact, there was some depression of incorporation as a result of incubation with the erythropoietin-containing material

Among the differences between the experiments reported previously and those here, the most important may be differences in the amounts of erythropoietin used and in the non-erythropoietic contaminants in the two preparations Although we do not know how much erythropoietin activity was used by Perretta and Thomson, our own experience in another experiment in which 5 and 10 units per ml were used, rather than 15 units per ml as in the experiment reported in Table 1 suggests no consistent dose effect

Table 1 EFFECT OF ERYTHROPOIETIN ON INCORPORATION OF LABELLED FORMATE INTO NUCLEIC ACID BASES *in vitro*

		RNA		DNA	
		A	*B*	*A*	*B*
Tissue	Base	c p m /μmole		c p m /μmole	
Liver	Guanine	676	792	36	49
	Adenine	2,087	2,645	52	56
	Thymine	—	—	275	256
Spleen	Guanine	548	175	29	36
	Adenine	1,330	576	18	21
	Thymine	—	—	56	69
Marrow	Guanine	735	180	364	201
	Adenine	1,838	807	115	70
	Thymine	—	—	628	413

0 15 ml packed marrow cells, 270 mg of slices of liver or spleen per sample , formate concentration, 9 6 μmoles/ml (4 85 μc/ml), incubation time 130 min

A, no added erythropoietin *B*, 15 units/ml erythropoietin

We feel that the clue to the discrepancies we have seen may lie in the nature of the contaminants present in the preparations The results reported by Perretta and Thomson show an appreciable depression of bone marrow formate incorporation caused by the control preparation from normal urine This same extract caused no stimulation when incubated with spleen or liver slices When the 'anæmic urine extract' was used the incorporation of formate of the marrow suspension was at about the normal level while there were large increases in the specific activities of the purines and thymine of spleen and liver These results suggest that the 'anæmic urine extract' contained a substance which stimulated formate incorporation in all three tissues, that the marrow was sensitive to an inhibitory material present in both urine extracts, and that the 'anæmic urine extract' was able to overcome the inhibitor action If these inferences are correct, then the difference Perretta and Thomson observed between spleen and liver on one hand and marrow on the other was quantitative, and the stimulation observed was not the result of erythropoietin action

P P DUKES
E GOLDWASSER

Argonne Cancer Research Hospital* and
Department of Biochemistry,
University of Chicago

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* Operated by the University of Chicago for the United States Atomic Energy Commission

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ENTOMOLOGY

Neural Transplants and Insect Diapause

THE induction and maintenance of larval and pupal diapause are at present thought to be under the direct control of the neuro-endocrine complex associated with the insect brain Williams[1] found that diapausing pupæ of the cecropia moth, *Hyalophora cecropia*, could be induced to resume development by either a prolonged (6–12 weeks) exposure to a low temperature (4–6° C) or by the implantation of the brain of a previously chilled pupa Unchilled diapause pupal brains were found to be incompetent to induce development when implanted into an unchilled pupa in diapause The competence or incompetence of a pupal brain appeared to depend on the activity or inactivity of its neurosecretory cells When active, the neurosecretory cells are responsible for the production of a hormone that is released via the corpora cardiaca and activates the prothoracic glands, which then produce the growth hormone, ecdysone, responsible for the initiation of development[2] In the diapause pupa, the neurosecretory activity of the brain is suppressed[3], as is its neural impulse activity[4]

Highnam[5] reported that the neurosecretory cells of a sphingid moth contained no secretory granules early in the pupal diapause period During three weeks of exposure to low temperature, the cells began to secrete granules that passed down the axons into the corpora cardiaca After three weeks of such secretory activity, the pupal brain was competent to induce development when implanted into an unchilled pupa in diapause Secretory granules have been detected in the neurosecretory cells throughout the period of diapause in several species[6,7] It has not been demonstrated, however, that the presence of such granules indicates concurrent secretory activity, rather than storage of the products of previous elaboration[8]

The present investigation was undertaken in an effort to elucidate some of the humoral factors operative in the initiation, maintenance, and termination of the photoperiodically induced larval diapause of the European corn borer, *Ostrinia nubilalis* The methods used were similar to those previously reported in regard to culture medium, rearing conditions, and diapause induction[9] Surgical procedures were carried out aseptically, and tissue implantations were made into the middle abdominal segments of mature borer larvæ

Table 1 EFFECTS OF TISSUE TRANSPLANTS ON THE TERMINATION OF DIAPAUSE IN THE EUROPEAN CORN BORER

Transplant	Physiological state Host	Physiological state Donor	Larvæ (No)	Survivors (No)	Pupæ (No)	Average days to pupation
Brain	D*	ND†	30	18	18	9 2
Thoracic ganglia	D	ND	14	9	0	—
Testes	D	ND	10	5	0	—
Brain	ND	D	13	9	9	3 2
Thoracic ganglia	ND	D	15	7	7	3 5
Testes	ND	D	18	9	9	2 9
Brain	D	D	105	52	43	16 1
Sub-œsophageal ganglion	D	D	27	19	0	—
Prothoracic ganglion	D	D	4	3	0	—
Sub-œsophageal + prothoracic ganglia	D	D	20	8	0	—
Prothoracic glands	D	D	10	5	0	—
Brain + sub-œsophageal ganglion	D	D	6	6	5	15 4
Brain + corpora allata-cardiaca + sub-œsophageal ganglion	D	D	14	2	2	13 5
Testes	D	D	8	8	0	—
Controls	D	—	18	16	0	—

* D, Diapause
† ND, Non-diapause.

The role of the neuro-endocrine complex in the initiation and maintenance of larval diapause was investigated by means of a series of tissue transplants (Table 1) The implantation of the brains of diapause larvæ into mature non-diapause larvæ did not induce diapause or delay pupation Implantation of brain and neuroendocrine tissues of non-diapause larvæ into diapause larvæ resulted in the initiation of pupal and adult development Implants of sub-œsophageal ganglia, prothoracic ganglia, fat body, or testes did not cause diapause to be terminated These results are fully consistent with the concept that diapause is an arrested state that is enforced by the absence (or low titre) of the brain hormone required for the initiation of morphogenesis

Cytological examination of the corpora allata, corpora cardiaca, and neurosecretory cells of diapause and non-diapause larvæ failed to disclose any diapause-associated differences in granule inclusions, staining reactions, and cell size Although admittedly inconclusive, the neurosecretory cells of diapause larvæ did not look incompetent from the standpoint of their cytological state This finding led us to consider the hypothesis that the neurosecretory cells of diapause larvæ were competent from a synthetic standpoint, but that the elaborated products were not being released into the circulatory system This suggested that a physical or physiological brain barrier might be involved in the maintenance of the diapause state

The 'brain barrier' hypothesis was tested by transplanting tissues from diapause larvæ into other diapause larvæ of the same age and previous treatment (Table 1) Diapause brains implanted into diapause larvæ caused termination of diapause and pupation after about two weeks That the effect was caused by the parts of the brain containing the medial and lateral neurosecretory cells was demonstrated by the failure of other implanted structures to evoke development We have implanted brains of diapause larvæ 12–60 days old, and have found them all quite competent to induce development However incompetent the diapause brain may be *in situ*, it becomes competent when extirpated and implanted into another diapause larva

Although it has not been put on a quantitative basis, we have observed a distinct tendency for the number of days elapsing between brain implantation and pupation to be inversely proportional to the amount of tissue damage inflicted on the implanted brain during the surgical procedure The results of these experiments are interpreted as indicating that diffusible substances are released from the implanted brain following surgical destruction of the barrier between the neurosecretory cells and the circulatory system

Other interpretations of the experimental results are possible One is that the diapause brain normally elaborates and liberates neurohormones at a rate too low to induce development, the implanted brain simply raises the hormone titre above the developmental threshold This possibility has been eliminated by experiments in which the host brain was removed by ligation and a diapause brain implanted into the abdominal cavity Larvæ so treated contained but one brain, but diapause was terminated, the larvæ pupated The hormonal output of the implanted brain was apparently sufficient to induce pupation Another possible interpretation of these results is that injury metabolism in the implanted brain tissue was sufficient to elevate the rate of neurosecretion and induce development This seems unlikely because such a non-specific effect has not been found in other species Williams[1] reported that as many as eight incompetent diapause brains implanted into a single diapausing pupa of cecropia failed to induce a resumption of development

It seems likely that the 'brain barrier' is an absence of impulse transmission in the neurosecretory axons, as there is some evidence supporting the idea that impulse transmission is necessary for the intraaxonal translocation of neurosecretions[10] Tissue injury during surgery may result in (1) a resumption of neural activity, (2) mechanical release of secretory products, or (3) facilitate the diffusion of blood-borne substances into the implanted brain tissue. In any case, the barrier between neurosecretion and the circulatory system is eliminated, and the neurosecretory products are able to exert their physiological action of evoking metamorphosis.

Publication of this communication has been approved by the Director of the Wisconsin Agricultural Experiment Station.

This work was supported in part by research grant *RG* 7557 from the U.S. National Institutes of Health.

ELMER J. CLOUTIER
STANLEY D. BECK
D. G. R. MCLEOD
D. L. SILHACEK

Department of Entomology,
University of Wisconsin,
Madison 6, Wisconsin.

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Effect of 'Rogor' (Dimethoate) and DDT on Cotton Mites in Uganda

THREE species of mites are known to attack cotton in Uganda. These are yellow tea mite, *Hemitarsonemus latus* (Banks)[1], red mite, *Tetranychus telarius* (L.)[2], and 'green mite', *Eotetranychus* sp. nr. *deflexus* (McGregor).

Tea mite is widespread and common, and though it can cause considerable leaf damage it has not been shown to reduce yields in Uganda[3], it is readily controlled by DDT-miscible liquid sprays[3]. The cotton red mite is of doubtful taxonomic status[2,4,5], and it would seem advisable to leave it as 'the *T. telarius* complex'. It is widespread and common on cotton, particularly at senescence, though excessive applications of DDT can cause severe attacks and complete defoliation[6]. *Eotetranychus* sp. is a new record for cotton in Uganda. It was first discovered at Kawanda Research Station early in 1961 and afterwards at widely scattered sites over the whole country. It may be a potential new pest or it may be that an exceptionally wet growing season provided a suitable breeding environment.

In view of the possibility of an increase in green mite and the fact that it can occur on the same plant as red mite it was decided to work out a joint control. Coaker[6] showed that 0·05 per cent parathion and 0·025 per cent 'Aramite' gave good control of the *T. telarius* complex in Uganda and McKinlay[7] that both 'Rogor' and 'Metasystox' at 0·0125 per cent gave good control in Tanganyika. Field application of 0·0125 per cent 'Rogor' at ten-day intervals does not give satisfactory control in Uganda but 0·06 per cent does (unpublished work).

Cotton leaves infested with both red and green mite were maintained in the laboratory with their petioles dipped in water. Active stages of both tetranychids were dead or dying within 4 h of spraying with 0·1, 0·05 or 0·025 per cent 'Rogor', while tea mites, which were also present on the leaves, remained perfectly healthy. Larvæ of both red and green mite hatching after 24 h were killed, but those after 48 h lived and fully re-established the colony after a week.

A further trial was laid down using leaves infested with all three species of mites in which 1 per cent DDT-miscible liquid, 0·02 per cent 'Rogor'-emulsifiable concentrate and a mixture of both 1 per cent DDT and 0·02 per cent 'Rogor' were sprayed on to either the upper side or the lower side of the leaves only. The 1 per cent DDT m.l. applied to the lower sides of the cotton leaves killed the active and the quiescent stages of all three species of mites, tea mite disappeared completely within 4 h while the tetranychids remained twitching on their webs for days before finally dying. Healthy larvæ of all three species re-appeared within 4 days and the colonies were re-established within 8 days. The same spray applied to only the upper surface of the leaves gradually reduced the numbers of actively wandering tea mites but had no effect on either red or green mite. The 0·02 per cent 'Rogor' applied to either surface had no effect on tea mite. Applied to the lower leaf surface it killed both red and green mite within 4 h and hatching larvæ died over the following 2 days but after 4 days there were no visible toxic effects. 'Rogor' sprayed on to the upper surface only had no effect on red or green mite living on the underside of the leaf and so cannot be absorbed by the cotton leaf under these conditions. With the mixture of DDT and 'Rogor', all three species were dead within 2 h, and no living larvæ were seen until the fourth day and re-establishment of the colonies was delayed until the twelfth day. The same mixture applied to the upper leaf surface gradually reduced the tea mite numbers, but the tetranychids increased. On the control leaves both the red and green mite numbers increased continuously but the tea mite population gradually decreased, as they either died naturally or wandered off the leaves. It was interesting to note that a large, active, predatory mite, as yet unidentified, was unaffected by either insecticide.

After observing the failure of 'Rogor', a known systemic insecticide, to kill red or green mite living on the underside of the leaves when applied to the upper, another trial was laid down in which unsprayed leaves had their petioles dipped into 'Rogor' instead of water. 0·1 and 0·05 per cent 'Rogor' were used and within 24 h both red and green mite were dead but tea mite was still alive. 'Rogor' absorbed into the leaves in this way proved to be highly phytotoxic and within three days all the treated leaves were dead or dying.

It is concluded that 0·02 per cent 'Rogor' as an underleaf spray is an effective contact poison against red mites of the *T. telarius* complex and green mite, *Eotetranychus* sp. nr. *deflexus*, but that it does not appear to be absorbed by the cotton leaf when sprayed on to the surface. 'Rogor', even as strong as 0·1 per cent, has no effect on tea mite, *H. latus*. A good wetting spray of 1 per cent DDT is a slow-killing poison to both red and green mite and is extremely lethal to tea mite. The residual toxicity appears to be no more than 4 days for either insecticide under the conditions of the experiment. There is no evidence of either toxicant having any action on the eggs of any of the three mite species. A good field control should be obtained with a mixture of 1 per cent DDT m.l. and 0·02 per cent 'Rogor' e.l. applied as an 'up and under' spray, but from the laboratory evidence a second application would be required about 12 days later.

I thank the Commonwealth Institute of Entomology for its help in identifying the *Eotetranychus* species.

W. R. INGRAM

Section of Entomology,
Kawanda Research Station,
Department of Agriculture,
Uganda.

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Anæsthesia and the Action of DDT on *Anopheles* Larvæ

DR. J. C. JONES[1] has reported that "anæsthesia . . . interferes in some important way with the obtainment of a lethal dose of DDT" by larvæ of *Anopheles quadrimaculatus* Say. If this effect were involved with the metabolic processes of the insect, one might make a case for the intriguing concept of 'active' uptake of this poison. If, however, the effect is simply one of producing a lower exposure to the insecticide by decreasing the physical activity of the larvæ, it is, obviously, worth no further investigation. On the basis of the following evidence: ". . . anæsthetized larvæ which are drawn about within the [DDT] suspension by cardboard tips are entirely comparable to those left completely quiescent", Jones ruled out the latter possibility.

Since the hypothesis of 'active' uptake is an attractive one, and has generally been neglected, we have endeavoured to expand Jones's work. It can, indeed, be shown that fourth-stage larvæ of *A. quadrimaculatus* are protected from the insecticidal effects of DDT by the action of any of several anæsthetics (Table 1). Further experimentation, however, occasions our assertion that this effect is, in spite of Jones's views to the contrary, a physical one.

Table 1. EFFECTS OF ANÆSTHETICS ON INSECTICIDAL ACTIVITY OF DDT

		Alive	Dead
		at 24 h*	
(1)	Ether–DDT	15	5
	DDT	0	20
	Ether–water	18	2
(2)	CO_2–DDT	15	5
	DDT	7	13
	CO_2–water	18	2

(1) 20 larvæ immersed in 3·5 per cent ether in water for 10 min, washed twice for 2 min with water, exposed to DDT (4 mg of *p,p*-DDT dissolved in 4 ml. of acetone and suspended in 100 ml. water) for 10 min, washed twice for 2 min with water, and placed into individual shallow water-filled containers for 24 h at 80° F.

(2) 20 larvæ exposed to carbon dioxide-saturated water for 10 min then placed in carbon dioxide-saturated DDT (made up as above) for 10 min, washed twice for 2 min with water, and incubated as above.

* Each result is an average of two replicates of 20 individuals.

Four groups of twenty larvæ were anæsthetized with carbon dioxide by bubbling the gas for 10 min through water in which they were immersed. Each group was then transferred to a separate container of DDT of sufficient strength to kill within 24 h 75–100 per cent of the animals exposed to it for 10 min. Group 1 was permitted to remain completely motionless for the 10-min interval; carbon dioxide was bubbled for 3 min through the DDT suspension in which group 2 had been placed, after which these larvæ were left undisturbed for the remaining 7 min (although completely anæsthetized, they were in motion in DDT for 3 of the 10 min); group 3 was kept in motion by the bubbles of carbon dioxide for 7 min, then left immobile for 3 min; group 4 was disturbed for the whole period. The experiment was completely carried out in an atmosphere of carbon dioxide, so there was no possibility of any larva becoming active during the time it was undisturbed by the bubbling gas. Table 2 clearly shows that larvæ which are kept in flux are unprotected by carbon dioxide even though metabolically torpid. To substantiate this, an experiment was done in which larvæ were anæsthetized with ether and subsequently stirred vigorously in a DDT solution while still in a flaccid state. The results obtained with this anæsthetic were the same as those reported above.

Table 2. EFFECTS OF MOVEMENT OF ANÆSTHETIZED LARVÆ ON ACTIVITY OF DDT

				Alive	Dead
				at 24 h*	
(1)	CO_2–DDT	quiescent	10 min	13	7
(2)		in motion	3 min	6	13
(3)		in motion	7 min	3	17
(4)		in motion	10 min	2	18
(5)	DDT			0	20

(1)–(4). Four groups of 20 larvæ exposed to carbon dioxide, as in Table 1, section 2.

* Each result is an average of two replicates of 20 individuals.

Whether the protective effect of anæsthesia is attributable to the very low rate of diffusion of DDT, limiting the amount of contact with the immobile larval surface in the time period involved, or to the decreased larval surface exposed as a result of cessation of movement, is not known at this time. In accord with the overall findings, however, is the work of Lewis[2], who has shown that Risella oil spreads quite rapidly over the cuticle of blowflies only when they are moving, and assigns this effect to elastic submicroscopic movements in the cuticular surface associated with walking.

This work was partially supported by grant No. *AI*-0286703 from the U.S. National Institutes of Health.

S. FRIEDMAN*
J. CHALKLEY

Ross Institute of Tropical Hygiene,
Keppel Street,
Gower Street,
London, W.C.1.

* On leave from the Department of Entomology, Purdue University, Lafayette, Indiana.

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MICROBIOLOGY

Origin of the Endoplasmic Reticulum

To propose a mechanism of origin of the endoplasmic reticulum based on a study of yeast cytology may seem presumptuous because there is no field in which so many different opinions prevail. The variety of opinions have arisen because yeasts, like all acellular (single-celled) organisms, manifest a maximal diversity of internal organization, and a limited number of these manifestations are considered by different individuals to be typical, and because yeast cytology has engaged the interest of those who are primarily interested in yeast and only secondarily interested in cytology. But the fact that almost the entire internal organization of a small (5μ) structure may be revealed by as few as 20 serial sections, while thousands of comparable sections (each much larger) would be required to trace the internal organization of the more familiar liver cell, makes the smaller yeast cell a more useful object in respect to tracing the relationship of the reticulum to the nucleus.

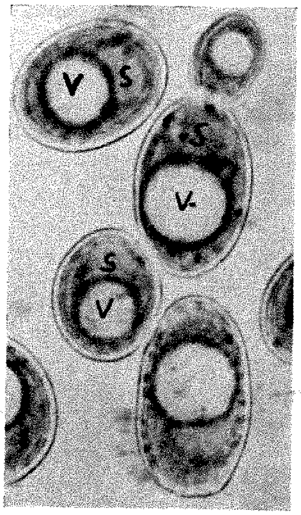

Fig. 1. Ultra-violet photomicrograph of yeast cells which were removed from a rapidly growing culture and starved overnight in phosphate buffer. The synthesis of nucleic acid is greatly diminished. The ultra-violet absorbing nucleic acid (soluble transfer of RNA) is streaming into the cytoplasm from the nuclear vacuole (*V*) and not from the spindle (*S*)

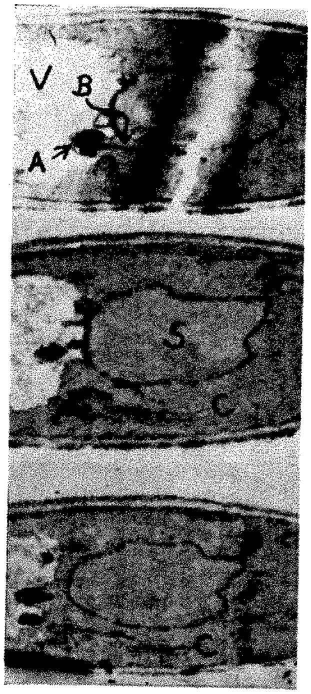

Fig. 2. Three serial sections of a single yeast cell showing the endoplasmic reticulum (*C*) folded in the cytoplasm. The vestibule (*A*) of the assembler has been retracted into the vacuole by fixation. The assembler is the Feulgen-positive structure which is revealed by light microscopy as a crescent closely applied to the surface of the spindle reservoir (*S*) (ref. 9). The vestibule and the extruder duct (*B*) are electron-dense when stained with uranyl acetate, which is specific for nucleic acid

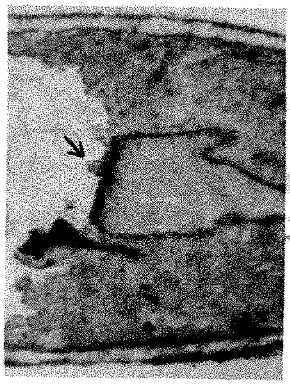

Fig. 3. A section of the yeast cell shown in Fig. 2 revealing the vestibule and the extruder duct, and also the vent (at arrow) leading from the spindle reservoir into the nuclear vacuole

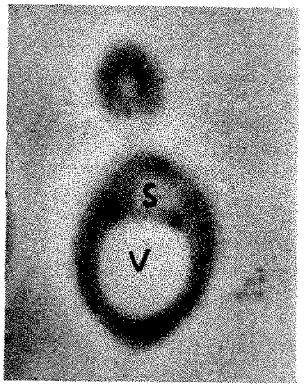

Fig. 4. A phase-contrast (visible-light) photomicrograph showing the assemblers in a living yeast cell

Caspersson's original proposal[1] that all nucleic acid originates from the nucleus has been abundantly confirmed, and ultra-violet photographs of living yeast cells from his laboratory[2] and from ours[3] have been interpreted by me as indicating that soluble (amino-acid carrier) nucleic acid passes directly through the wall of the nuclear vacuole into the cytoplasm (Fig. 1). Analysis of electron micrographs of serial sections of yeast cells, made by Dr. Tadashi Hirano, suggest that the endoplasmic reticulum[4] (and the attached ribosomes[5]) also arise from the nuclear vacuole, passing into the cytoplasm through a specialized organelle, the assembler (Fig. 2) comprising: (1) a funnel-shaped vestibule (*A*) leading from the nuclear vacuole (*V*) into (2) an extruder duct (*B*), through which the endoplasmic reticulum (with its ribosomes) passes into the cytoplasm. While the soluble nucleic acid (transfer RNA) is streaming directly through the membrane of the nuclear vacuole into the cytoplasm, the Feulgen-positive chromosomes and the ribonucleic acid and other components, destined to become ribosomes and endoplasmic reticulum, are packed into the vestibule of the assembler. The presence of the chromosomes in the assembler suggests on one theory of gene action[6] that the messenger RNA is transferred from the gene locus to the ribosome in the assembler. On this concept, the endoplasmic reticulum (*C*) (carrying the ribosomes coated with messenger RNA) issues from the extruder duct in the form of a long ribbon which folds as it emerges into the cytoplasm. The most distal extension of the ribbon carries the oldest ribosomes, and the most proximal region, near the exit of the extruder duct, carries the most recently synthesized ribosomes. A vent from the spindle reservoir (*S*) leads into the nuclear vacuole to permit the passage of the chromosomes from the spindle reservoir into the vacuole (at arrow in Fig. 3).

There are two assemblers attached to each nuclear vacuole, thus explaining the long-standing misconception, originating with Badian[7,8], that the yeast cell contains two Feulgen-positive chromosomes. Fig. 4 is a phase-contrast (visible light) photograph showing both assemblers occupying their characteristic positions in the living cell. The turgid vacuole and the fainter but equally turgid spindle are characteristic of the rapidly growing yeast cell and suggest that hydrostatic pressure pushes the chromosomes into the vestibule and provides the current which moves the soluble nucleic acid through the membrane.

This work was supported by a research grant from the National Science Foundation G–15619.

CARL C. LINDEGREN

Biological Research Laboratory,
Southern Illinois University,
Carbondale, Illinois.

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Occurrence of Sialic Acid in some Gram-positive and Gram-negative Pathogenic Bacteria

Aaronson and Lessie[1] searched for sialic acid in ten species of bacteria and detected it only among the Gram-negative organisms. In continuation of our findings with *Pasteurella pestis*[2], we have screened many pathogenic bacteria for the presence of sialic acid.

The micro-organisms were grown in the conventional liquid media or nutrient agar as indicated. After testing for the purity of the cultures, they were washed out with saline in the case of agar growths and the bacterial mass collected by centrifugation. After washing once with water, the cells were triturated with cold acetone (– 20° C.) and acetone powders prepared as usual. Fractionation of these were made as reported earlier[2] and the sialic acid estimated by the thiobarbituric method[3] after extraction of the chromogen with isoamyl alcohol to remove the colour produced by deoxyribose. We did not meet with the difficulties referred to by Forbes and Ruck[4]. The results obtained are given in Table 1. There is some destruction of sialic acid in the acid treatment and on hydrolysis.

In its sialic acid content also *Past. pseudotuberculosis* resembles closely *Past. pestis*. The *Salmonella* organisms, *Bord. pertussis* and *Ps. pyocyaneus*, contain sialic acid. The case of *V. cholerae* is of interest in that the sialic acid content is negligible or very low in the four strains studied. It is of interest to find out whether the presence of the receptor-destroying enzyme in this organism, which is a sialidase[5], has anything to do with the low sialic acid content. Among the Gram-positive bacteria studied, sialic acid was not detected in *C. diphtheriae*, and there seems to be a trace in *Staph. aureus*. The definite presence of sialic acid in the Gram-positive *Strep. faecalis* does not confirm the belief[1] that sialic acid is present only in Gram-negative organisms.

We thank Dr. M. K. Habbu, Mr. R. S. Karbhari, and Mr. A. S. Narasimhan for help in growing the cultures and checking the purities. We also thank the Haffkine Institute Diamond Jubilee Research Committee for the award of a senior fellowship to one of us (R. J. I.).

ROSHAN J. IRANI
K. GANAPATHI

Haffkine Institute,
Bombay, 12.

Table 1. SIALIC ACID CONTENT OF VARIOUS BACTERIA

No.	Bacteria under test	Sialic acid content in μgm./100 mgm. of acetone powder of the bacteria: Hot aqueous extract	Aqueous extract after hydrolysis	Hydrolysate of residue	Hydrolysate of cell mass	Hot TCA extract	Hydrolysate of residue
1	*Pasteurella pseudotuberculosis*	32	54	167	337	506	30
2	*Salmonella typhi*	58	192	73	117	62	0
3	*S. paratyphi A*	88	116	0	183	380	66
4	*S. paratyphi B*	12	22	141	187	365	65
5	*S. typhimurium*	53	103	126	188	222	40
6	*Bordetella pertussis*	14	22	0	66	97	34
7	*Pseudomonas pyocyaneus*	66	192	108	110	150	23
8	*Vibrio cholerae Inaba*	0	0	0	15	42	16
9	*V. cholerae Inaba*	0	0	0	0	tr.	tr.
10	*V. cholerae Ogawa*	4	5	0	13	37	12
11	*V. cholerae*, non-agglutinable	2	16	10	15	29	14
12	*V. cholerae El Tor*	16	7	5	17	0	4
13	*Corynebacterium diphtheriae*	0	0	0	0	0	0
14	*Staphylococcus albus*	0	0	1	0	0	0
15	*Staph. aureus*	2	2	5	4	0	0
16	*Streptococcus faecalis*	64	187	74	104	76	2

Past. pseudotuberculosis (No. 1) was grown in liquid medium at 37° C. for 13 days. *V. cholerae Inaba* (No. 9) was grown in casein hydrolysate liquid medium at 37° C. for 48 hr. *C. diphtheriae* (No. 13) was grown in liquid medium for 7 days at 37° C. All the others were grown in nutrient agar and incubated at 37° C. for 48 hr. The cold trichloroacetic acid extract of the acetone powder did not contain any sialic acid except in No. 3 (48 μgm./100 mgm.).

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Spermicidal Activity of Bacterial Endotoxin

THE endotoxins of Gram-negative bacteria are a group of macromolecular lipopolysaccharide complexes which, on administration to experimental animals, produce a wide variety of physiological and pathological alterations[1,2]. Despite the similarity of some of the pathological findings in these varied reactions neither a common factor nor a basic mechanism responsible for the pathogenesis of these reactions has been established, and there is evidence that significant underlying physiological differences exist in some of these phenomena.

Endotoxins have been isolated from a wide range of Gram-negative bacteria. Little attention has been given to micro-organisms from the genital tract although Gram-negative organisms comprise a large part of the pathogens involving this system in man and animals. Brucellosis of cattle, sheep and swine, and vibriosis of cattle, for example, are at present responsible for heavy annual losses in the livestock industries. Recently, a toxic lipopolysaccharide has been isolated from *Vibrio fetus* by the phenol–water extraction method[3] and has been shown to possess many of the chemical and biological properties of the classical Gram-negative bacterial endotoxin[4]. In the course of examination of the pathogenesis of *V. fetus* infection this lipopolysaccharide has been found to be spermicidal *in vitro* to both ram and bull spermatozoa.

In these experiments the semen of rams was collected by electro-ejaculation[5], and only apparently normal specimens with good initial motility were used. The semen was diluted 1 : 20 in calcium-free Ringer's phosphate solution in small tubes for motility observations which were made at 37° C. The lipopolysaccharide solution was dialysed against distilled water at 2°–4° C. for 14 days prior to use. All the tubes were checked for any alteration in *p*H. Motility was scored at hourly intervals by the system of Emmens[6].

The ram spermatozoa were immobilized at the end of the first hour at the concentration of 100 μgm./ml. and higher and only the tails were moving. All activity had usually ceased after 3 h (Fig. 1).

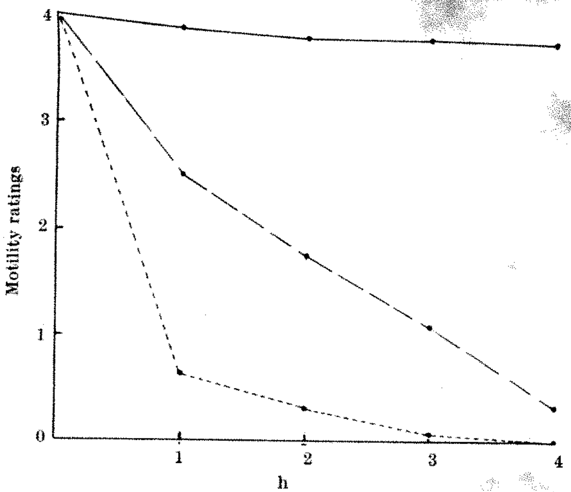

Fig. 1. Motility decreasing effect of lipopolysaccharide of *V. fetus* on ram spermatozoa *in vitro* at 37° C. – – –, 100 μgm. lipopolysaccharide; - - -, 1 mgm. lipopolysaccharide; ——, control

The mean effective dose was shown to be 227 μgm./ml. Preheating the lipopolysaccharide increased the activity up to five-fold, a finding in keeping with the other specific activities of Gram-negative bacterial endotoxins[7]. The control tubes showed little or no diminution in motility over the 4-h test period. Limited studies with bovine spermatozoa suggested that the lipopolysaccharide of *V. fetus* appeared to be more spermicidal in this species, by decreasing the motility in a shorter period of time and at a lower concentration. This suggestion is in keeping with the epidemiology of both bovine and ovine vibriosis.

The effect of lipopolysaccharide on the motility of spermatozoa is non-specific for *V. fetus* as the lipopolysaccharide of both *Pasteurella multocida* and *Serratia marcescens* were also shown to be effective, indicating that the spermicidal activity is, in general, another specific effect of Gram-negative bacterial endotoxins. The lipopolysaccharide of the non-pathogen, *Serr. marcescens*, was the most spermicidal of the three to the ram spermatozoa and the mean effective dose was calculated to be 78 μgm./ml.

Thomas[8] considers that the only known primary actions of endotoxin are those arising from injection into the sub-arachnoid space and the release of a pyrogen by the addition of endotoxin to leucocytes. With these two exceptions, Thomas[8] states that to his knowledge no other phenomena can be ascribed to the direct effects of endotoxin. In addition, he states that he does not believe that anyone has succeeded in showing a direct effect of endotoxin *in vitro*. It is proposed that the spermicidal activity of bacterial endotoxin is a specific type of endotoxin-induced pathological alteration having a direct effect upon a mammalian cell *in vitro* and exhibits certain significant differences from several of the other endotoxin-induced lesions.

I thank Mr. R. V. S. Bain and Dr. I. G. White for advice.

STANLEY M. DENNIS*

Department of Veterinary Pathology
and Bacteriology,
University of Sydney.

* Present address: College of Veterinary Science, University of Minnesota, St. Paul 1.

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GENETICS

Twinning and Haploidy in *Cocos nucifera* Linn.

IN the coconut, *Cocos nucifera* Linn., the ovary is tricarpellary and each carpel contains an ovule. Usually only one ovule develops and two abort, and although there are three depressions or 'eyes' in the endocarp wall of the mature fruit only one of these is a functional germ pore. From this eye normally a single shoot emerges.

Exceptions to this pattern include the functioning of extra germ pores[1-3], and the production of additional shoots from one germ pore[4,5], a rare occurrence according to Menon and Pandalai[6].

Most observations have so far been on seedling and mature palms[7] and Harland[8] has discussed the value of polyembryony in physiological and breeding studies. Recently, however, Guevara[9] has described two twin embryos in both instances lying beneath a single germ pore. In one pair the embryos were completely separated and in the other were joined. The extra embryo in the first pair he considered to be most likely of synergid origin but it could have resulted from cleavage of one embryo.

In Jamaica, from a collection of 75 seed nuts each with more than one shoot, 71 were twins and four were triplets. When the husks of 33 of these nuts

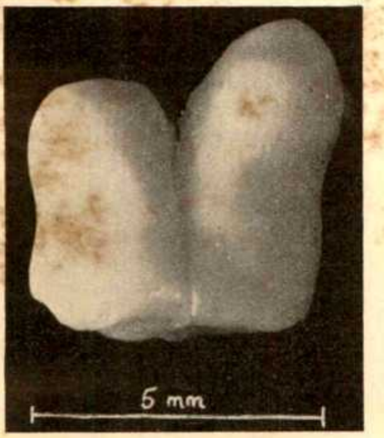

Fig. 1. A twin embryo of *Cocos nucifera* Linn. from a mature ungerminated nut

including two triplets were removed it was found in each case that all shoots emerged from one germ pore. Further, although members of a pair often differed in size there was generally no other marked morphological difference.

Studies of these 31 twin seedlings showed all states between complete fusion of the haustoria within the nut and complete separation, and the majority were apparently diploid–diploid.

These observations suggested that twins often arise as a result of cleavage in a single embryo, and the discovery of a partially cleaved embryo pair from an ungerminated nut shown in Fig. 1 tended to support this. That separation of this embryo pair was incomplete is shown in Fig. 2, where a small area (*C*) of cells common to both is evident.

In only one pair of twins so far examined have marked differences between partners been observed. The twins were found to be completely separated and further showed considerable disparity in vigour. The suspicion that the less-vigorous plant was haploid was confirmed when it was shown to have 16 rather than 32 chromosomes per cell. The diploid plant had a bronze rachis whereas that of the haploid was green and the latter had noticeably more slender leaves. Unlike the diploid the margins of the haploid rachis were fused together.

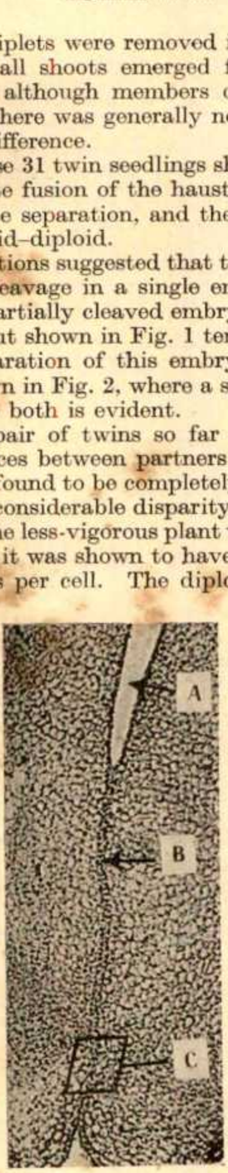

Fig. 2. Photomicrograph of a section of the region between the embryos in Fig. 1. In *A* complete separation has occurred; *B* shows the line of cleavage; *C* is an area of tissue common to both embryos

This is apparently the first record of haploidy in *Cocos nucifera* and in addition is an example of a second kind of twinning.

The value of haploids in coconut cytogenetics and breeding will naturally depend on their frequency and the ease with which they can be converted into diploids. At present it appears that haploid–diploid twins are much less frequent than diploid–diploid twins.

R. A. Whitehead

Research Department,
Coconut Industry Board,
Jamaica.

G. P. Chapman

Department of Botany,
University of the West Indies,
Jamaica.

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CYTOLOGY

Non-Random Distribution of Certain Homologous Pairs of Normal Human Chromosomes in Metaphase

Developments over recent years in the technique of cell culture and chromosome analysis have made available considerable numbers of photographs of mitoses. From these, information can be gained rather simply as to the distribution of chromosomes in metaphase, whether it is random, and more particularly whether certain chromosomes tend to lie close together.

Barton and David[1] and Stewart[2] have developed methods for studying this problem, the former finding a slight suggestion of homologous association of all chromosomes within a single cell, the latter finding random spread of all chromosomes within ten cells. Difficulty exists, however, in assigning most chromosomes to the correct pairs, and wrong assignment would tend to swamp any evidence of non-randomization.

We wish to report results obtained on several chromosome pairs which were chosen because they can be unequivocally identified. These showed significant evidence of non-random dispersion in metaphase and of homologous association.

Thirty photographs, measuring approximately 15 cm.^{2}, of cultured peripheral blood and skin cells of normal males and females were obtained from members of the Galton Laboratory. (We thank R. Chitham, J. Delhanty, J. Ellis, K. Lele, R. Marshall and U. Mittwoch for providing photographs and karyotype analyses.) Distances were measured between the centromeres of homologous members of pairs 1, 2, 3 and 16 (Denver System) and compared with distances between a randomly chosen member of each pair and three randomly chosen chromosomes. Thus, for example, the distance, $X(1,1')$ between homologous members of pair 1 was measured and compared with three distances, $X(1,\alpha)$, $X(1,\beta)$, $X(1,\gamma)$, between a member of the pair and three randomly chosen chromosomes, α, β, γ. The difference $\bar{d} = \frac{1}{3}[X(1,\alpha) + X(1,\beta) + X(1,\gamma)] - X(1,1')$ will tend to be positive on the average if there is association between homologous chromosomes; this was tested statistically by comparing the mean $\bar{d}$ over all 30 plates with its standard error σ, according to the usual *t* test (with 29 d.f.). This test does not use all the available information, but it has the convenience of being easy to compute.

Table 1

Pair	$\bar{d}$ (cm.)	*S.E.*, σ	$t = \bar{d}/\sigma$	*P* (two-tail significance-level)
1	0·63	0·42	1·50	0·15
2	0·30	0·45	0·67	0·48
3	1·20	0·47	2·55	0·017
16	0·82	0·36	2·28	0·03
Total	2·95	0·86	3·43	0·002

As can be seen, a tendency toward homologous association was present in all four pairs. In pairs 1 and 2, this effect did not reach levels of significance; in pairs 3 and 16, the effect was significant at the 5 per cent level. Pooling the data shows an overall tendency of homologous association with a high degree of significance.

As a control, the same measurements were made between randomly chosen members of each pair under examination and a randomly chosen non-homologous chromosome. Similarly, these distances were then compared with those between the chosen member of the pair under study and three other chromosomes chosen at random. Values for the mean $\bar{d}$ and its standard error σ were obtained as before. These results showed no significant deviation from the null hypothesis, and thus give further support to the significance of the above findings.

Table 2

Pair	$\bar{d}$ (cm.)	*S.E.*, σ	$t = \bar{d}/\sigma$	*P* (two-tail significance-level)
1	0·42	0·71	0·59	0·56
2	−0·31	0·90	−0·34	0·74
3	0·31	0·80	0·39	0·70
16	−0·10	0·89	−0·11	0·91
Total	0·32	1·7	0·85	0·40

On the suggestion of Prof. L. S. Penrose, a further test of the study method was made, utilizing the observation that certain acrocentric chromosomes, for example those of the 21–22 group, are known to exhibit terminal associations and would be expected to lie close to one another[3]. From twenty mitotic plates of male cells a mean of all six distances between the members of chromosome pairs 21 and 22 was obtained. This was compared with the mean distance between six randomly chosen pairs of chromosomes. The differences, $\bar{d}$, were subjected to *t* test analysis and the results confirming the presence of association within the group were consistent with cytological observations.

Table 3

Group	$\bar{d}$ (cm.)	*S.E.*, σ	$t = \bar{d}/\sigma$	*P* (two-tail significance-level)
21–22	1·4	0·68	2·08	0·054

This investigation offers evidence that homologous members of chromosome pairs 1, 2, 3 and 16, which were chosen because of their ease of identification, tend to lie closer together than would be expected by chance in metaphase plates. That pairs 1 and 2 did not show the tendency so significantly as pairs 3 and 16 might be due to real differences of behaviour during mitosis or might simply be a result of their greater size. All cells studied had been subjected to metaphase arrest with colchicine, a substance which disrupts spindle formation. It is possible, therefore, that more conclusive results might be obtained from cells in which colchicine is not used.

LAWRENCE J. SCHNEIDERMAN*
CEDRIC A. B. SMITH

The Galton Laboratory,
University College,
London, W.C.1.

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* Postdoctoral Fellow, National Heart Institute, U.S. Public Health Service.

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VIROLOGY

Control of Virus Yellows in Sugar Beet Crops

VIRUS yellows, transmitted by aphids, causes great damage to sugar beet crops in north-west Europe. In Britain its incidence at the end of August in the years 1946–61 is shown in Fig. 1.

This incidence has been converted into estimates of sugar beet tonnage lost[1]; in the exceptional year 1957 this amounted to 1,039,000 tons, and in the three years 1958–60 it averaged 427,000 tons.

As we now know that two separate viruses are responsible for virus yellows[2], and that mild yellowing virus, the more common one, causes only 50–60 per cent of the loss of yield produced by severe yellows[3], it would be prudent to reduce these estimates of loss by 25 per cent.

In the four years 1957–60 the addition of these tonnages, so reduced, would have increased farm incomes in Britain by an average of £2,610,000 per annum. Moreover, large-scale spraying with systemic insecticides commenced in 1957, and in the three years 1959–61 a mean of 309,000 acres out of the total of 411,000 acres of the sugar beet crop were sprayed one or more times with aphid-killing insecticide, at an average cost of more than £1,000,000 per annum. This new control measure had no beneficial effect on the overall incidence of the disease (mean August incidence, 1946–56 = 13·2 per cent; 1959–61 = 17·5 per cent; see Fig. 1).

There is much evidence[4–12] that overwintering sugar beet and mangold seed crops are the important

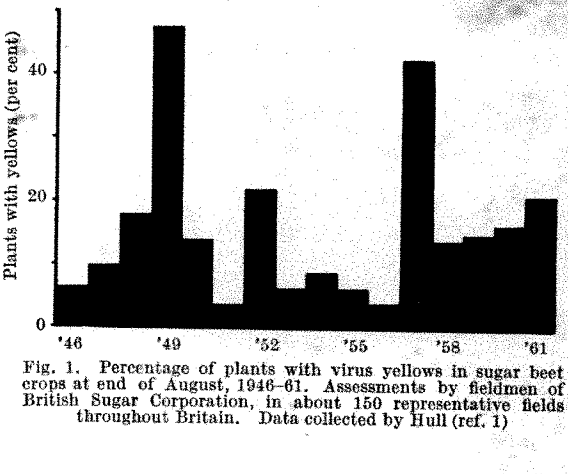

Fig. 1. Percentage of plants with virus yellows in sugar beet crops at end of August, 1946–61. Assessments by fieldmen of British Sugar Corporation, in about 150 representative fields throughout Britain. Data collected by Hull (ref. 1)

winter reservoirs of these viruses. Therefore the disease would be greatly reduced if the seed crop could be eliminated.

Beet seed is readily stored and keeps its germinating capacity for a long time; under ordinary warehouse conditions this capacity is only reduced by 50 per cent after storage for 5–9 years[13]. Moreover, beet and mangold seed crops occupy less than 3,000 acres of Britain. If seed crops of sugar beet and mangold were grown, throughout Britain, only in alternate years, the virus cycle would be broken and the disease controlled. Under the Destructive Insects and Pests Acts 1877 to 1927, the Minister of Agriculture possesses general powers to make Orders to prevent the introduction or spread of any disease or pest destructive to agricultural crops in England and Wales, and such Orders may include provisions to control and prohibit the growing of specified crops.

C. R. Ribbands

School of Agriculture,
Cambridge.

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Sugar Beet Mild Yellowing Virus: a Persistent Aphid-transmitted Virus

I have shown[1-3] that there are two aphid-transmitted viruses which commonly cause yellowing of sugar beet leaves in eastern England. One of these is beet yellows virus (SBYV), first described by Roland[4], and the other has been called sugar beet mild yellowing virus (SBMYV) by me[1]. The two viruses are apparently unrelated.

The virus–vector relationships of SBYV and *Myzus persicae* (Sulz.) have been examined in detail by Watson[5,6] and later by Sylvester[7]. *M. persicae* acquired SBYV by feeding on infected sugar beet plants for 5–10 min., but the probability of successful transmission was increased by longer feeding times up to 12 hr.; the virus persisted in the aphid for less than 72 hr. in aphids feeding on virus-free plants and Sylvester consequently classified SBYV as a semi-persistent virus. Viruliferous aphids only needed to feed on healthy plants for a short time to cause infection, although the proportion of successful transmissions increased with longer test feeding times up to 6 hr. Watson[8] has reported that viruliferous aphids are no longer able to transmit SBYV after moulting.

The virus–vector relationships of SBMYV have now been studied at the Plant Breeding Institute, Cambridge, and the purpose of this communication is to report that this virus, unlike SBYV, is a true persistent virus. Very few successful transmissions were obtained when *M. persicae* was allowed to feed for 24 hr. or less on infected plants; the aphids appeared to reach maximum transmission efficiency after three or more days infection feeding. Test feeds of 24 hr. or more were necessary for efficient transmission, the best results being obtained by test feeds of 48 hr. or more. The results of one experiment in which the virus–vector relationships were studied are shown in Table 1. Other experiments have given similar results.

Table 1. Number of Successful Transmissions of SBMYV to Sugar Beet (20 Plants per Treatment) using Five *Myzus persicae* per Plant

Time on healthy plants (hr.)	Time on infected plants (hr.) 2	8	24	48	72	96	120	Total
2	0	0	0	4	9	10	11	34
8	0	0	3	7	10	11	8	39
24	0	0	6	10	16	15	19	66
48	0	0	6	14	20	19	20	79
72	0	0	5	17	20	20	20	82
Total	0	0	20	52	75	75	78	

SBMYV persisted for long periods in *M. persicae*; a large proportion of aphids which had fed for 4 days on infected sugar beet were still able to transmit the virus after 9 days on *Brassica pekinensis*, which is immune to SBMYV. It has also been shown that, unlike SBYV, aphids which have acquired SBMYV could still transmit the virus after moulting without access to further sources of virus.

In experiments to find better test plants than sugar beet for SBMYV in the glasshouse, *Claytonia perfoliata* Donn. and *Capsella bursa-pastoris* Medik. were found to be very suitable for this purpose. Bjorling[9] showed that *C. perfoliata* is susceptible to SBYV, the leaves of infected plants showing numerous red spots; however, in plants infected with SBMYV the distal halves of the older leaves became a deep pink colour but without red spots within 2–3 weeks after inoculation. SBMYV was recoverable from *C. perfoliata* without difficulty. The symptoms of SBMYV infection in *C. bursa-pastoris* were a yellowing or purpling and thickening of the older leaves, often associated with marked stunting.

SBMYV is a persistent virus and SBYV a semi-persistent virus; this difference might influence the pattern of spread of the two viruses in the field, which could explain the observed differences in their distribution in East Anglia[1-3].

G. E. Russell

Plant Breeding Institute,
Trumpington, Cambridge.

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A New Living Vaccine against Fowl Plague Disease

In a previous investigation[1] three attenuated substrains from a pea fowl strain were obtained by serial passages mainly in pigeon embryos. One of these substrains ($PFC_2\ P_{11}\ C_{30}$)—that is, pea fowl strain subjected to 2 passages in chick-embryos, 11 in pigeon-embryos and 30 in chick-embryos successively—was subjected for further attenuation to more than 400 passages in pigeon embryos using either the intra-allantoic or the intra-yolk route of inoculation. During these passages 33 desiccated batches were prepared from the pigeon embryos. These batches were titrated in chick-embryos, fowls, and afterwards tested for safety and potency in laboratory and field experiments.

About 15,000 fowls more than 4 months old, of different foreign, native and cross-breeds, were vaccinated during 1952–62, intramuscularly with 1 c.c. dose using either 1 : 500 or 1 : 100 dilution from the desiccated material. The deaths attributable to the virus reaction after excluding other disease factors usually range from 0·5 to 1·8 per cent, according to the condition of the inoculated fowls. Some groups of the vaccinated fowls, each of more than 100, were immunized without any resultant mortality.

Immunization of 2–4 month old chickens was attempted using the intra-ocular and the intra-nasal routes of inoculation without so much success. The wing web method was consequently tried with satisfactory results. More than 5,000 of the young chickens were vaccinated with 2-stabs dose, applying the Liderly-model stab, using either 1 : 100 or 1 : 60 dilution from the desiccated material by pricking through the fold of the wing web, with safe and excellent immunizing results.

Active immunity starts in 48 hr., becomes complete in 2 weeks after inoculation, covering approximately 80–98 per cent of the vaccinated birds. Immunity proved to persist for 16 months in about 60 per cent of the vaccinated birds, and might be prolonged for the life-time in some of them.

This new vaccine proved to have remarkable advantages compared with the oil-base inactivated vaccine, since it has a highly antigenic potency producing solid lasting immunity; also it is easy to be prepared, preserved and applied. Furthermore, it is portable and can be issued to remote places without losing its efficacy or potency. Its cost is negligible compared with that of the oil-base inactivated vaccine.

George Is-hak Mickail

Serum and Vaccine Institute,
Abbassia, Cairo.

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SOIL SCIENCE

Biological Destruction of 2,3,6-Trichlorobenzoic Acid in Soil

Those herbicides which are composed of elements not intrinsically toxic are mostly broken down in the soil sufficiently rapidly for them to constitute no hazard to crops grown in the season following their use at selective dosages. Destruction by micro-organisms is often assumed responsible and has, in the case of phenoxyacetic acids and some other compounds, been clearly proved[1,2].

2,3,6-trichlorobenzoic acid has been found much more stable in the soil than most herbicides[3,4], and it has been questioned whether loss other than by leaching[4] or reaction in the crops or weeds[5] occurs.

We therefore carried out experiments on a sample prepared by our colleague, Dr. G. F. Laws, incorporating chlorine-36 generally distributed in the 2,3,6 positions. The purity of the sample was confirmed by radio assay on crystals and mother liquor after addition of an authentic non-radioactive specimen and crystallization from water. All subsequent radio assay procedures were calibrated from the standard stock solution used for the tests, ionic chloride being obtained by conventional micro-combustion.

1 kg of treated soil was agitated with 1,000 ml. of 50 per cent aqueous methanol containing 0·5 per cent sodium nitrate and filtered. The process was repeated with two further volumes of 750 ml. Filtration was much quicker than from water only and yielded less yellow resinous matter on evaporation. The combined filtrate was made alkaline with caustic soda and reduced to small volume by evaporation at reduced pressure in a steam-heated stripping still. The concentrated extracts were acidified with nitric acid and shaken with ether.

Ether layers were evaporated on to filter paper cylinders and assayed in an annulus counter. The papers were then extracted by aqueous alkali and a standard weight of pure inactive 2,3,6-trichlorobenzoic acid was added to the extract, which was then acidified and allowed to crystallize. The crystals were collected on a 1·2-cm diameter circle of Whatman No. 42 paper, dried, weighed and assayed at an end-window counter to determine the amount of unchanged starting material.

The original aqueous liquor after ether extraction was treated with excess silver nitrate solution. The contaminated precipitate was not easily filtered and was therefore centrifuged, dissolved in aqueous ammonia and reprecipitated by nitric acid before filtration and assay at the end window for ionic chloride. The counting-rate for standard radio ionic chloride was found not to be significantly lowered by total weight of silver chloride on the filter if this did not exceed 100 mg. The amounts precipitated from soil extracts were all below this.

The procedure applied to freshly treated soil gave about 80 per cent recovery and no radioactivity was found in any fraction except unchanged starting material. No significant radioactivity was found in the ionic chloride from soils sterilized before storage or stored frozen. The highest yield of radioactive chlorine in ionic form was 60 per cent of that supplied initially in covalent form. This was obtained after 5 months in fen soil. Mostly the yields were between 30 and 50 per cent at 1, 2 and 5 months. Erratic variation between samples was too great to provide evidence of bacterial adaptation leading to accelerated decomposition or of systematic variation of rate over dosages corresponding to between 1 and 16 oz. per 2″ acre.

The recrystallization assay indicated the ether extract in all cases to be unchanged 2,3,6-trichlorobenzoate. We found, therefore, no chlorine in an intermediate stage between this and ionic chloride. Any compound formed by opening of the benzene ring is presumably too unstable to accumulate.

Total recoveries varied from 35–100 per cent so that significant activity was often not accounted for, but the conclusion is certain that destruction of the 2,3,6-trichlorobenzoic acid molecule does occur in a biologically active soil.

O. R. Dewey
Ruth V. Lyndsay
G. S. Hartley

Chesterford Park Research Station,
Nr. Saffron Walden,
Essex.

[1] Audus, L. J., *Plant and Soil*, 3, 170 (1951).

[2] Audus, L. J., in *Herbicides in the Soil*, edit. by Woodford, E. K., and Sagar, D., 1 (Blackwell, Oxford, 1961).

[3] Dewey, O. R., and Pfeiffer, R. K., *Eleventh Intern. Symp. Crop Protection*, 899 (Ghent, 1959).

[4] Phillips, W. M., *Weeds*, 7, 284 (1959).

[5] Mason, G. W., thesis, Univ. of California (March 1960).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, September 24

Society of Chemical Industry, Food Group (at Church House Westminster, London, S W 1), from 9 30 a m to 5 p m —International Symposium on "Food Regulations in Relation to International Trade"

Tuesday, September 25—Thursday, September 27

Institute of Physics and Physical Society (at Imperial College of Science and Technology, London, S W 7)—Introductory Seminar on "The Theory and Practice of Thermal Neutron Scattering Diffraction, as Applied to the Study of Solids and Liquids', followed by a one-day conference on current neutron work

Tuesday, September 25

Society of Instrument Technology, Control Technology Section (at Manson House, 26 Portland Place, London, W 1), at 5 p m —Exposition—"Control Applications of Static Switching"

Wednesday, September 26

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Discussion on "The Technical Implications of the Report of the Committee on Broadcasting"

Institute of Fuel (at the Institution of Civil Engineers, Great George Street, London, S W 1), at 6 p m —Dr J Burns Presidential Address *

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W C 1), at 6 15 p m —Mr R A Fairthorne and Mr J Farradane "Information Retrieval in America To-day" *

Oil and Colour Chemists' Association, London Section (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr A C B Matthews "The Use of Heat to Accelerate Drying"

Thursday, September 27

Institution of Mechanical Engineers, Railway Engineering Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 10 a m —Convention on "Braking"

Thursday, September 27—Friday, September 28

Society of Chemical Industry, Plastics and Polymer Group (at the Institution of Electrical Engineers, Savoy Place, London, W C 2)—Symposium on "Techniques of Polymer Science"

Monday, October 1

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 30 p m —Mr A J Davenport "Plastics as a Structural Material"

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Scientific Films

Monday, October 1—Tuesday, October 2

Plant Phenolics Group and the British Mycological Society (at the School of Pharmacy, Brunswick Square, London, W C 1) at 10 a m daily—Symposium on "Fungi and their Metabolites"

Monday, October 1—Wednesday, October 3

British Society of Rheology (at the Hotel St George, Harrogate) —Symposium on "The Rheology of Emulsions"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Research Assistant (with a university degree, preferably in history, geography or other appropriate subject, and with an interest in maritime affairs) at the National Maritime Museum, Greenwich—Civil Service Commission (2), Burlington Gardens, London, W 1, quoting 5538/62/7 (September 27)

Assistant Librarian (graduate) in the University Library—The Secretary of the University, Northcote House, The Queen's Drive, Exeter (September 28)

Assistant Lecturer in Chemistry within the Department of Physiology—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N W 1 (September 29)

Lecturer (man or woman, with good academic qualifications, and preferably school experience) in Geography—The Principal, Avery Hill Training College, Bexley Road, Eltham, London, S E 9 (September 29)

Research Assistant (university graduate, either biologist with biophysical training or a physicist with biological interests) in the Wellcome Laboratories of Pharmacology, for investigations into the effects of hormones on the permeability of membranes—The Secretary, The University, College Gate, St Andrews (September 30)

Senior Lecturer in the Department of Veterinary Bacteriology—The Secretary, The University, Edinburgh (September 30)

Lecturer or Assistant Lecturer in Mathematical Economics—The Registrar, The University, Sheffield (October 1)

Scientific Officer (unestablished) (with a first- or second-class honours degree or equivalent in mathematics or science), for work in a section dealing with information and publicity through exhibitions, press, etc , secretarial work for home and European scientific committee, patents and general central administration on scientific matters—The Director, Building Research Station, D S I R , Garston, Watford, Herts, quoting BRS 47S (October 1)

Senior Experimental Officer (with a sound knowledge of electronics, a working knowledge of chemistry, and ability to accept responsibility) in the Department of Chemistry, to initiate and supervise the design and development of electrical, mechanical and glass equipment of all types for use in chemical laboratory research, and to assist in the control of technical staff in the department—The Registrar, Manchester College of Science and Technology, Manchester, 1 (October 1)

Electron Microscopist, Scientific Officer or Senior Scientific Officer (with a first- or second-class honours degree in chemistry or physics with postgraduate research experience in X-ray or electron diffraction, and with a good knowledge of diffraction theory as applied to structural crystallography) in the Department of Pedology—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (October 5)

Lecturer in Pharmacology—The Secretary, University College Gower Street, London, W C 1 (October 8)

Research Assistant (graduate, preferably in biochemistry with a special interest in pharmacology), to continue investigations concerned with the isolation of a polypeptide from urine which inhibits gastric secretion—The Professor of Pharmacology, University College, Gower Street, London, W C 1 (October 8)

Senior Assistant Librarian (graduate, with experience in librarianship, preferably in a university library)—The Registrar, University of East Anglia, 4 Cathedral Close, Norwich, NOR 16 P (October 8)

Lecturer in Psychology—The Secretary, The University, Aberdeen (October 10)

Lecturer (with a good honours degree and preferably a higher degree, and also a special interest in structural geology, mineralogy and petrology) in Geology—The Secretary, Sir John Cass College, Jewry Street, London, E C 3 (October 10)

Lecturer in Applied and Industrial Microbiology in the Department of Chemical Engineering—The Secretary, University College, Gower Street, London, W C 1 (October 15)

Lecturer in the Department of Pathology—The Secretary, The University of Edinburgh, Edinburgh (October 15)

Lecturer or Senior Lecturer (with teaching and research experience in pharmaceutical chemistry, and preferably with working experience in the field of chemical mechanisms of drug action) in Pharmaceutical Chemistry at the University of Sydney, Australia —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Senior Lecturer in Pharmacology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 15)

Senior Lecturer or Lecturer (with an interest in electrical communications) in Electrical Engineering at the University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (October 15)

Plant Anatomist (with at least a university honours degree with botany as a major subject, and at least two years research experience in some field of plant morphology or anatomy) in the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, A C T , Australia, to collaborate in research on the floral histogenesis of the monocotyledons—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2, quoting Appointment No 130/556 (October 20)

Senior Lecturer or Lecturer in Chemical Pathology and Medicine—The Dean, St Thomas's Hospital Medical School, London, S E 1 (October 22)

Lecturer or Senior Lecturer in Biochemistry in the Faculty of Medicine, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 27)

Lecturer (preferably with an interest in systematics and anatomy) in Botany in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 29)

Chair of Biology in the School of Biological Sciences—The Registrar, University of East Anglia, 4 Cathedral Close, Norwich, NOR 16P (October 31)

Senior Research Officer (medical or science graduate with experience in medical entomology) attached to the Department of Microbiology in the Medical School, for whole-time research on arthropods of medical importance found in New Zealand and the Pacific Islands to the North—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, The University of Otago, Dunedin, New Zealand (New Zealand and London, November 1)

Agricultural Economist (with experience in tropical agriculture and a genuine interest in the problems of developing countries), for a technical assistance appointment in the Federation of Malaya, to make a detailed survey and analysis of land use among aborigines—The Director of Recruitment, Department of Technical Co operation, Sanctuary Buildings, Great Smith Street, London, S W 1, quoting RC 213/87/02

Assistant Lecturers (2) (with a good honours degree) in Microbiology—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C 1

Assistant Professor (preferably with a Ph D in physiology) of Physiology—Prof L B Jacques, Department of Physiology and Pharmacology, University of Saskatchewan, Saskatoon, Canada

Biochemist to work in the Department of Surgery on problems associated with the use of chemotherapy in the treatment of cancer—The Registrar, The University, Bristol 8

Curator in the Geology Department—Head of the Geology Department, Chelsea College of Science and Technology, Manresa Road, London, S W 3

Educational Psychologist (with an honours degree in psychology or equivalent qualification with appropriate clinical training, preferably with teaching training and experience) for the Hertfordshire Child Guidance Service and Associated School Psychological Service—The County Education Officer, County Hall, Hertford

Head (with good academic qualifications, and teaching and research experience) of the Department of Physics, Grade III, West Ham College of Technology, Romford Road, Stratford, London, E 15—Chief Education Officer, Education Department, 95 The Grove, Stratford, London, E 15

Physicist or Physiologist (with a good honours degree) for postgraduate research in pulmonary physiology—The Finance Officer, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1

Plant Nematologist (under 45, with several years research experience in plant and soil nematology) in the East African Agricultural and Forestry Research Organization, to take charge of the Plant Nematology Division—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S W 1, quoting T/RC 264/348/05

Principal Lecturer (with a university degree and preferably a corporate member of the I E E , with teaching and industrial experience) in the Department of Electrical Engineering and Electronics, with special responsibility for the field of electrical machines and power—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W 3

Registrar (graduate) of the University of Essex—The Vice-Chancellor, University of Essex, c/o Education Office, County Gardens, Rainsford Road, Chelmsford, Essex

Research Assistant (Academic) (preferably with previous research experience in vacuum physics, optics, spectroscopy or electron-optics), for a programme of research in ultra-violet interference spectroscopy for space research employing image tube detectors—Dr D J Bradley, Physics Department, Imperial College of Science and Technology, London, S W 1

Research Associate in the Institute of Education, to assist with a study of relations between universities and the general public—Prof M V C Jeffreys, Education Department, University of Birmingham, Edgbaston, Birmingham 15

Research Students (2) in Solid State Physics to study semiconducting compounds of interest for thermoelectric applications—Prof D A Wright, Department of Applied Physics, The University, South Road, Durham

Statistician (with a first- or upper second-class honours degree, together with an interest in biological research) in the Department of Biometrics—The Secretary, Grassland Research Institute, Hurley, Maidenhead, Berks

Tutor (graduate with good teaching experience) in Mathematics and/or Physics—The Bursar, College of Air Training, Hamble, Southampton

Woman Graduate (with a degree in geology, and preferably with research experience and artistic ability), to have special responsibilities for museum display under the Curator of the Geological Collections—Mr J M Edmonds, University Museum, Oxford

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Influence of Animal Strain Selection and Conditioning on Biological Experiments and Assays Report of a Symposium organized by the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain, January 16, 1962 Pp iv + 32 (London The Pharmaceutical Society, 1962) 5*s* [307

The International Nickel Company (Mond), Ltd Nickel/Chromium Plating Corrosion Data (Based on a review "The Present Status of Decorative Nickel/Chromium Coatings" by William Blum Published in *Plating* 1961, Vol 48) Pp 19 (London The International Nickel Company (Mond), Ltd , 1962) [307

Bulletin of the British Museum (Natural History) Zoology Vol 8, No 7 A Study of the Rare Birds of Africa By B P Hall and R E Moreau Pp 313–378 20*s* Vol 8, No 8 Hydroids of the Family Cytaeidae L Agassiz, 1862 By William J Rees Pp 379–400 + plates 10 and 11 19*s* (London British Museum (Natural History), 1962) [307

The General Electric Company, Ltd Report and Accounts for the year ended 31st March, 1962 Pp 28 (London The General Electric Company, Ltd , 1962) [28

Map of Southern Britain in the Iron Age Scale 1 625,000 (About Ten Miles to One Inch) Map and 56 page booklet 17*s* 6*d* net , Map only 7*s* 6*d* net , Text only 8*s* net (Chessington, Surrey Director General of the Ordnance Survey, 1962) [28

Gateways Guide to courses of Further Education in Middlesex, Part 1—Vocational Courses, 1962–1963 Pp 55 + xi (London Middlesex County Council Education Committee, 1962) [28

The Institution of Electrical Engineers 1962 Amendments to the Thirteenth Edition of the Regulations for the Electrical Equipment of Buildings Pp 7 (London The Institution of Electrical Engineers, 1962) [28

B B C Engineering Monograph No 42 Apparatus for Television and Sound Relay Stations—Translators, Receivers, and Drive Equipment By F A Peachey, R Toombs and D L Smart Pp 23 (London British Broadcasting Corporation, 1962) 5*s* [28

Scientific Documentation Centre, Dunfermline Spectra Index Pp 9 (Dunfermline Scientific Documentation Centre, 1962) [38

Other Countries

Comptes Rendus des Travaux du Laboratoire Carlsberg Vol 32, No 26 An Apparatus Capable of Rapid Dialysis of 0 1 ml Samples By K K Hjelm Pp 409–423 3 kr 25 øre Vol 32, No 27 The Heterogeneity of Prorennin and Its Transformation into Rennin By Bent Foltmann Pp 425–444 3 kr 60 øre Vol 32, No 28 Sodium and Bromine Fluxes in the Amoeba *Chaos chaos* L By Cicily Chapman-Andresen and D A T Dick Pp 445–468 4 kr 30 øre Vol 32, No. 29 On the Nature of Stentorin By K Max Møller With an Appendix on the Photodynamic Action of the Pigment by K Max Møller and Arthur H Whiteley Pp 471–498 4 kr 75 øre (Copenhagen Danish Science Press, 1962) [28

Institut de Recherches pour les Huiles et Oléagineux, Paris Vingt ans d'Activité 1942–1962 Pp xliv + 239–450 (Paris Institut de Recherches pour les Huiles et Oléagineux, 1962) [28

Fourteenth Annual Report of the Pacific Marine Fisheries Commission for the year 1961 Pp 120 (Portland, Oregon Pacific Marine Fisheries Commission, 1962) [38

National Academy of Sciences—National Research Council Nuclear Science Series Radiochemical Techniques NAS-NS 3105 Detection and Measurement of Nuclear Radiation By G D O'Kelley Pp vii + 138 (Washington, D C Office of Technical Services Department of Commerce, 1962) 1 50 dollars [38

Bulletin of the American Museum of Natural History, Vol 124, Article 2 On the Breeding Behavior of the Cock-of-the-Rock (Aves, *Rupicola rupicola*) By E Thomas Gilliard Pp 31–68 + plates 29–38 (New York American Museum of Natural History, 1962) 1 75 dollars [38

United States Department of the Interior Geological Survey Bulletin 1031-E Geology of Waterpower Sites on Scenery, Cascade and Delta Creeks near Petersburg, Alaska By John Charles Miller Pp iii + 103–125 + plates 8 and 9 Bulletin 1112-D Distribution and Thickness of Devonian Rocks in Williston Basin and in Central Montana and North-Central Wyoming By Charles A Sandberg Pp iii + 105–127 + plates 6–11 Water-Supply Paper 1619-I Pumping Tests in the Los Alamos Canyon Wellfield near Los Alamos, New Mexico By C V Theis and C S Conover Pp iii + 24 + 3 plates Water-Supply Paper 1704 Surface Water Supply of the United States 1960 Part 2-B South Atlantic Slope and Eastern Gulf of Mexico Basins, Ogeechee River to Pearl River Prepared under the direction of E L Hendricks Pp x + 465 Water-Supply Paper 1711 Surface Water Supply of the United States 1960 Part 7 Mississippi River Basin Prepared under the direction of E L Hendricks Pp xii + 594 Water-Supply Paper 1719 Surface Water Supply of Hawaii, 1959–1960 Prepared under the direction of E L Hendricks Pp vii + 211 75 cents (Washington, D C Government Printing Office, 1961 and 1962) [38

United States Department of the Interior Geological Survey Professional Paper 354-H Foraminifera from Onotoa Atoll, Gilbert Islands By Ruth Todd Pp iii + 171–191 + plates 22–25 Professional Paper 378-B Analysis of Plastic Deformation According to Von Mises' Theory, with Application to the South Silverton Area, San Juan County, Colorado By David J Varnes Pp iv + 49 + 2 plates Professional Paper 411-C Transverse Dispersion in Liquid Flow Through Porous Media By Eugene S Simpson Pp iii + 30 Professional Paper 418-A Late Bajocian Ammonites from the Cook Inlet Region, Alaska By Ralph W Imlay Pp iii + 15 + 5 plates Professional Paper 450-B Short Papers in Geology, Hydrology and Topography, Articles 1–59 (Geological Survey Research 1962) Pp vi + 145 Professional Paper 500-A The Concept of Entropy in Landscape Evolution By Luna B Leopold and Walter B Langbein Pp iii + 20 (Washington, D C Government Printing Office, 1962) [38

Canada Department of Mines and Technical Surveys Geological Survey of Canada Geological Maps Index Sheet 65 District of Keewatin—District of Mackenzie Index Sheet 66, 76 District of Mackenzie—District of Keewatin Index Sheet 75 District of Mackenzie Index Sheet 67, 77 District of Franklin—District of Mackenzie—District of Keewatin Index Sheet 85 District of Mackenzie Index Sheet, 86, 96 District of Mackenzie Index Sheet 87, 97 District of Franklin—District of Mackenzie Index Sheet 95 Yukon Territory—District of Mackenzie Index Sheet 105, 115E Yukon Territory—District of Mackenzie Index Sheet, 106, 116 Yukon Territory—District of Mackenzie Index Sheet 107, 117 Yukon Territory—District of Mackenzie (Ottawa Director, Geological Survey of Canada, 1960) [78

United States Naval Observatory Circular No 92 Ephemeris of the Radio Longitude of the Central Meridian of Jupiter, System III (1957 0) By B L Morrison Pp 4 (Washington, D C United States Naval Observatory, 1962) [78

United States Department of the Interior Fish and Wildlife Service Bureau of Commercial Fisheries North American Fauna No 64 Pelage and Surface Topography of the Northern Fur Seal By Victor B Scheffer Pp iv + 206 (112 plates) (Washington, D C Government Printing Office, 1962) 1 dollar [78

National Academy of Sciences—National Research Council NAS–NS 3051 The Radiochemistry of Nickel By L J Kirby Pp iv + 52 (Washington, D C Office of Technical Services, Department of Commerce, 1961) 50 cents [78

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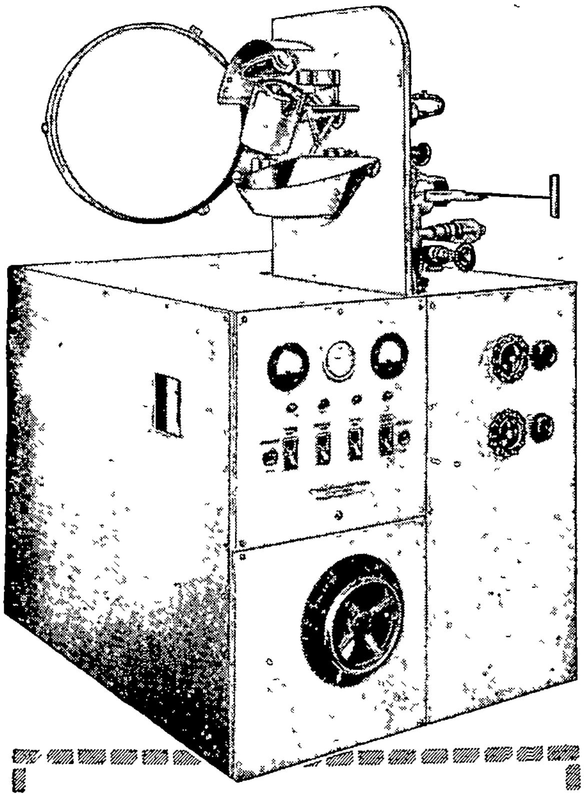

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UNIVERSITY OF NATAL

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Applications are invited from suitably qualified persons for appointment to the post of Senior Lecturer in Geology (Petrology) Durban, on the salary scale R 3,180 by R 120 to R 4,140 (£1,590 by £60 to £2,070) per annum The commencing notch will be dependent on the qualifications and/or experience of the successful candidate, who will be expected to assume duty on January 1, 1963, or as soon as possible thereafter

Application forms and further particulars of the post, including commencing dates, leave conditions, staff bursaries, etc , are obtainable from the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 Applications close in South Africa and London on October 15, 1962

LABORATORY TECHNICIAN REQUIRED to assist in biochemical research on the biosynthesis of proteins Two passes in an experimental scientific subject at Advanced level G C E or Associateship of I M L T or equivalent qualification required Salary, for suitable applicants, on Whitley Council Medical Laboratory Technician scale (£750 to £850 per annum) —Applications to Dr P N Campbell, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W 1

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CALCUTTA

PRIVATE FOUNDATIONS AND THE SCIENTIFIC ERA

THE seventeenth annual report of the Nuffield Foundation, covering the year ended March 31, 1962, which for the most part deals with projects in fields in which the Foundation has worked for many years, is of special interest as illustrating the flexibility which is one of the most valuable features of the programmes of the private foundations* The introduction to the report itself directs attention to this feature of the Foundation's work, noting how in the first few years after the War grants for research in the physical sciences, notably in nuclear physics, figured prominently in the Foundation's activities, until the university departments concerned became fully established and began to attract sufficient permanent finance Then the Foundation, a few years back, noted the opportunity for substantial support of fundamental researches in biology, then much narrower in scope than it is to-day Now the subsequent progress in biological science and the growing support it can claim allows the Foundation to look elsewhere for growing points to be nurtured, and this year it has initiated a large-scale programme which it hopes will lead to improvements in the teaching of school science and mathematics

Over the years the Foundation has made grants for educational research and experiment totalling nearly £300,000 and covering general surveys of university, technical and adult education, sociological studies of teachers and pupils in schools, some broadly psychological investigations, and work on curricula and teaching methods During 1961–62 alone the Foundation allocated some £340,000 for this purpose, reflecting the criticism of the Crowther Report and a memorandum of the Parliamentary and Scientific Committee of the inadequacy of the present national expenditure on educational research, as well as the Foundation's growing interest in education and the existence of people able and willing to try out new ideas in school teaching The greater part of this sum —£250,000—has been allocated to a comprehensive long-term programme on the teaching of science and physics, concentrating initially on five broad sections, the Foundation acting as prime sponsor and co-ordinator, but collaborating fully with other interests in this field

The five sections chosen as starting-points are first, physics for 11–15-year-olds, second, chemistry, third, biology for the same age groups, fourth, secondary school mathematics, and fifth, primary and secondary school science in general The aim in the first three sections will be to provide a complete range of aids in physics, chemistry and biology, designed by teachers for teachers in the first four or five years in grammar schools, and the upper streams of some secondary modern schools, and the programmes will be based on, and are intended to consolidate, the work which has already been done in revising school science curricula The physics project is already under way and is expected to be complete within three years Concurrently with the general science teaching programme, a grant of £1,000 has been made to enable the physics masters at King's College School, Wimbledon and Westminster School to spend four weeks working with the senior science master at Malvern College in the development of new ideas in the teaching of modern physics

The informed interest aroused by the small experiment which the Foundation fostered in the teaching of French at primary-school level at East Ham Grammar School for Boys and Talbot County Primary School, Leeds, has encouraged the Foundation to support a proposal from the Ealing Technical College for developing rapid language courses for adults The proposal fits in well with the Foundation's general interest in language teaching, and a grant of up to £26,200 over three years has been offered The aim is to produce materials for a full-time course of from 8 to 10 weeks duration which would give the adult beginner an understanding of the language—initially German and Spanish—and enable him to use it for most general purposes Another grant of £14,000 over six years has been made to the Institute of Education, University of London, towards an extended inquiry by the Reading Research Unit into the effectiveness of using an augmented alphabet in teaching children to read A grant of £45,000 was also made towards the building of a graduate house at Somerville College, Oxford The supplementary grant of £8,500 made to the Department of Anatomy, University College, London, largely for necessary units in connexion with the use of the learning computer, further illustrates the Foundation's interest in new educational developments

Two other illustrations of the value of flexibility are to be found in this report The Foundation was early concerned with the health of people at work, and in its first decade did much to establish the study of industrial health as an academic discipline, it also helped to set up the first practical experiments in group health schemes for smaller factories and to provide an occupational hygienic service for industry. Time was needed for these innovations to prove their worth, and meanwhile the Foundation's interest in industrial health lay dormant In 1960 it was actively revived, whereupon the Foundation allocated £250,000 for new developments in this field, and industry's present receptive attitude to industrial health schemes indicates that the Foundation's renewed support has been given at the right time The present report records the offer of a temporary grant of £20,000 over five years for the establishment by Prof R E Lane, professor of occupational hygiene, within the University of Manchester of an occupational hygiene unit to serve the needs of industry in north-west England and Wales, on the assumption that the unit will become self-supporting by the fifth year £60,000 has also been promised for capital needs and recurrent expenses on a tapering basis to experiments in Rochdale and in Dundee in establishing group health schemes for local firms

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* The Nuffield Foundation Seventeenth Report for the year ended 31 March, 1962 Pp xiv+159 (London The Nuffield Foundation, 1962) (see also p 1,258 of this issue)

The second illustration is to be found in the work of the Foundation overseas While at the end of the present year the Foundation decided that, since South Africa had chosen to leave the Commonwealth it could not continue the special help it had been giving to the Union, the overall needs of Commonwealth territories are of lasting concern Existing schemes, mainly for research and training, will be run down during the next twelve months, after which the Foundation must regard South Africa, so far as eligibility for grants is concerned, on the same footing as other non-Commonwealth countries, and, although the Foundation is glad to have made a significant contribution to the development of the universities in South Africa, the free universities, in their stand for academic privileges and standards, will not wish it otherwise

In its overseas work to-day the Foundation rightly recognizes, however, that the newly developing countries have a major claim It is difficult to exaggerate the size and diversity of their need, and although no private foundation, however large its resources, can make much direct impression on the economic conditions, sound judgment in choosing the right projects can be of decisive effect Here, too, the selection of the growing points is what matters, and, in his Chancellor's lecture to the University of the Witwatersrand last April, Sir Eric Ashby indicated how wide a field there is for experiment with educational schemes and local community development The Foundation's interests in the developing countries are very much determined by the effective application of the principle of self-help, and of this the Centre for Educational Television courses towards which the Foundation has matched a Government contribution of £100,000 with a like sum, to be made available to the Centre over five years, are an outstanding example

In this connexion the grant of £30,000 a year for three years which the Foundation has made to the Overseas Development Institute should be mentioned, the Anglo-American study on general development problems which the grant is to support is concerned with the problems of emergent countries and the aid that might be given them and derives much of its value from its unofficial character

Of grants totalling more than £190,000 in 1961–62 for the Commonwealth Overseas it is pertinent to note that the Foundation has offered £4,000 to the Inter-University Council for Higher Education Overseas for the first year of a scheme for training technicians of overseas university institutions, in the hope that thereafter the Department of Technical Co-operation will support the scheme £50,000 has been set aside over three years towards the expenses of an organization concerned with the development of improved materials and curricula for African schools, a grant of £34,100 has been made to the University College of Rhodesia and Nyasaland for fundamental research at Lake Kariba, in co-operation with the University of the Witwatersrand, and also, it is hoped, in association with the zoology department of a university in Great Britain, covering the physical and chemical states of the water, the biology of the phyto- and zoo-plankton, and the biology of the invertebrate fauna and of particular plants A grant of £16,000 over five years has been made to enable Prof W J M Mackenzie of the Department of Government at the University of Manchester to assist in developing teaching and research in public administration in East Africa, particularly by creating a link between work in that field in Manchester and in Nairobi

Three other grants, also to Africa, are also particularly significant in this same connexion A grant of £12,500 has been made to the African Medical and Research Foundation covering the cost of fifty high-frequency radio sets to enable the Foundation to connect outlying district and mission hospitals in remote areas with headquarters With the Gulbenkian Foundation, the Nuffield Foundation is sponsoring a scheme of the Royal Commonwealth Society for the Blind for the education of blind children in Nigeria, providing £15,000 over the period from July 1962 to December 1965 by which time it will be locally self-supporting The investment could bring about a spectacular advance in the education of handicapped children, and if the scheme is successful in Nigeria it may provide a pattern for the education of blind children in other emergent Commonwealth countries

The third project also meets a need that has become acute since African territories reached independence A grant of up to £8,500 over three years to the Department of Zoology, Fourah Bay College, is to enable the College to pay the extra salaries involved in taking over the Fisheries Research Laboratory in Freetown, Sierra Leone, on the understanding that the College would finance these posts after 1964 There is at present no marine biological research activity between Dakar and Accra, and the position of Freetown on the sea and estuary makes it particularly suitable for the study of tropical marine biology Trained marine biologists are greatly needed in Africa, and the grant may also make it possible for the Department to develop research into fishing resources, about which little is known

In so far as this project may eventually contribute a valuable addition to the diet of the population in the shape of fish protein, it may be set beside a fourth project—the grant of £17,500 over three years to the Scientific Research Council of Jamaica in support of a Leaf Protein Unit set up by that Council to develop the production of protein from local leaves What is worth noting is that the Foundation is giving vital help in fields where the Governments of newly independent territories have largely failed to appreciate the significance of research or been unable as yet to find the necessary resources Long-term research is particularly liable to suffer in this situation, and the lead which the Nuffield Foundation is giving should be noted also by the Overseas Research Council and the Department of Technical Co-operation

There is a further kind of interest of the Foundation which is noted specially in the introduction to this report which equally illustrates the unique value of the independent foundation in the world of to-day This lies in new ventures which have as yet no practical expression When such a new direction of interest is established the Foundation must often wait its opportunities and seek out the right people, it may be necessary first to test out ideas on a small scale, while at other times a major concern may develop very rapidly The Foundation must be ready to seize such an opportunity and back a favourable judgment with adequate support, and it therefore welcomes, and encourages, the flow of ideas and proposals which come to it from outside This seventeenth annual report of the Nuffield Foundation fully demonstrates how invaluable a place the private foundation has in discovering and fostering the growing points in society to-day It can take risks which no Government institution could wisely do and which even the university might hesitate to accept—even if it had the resources No expansion of Government support for research and development or modification of Government organization in that field is likely to diminish, still less eliminate, the need for such pioneering, its emphasis on response enables the Foundation to do something that is much more difficult for a Government body to attempt

THE SUN DURING THE INTERNATIONAL GEOPHYSICAL YEAR

I G Y Solar Activity Maps, *D* I, and *D* II

Annals of the International Geophysical Year, Volumes 21 and 22 (International Council of Scientific Unions—Comité International de Géophysique) 1,116 maps (London and New York Pergamon Press, 1961) £54, 150 dollars

WHAT might very roughly and a little misleadingly be called solar meteorology, the study of all kinds of changes on the surface of the Sun, began in a primitive way some centuries ago with the observation of sunspots It was not, however, until the nineteenth century that much real progress was made in systematizing the results, and not until the twentieth that the close connexion between events on the Sun and subsequent events on the Earth began to be understood Some of the essential techniques are more recent still The Lyot filter did not come into general use until the 1930s, and radio-astronomy was virtually unknown until the middle 1940s Our appreciation of some of the related phenomena, for example, of cosmic rays, also depends on rather recently acquired knowledge

We now have a picture of a Sun in which somehow powerful magnetic fields are transported piecemeal to the surface, in a kind of 11-year tide, and where these fields produce, or have associated with them, not only sunspots but also a number of other phenomena flares, coronal changes, etc These in turn produce, or are associated with, irregular enhancements of radiation in the far ultra-violet and in the radio spectrum, sometimes of cosmic rays, too, and are accompanied by the ejection of clouds of ionized gas with velocities from hundreds to a thousand or more kilometres per second Some of these gas particles reach the vicinity of the Earth, giving rise to magnetic storms, auroræ, changes in the ionosphere, etc Within recent years the rise of artificial satellite research, and the possibility of manned flight outside our atmosphere, have stimulated much interest in what happens in interplanetary space during these disturbances

Some ten years ago, when plans were being made for the International Geophysical Year of 1957–58, it was natural to include an intensive campaign to record as completely as possible all events open to our inspection on the solar surface Routine solar observations, in contrast to many geophysical measurements, are prone to serious interruption from the alternation between day and night and from the weather, so that to obtain a solar record sufficiently continuous to give good correlations with geophysical data it is necessary to organize world-wide co-operation between observatories Further, after the observatories have done their part, the data have to be collected and published in some concise and convenient form, if they are to be of real value All this has been done, and we have here, edited by Dr M A Ellison, Volumes 21 and 22 of *Annals of the International Geophysical Year* They consist of two large filing cases, each containing for each day of the period from July 1, 1957, to December 31, 1958, a loose sheet on which the observed features of the surface of the Sun are entered in simple outlines of various colours, according to a given code

The diameter of the solar image on these maps is 15 cm and, to avoid confusion in the reproduction, heliographic co-ordinates (which are essential for the effective use of such maps) are provided, not on the maps themselves but on transparent sheets, in the form of a set of the well-known Stoneyhurst disks The appropriate sheet is selected from figures given on the *D* I maps

The maps of the first set (*D* I, Volume 21), the production of which was supervised by the High Altitude Observatory, Boulder, Colorado, give sketches of sunspot umbræ and penumbræ, mainly from the routine observations made regularly at Zurich and Locarno, and the outlines of calcium plages from observations made for the most part at the Crimea, Kodaikanal, Arcetri and McMath solar observatories In addition, flares observed in Hα light, of importance 1 + or greater on the usual scale, are entered with their position to ± 1°, and with their time of occurrence and importance This flare record is based on very numerous Hα patrol photographs provided by 26 stations located all over the world Finally, the *D* I maps also give all available information about active prominences and surges (A surge prominence is observed as a column of material expanding more or less radially outwards from the Sun and then returning along the same path, an intriguing and imperfectly understood phenomenon best seen on motion picture films)

In the *D* II maps of Volume 22, prepared at the Fraunhofer Institute, Freiburg, Germany, there is a similar presentation of sunspot *groups* (from daily maps prepared by the Fraunhofer Institute), giving their positions, magnitude, Zurich classification and the number of individual spots in the group. Limb prominences, disk filaments and indications of 'sudden disappearances' (of filaments or prominences) are also given, all based on observations in Hα with either Lyot filters or spectroheliographs. Around the disk, at intervals of 5°, intensities of the coronal green line, 5303 Å, are plotted in the usual convention in which distance from the limb is proportional to intensity. Another transparent disk is provided for reading off these intensities conveniently. The original measures, made at nine different observatories by slightly varying methods, refer to positions between 40″ and 1′ above the limb, and have been reduced as well as possible to a common scale. Finally, lines of equal intensity (radio isophotes) are given for 21-cm radiation, the measures being made with a multiple pencil beam, 3′ wide, at the Radio Physics Laboratory, Commonwealth Scientific and Industrial Research Organization, Sydney.

The two sets of maps cover between them most routine observations of the Sun during the International Geophysical Year which it was possible to make without too much difficulty by existing techniques. Measurements of solar magnetic fields are perhaps the most important exception and these are to be given separately in a later publication. The maps are an impressive example of what can be achieved by international scientific collaboration between five continents. They provide a permanent record of the behaviour of the solar surface during a period of very high sunspot activity, and astronomers, geophysicists and others should be able to refer to them with profit for many years to come. As is now generally agreed, it appears highly desirable to repeat many of the activities of the International Geophysical Year during a typical period of minimum solar activity. The hope is that observations made over the period April 1, 1964, to December 31, 1965, will eventually become available as an essential supplement to these volumes.

The maps are arranged and printed well. The explanatory preface is given in both English and French. The very high price is likely to restrict their sale to the bigger science institutions and libraries.

H. von Kluber
R. O. Redman

ASTRONAUTICS AND ASTRONOMY

Advances in Space Science and Technology

Volume 3. Edited by Frederick I. Ordway, III. Pp. xiii + 482. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 100s.

The first two volumes of this series were entitled *Advances in Space Science*: the addition of *Technology* provides a better description of their contents, but does not imply any change in editorial policy.

Volume 3 consists of 8 papers, of which the first five form an admirable review of the solar system (excluding the Sun and the Earth), regarded as an objective for astronautical exploration. The first paper, "The Role of Geology in Lunar Exploration", by J. Green and J. R. Van Lopik, is a 110-page survey of a rather neglected subject—the proper method of study and the uses of the lunar surface materials. The second, "Venus as an Astronautical Objective", by P. Moore and S. W. Greenwood, describes the little that is known of Venus and discusses possible transfer orbits by which it might be approached. The third paper, by S. L. Hess, considers "Mars as an Astronautical Objective", outlining our present knowledge of the planet and suggesting how it might be explored. The next paper, by R. L. Newburn, jun., is a similar review of Mercury, the asteroids and the outer planets. The fifth and last paper on the solar system is a short account of interplanetary matter by E. Manring, which emphasizes the gaps in present knowledge and the need for better calibration of the instruments for detecting interplanetary dust. All these papers are well written, and together they constitute a most useful preview of the space explorer's sphere of operations.

It is interesting that three of the papers advocate observations (of Mercury, Venus and Mars) with large telescopes carried by balloons to great heights, where the 'seeing' would be better than on Earth: perhaps this logical preliminary experiment will be classed as 'space exploration' and thereby qualify for a small fraction of the vast expenditure on rocket-launched vehicles.

The last three papers are primarily technological. A. Alberi and C. Rosenkranz discuss the "Structures of Carrier and Space Vehicles" in a 120-page monograph covering everything from pre-launch loads to the properties of structural materials; though thorough, the paper is less readable than it might be, chiefly because of an overload of abstract nouns. The next contribution, short but adequate, is by W. C. Cooley on "Advanced Nuclear and Solar Propulsion Systems". The last and least satisfactory paper is on "Human Factors Aspects of Weightlessness", by P. A. Campbell. The attempts to define weightlessness are unconvincing, and the tendency to ignore non-American work is carried too far: for example, "The author is not aware of any individual who has been constantly weightless for more than a few minutes", but since the author mentions Alan Shepard's 4 min. of weightlessness in May 1961, he should have been aware of Yuri Gagarin's 75 min. in April 1961.

D. G. King-Hele

CHEMICAL ENGINEERING

Advances in Chemical Engineering

Vol. 3. Edited by Thomas B. Drew, John W. Hoopes, Jr., and Theodore Vermeulen. Pp. x + 345. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 86s.

The third volume in this series appears after an interval of four years. Once again the individual contributions are of a high standard, but there does not appear to be a consistent editorial policy. Thus, the chapter on crystallization from solution would be appropriate in a general text-book on chemical engineering. It is in no sense a complete review, there being a number of surprising omissions, particularly in respect of recent work in Britain. The chapter on mixing and agitation is comprehensive, not only referring to recent work but also reviewing all the important work in the field.

High-temperature technology is a rapidly developing field in which much of the basic work has been carried out by the chemist and the physicist and may not be familiar to many chemical engineers This is the kind of subject which benefits from the treatment which can be given in a book of this type The methods of measuring and of attaining high temperatures are described in detail, and the authors look forward to fields of application which are of interest to combustion engineers and others

In much of the earlier theoretical section of the chapter on the design of packed catalytic reactors, matrix methods are used, but the advantages of so doing are not apparent, apart from brevity The authors summarize most of the recent work on velocity profiles and transport phenomena in packed beds and give practical design procedures, paying special attention to the approximations which may be introduced and the probable errors arising therefrom

In the final chapter, optimization methods are discussed The use of optimization techniques is becoming of increasing importance with the general downward trend of profit margins in the chemical industry The author discusses their application in relation to determining optimum conditions for an individual item of plant such as a chemical reactor, and for the more general problem of formulating construction and maintenance programmes to produce the maximum profit in a given period The treatment is concise and provides a necessary background for the chemical engineer concerned with either plant design or with forward planning

This volume contains much valuable material, and will be useful both to the practising chemical engineer and to the student who is taking an advanced course in any of the fields discussed J F. RICHARDSON

TREATISE ON ZOOLOGY

Traité de Zoologie

Anatomie, Systématique, Biologie Publié sous la direction de Prof Pierre-P Grassé Tome 4 (Premier Fascicule) Plathelminthes, Mésozoaires, Acanthocéphales, Némertiens Par J -G Baer, P de Beauchamp, M Caullery, L Euzet, M Gontcharoff, P -P Grassé, Ch Joyeux Pp 944+4 planches en couleurs (Paris Masson et Cie , 1961) 200 NF

THIS is the first part of the fourth volume of this well-known work It is divided into four approximately equal parts—one dealing with turbellarians, one with flukes, one with tapeworms and one with mesozoans, nemertines and acanthocephalans Nematodes and rotifers will be dealt with in the second part The authorship is French and Swiss, and is both authoritative and distinguished One has become accustomed, in earlier volumes of "Grassé", to excellent typography and illustrations, and the same high level is maintained here The volume seems to have more photomicrographs than previous ones they relate mainly to parasitic forms, and, excellent though they be, the complicated anatomy of these animals is better displayed by the beautiful line diagrams There are four coloured plates, one of polyclads and three of nemertines—one, indeed, a reproduction of McIntosh's well-known seventy-year-old figure of a tangled boot-lace worm. There are more typographical errors than one expects to find in a book of this sort they become particularly tiresome to the kind of reader that one hopes will use the book when they occur (as many do) in bibliographies. It is also disappointing to find references in the text—even as specific as "Voir en particulier Koehler—32 " (p 75)—and no corresponding bibliographical entry

This said, may it be added that the treatment is excellent, particularly in relation to parasitic groups, where the biology, life-history and evolution of an extended series of types is presented, as a zoological rather than a medical or veterinary matter. In dealing with free-living forms the approach is predominantly systematic, and many students might prefer the more comparative account by Hyman "Grassé" has never pretended to treat the physiology of animals except as an incidental, but it is disappointing that the outlook of functional morphology is so lacking—for example, of the two papers by Cowey and Clarke which make intelligible much of nemertine and turbellarian form one is not mentioned and the second receives only the briefest acknowledgment Given its own point of view, however, this is a valuable and welcome addition to zoological literature

ALASTAIR GRAHAM

GENETICS *AB OVO*

Cell Heredity

By Ruth Sager and Prof Francis J. Ryan Pp. xi + 411 + 16 plates (New York and London John Wiley and Sons, Inc , 1961) 60*s*.

MOST of the text-books of genetics published in the past decade still followed the traditional treatment set by the oldest and most persistently successful of them all Sinnott and Dunn's. This treatment started with the description of variation between individuals of a species, introduced Mendelian inheritance and its principles, went on to linkage and the chromosome basis of inheritance, branched out into the ways in which genes produce their effects and concluded with population genetics and evolution

Sager and Ryan make a brave attempt towards a novel approach they start from deoxyribonucleic acid and work their way up to the genetic control of cellular integration Because of its methodological divergence, they omit population genetics The greatest part of the book is based on examples and methods from microbial genetics The material is therefore largely of a kind which was barely represented in older text-books

This attempt deserves all respect, and most geneticists will welcome it, with minor reservations In its present form the book will be especially useful to those who—by profession or hobby—have already gone through a more traditional introduction to genetics Its usefulness lies in the wealth of well-summarized examples—though with a somewhat high rate of mistakes—from the genetics of bacteria, bacteriophages, fungi and unicellular algæ, and in its good review of the present state of knowledge on non-nuclear inheritance

Its shortcomings lie mainly in the logics of presentation and argumentation, at their weakest in the chapter on "What is a Gene ?" Yet, this same chapter will be found most useful in bringing together data on fine genetic analysis in several organisms

On the whole we have to be most thankful to the authors for taking up a difficult task, and wish them future editions in which their attempt will become as fully fruitful as it deserves to be

G PONTECORVO

SCIENCE AND WORLD AFFAIRS*

By The Right Hon Viscount HAILSHAM, Q C

Lord President of the Council and Minister for Science

IT is clear to-day that scientists have much to say to public men which the latter ought to know and perhaps do not It does not follow, however, that public men have nothing to say to scientists, or that both have not much material for mutual discussion What is clear is that the social and political problems of the day, and the technological and scientific questions are in a great degree closely related, perhaps inextricably connected, and neither the scientist nor the politician can afford either to disinterest himself in the discussions of the other, or not to feel himself wholly involved in the success of the other's attempts to solve his part of the puzzle

However, since I have come to fulfil my own part of the bargain and to consider what might be said appropriately by a Minister of H M Government on an occasion like this when distinguished scientists from many countries of the world are meeting, I almost came to repent what I had done

There is not, I believe, as yet, any genuinely scientific theory of politics Whether there ever can be I will not profess to argue, but let any who thinks that such a system would be easy to develop reflect at least that the parameters involved would be numerous and somewhat difficult to identify In the meantime, we dwell in the pre-scientific world of the craftsmanship, such as it may be, of the practical politician, and the insights, such as they may be, of the occasional political theorist

But it does not take a scientist or a politician to see, or to say, that the human race is in a state of crisis, and that, unlike all previous human crises, the nature of the crisis is such as to affect not simply human groups or institutions but the entire life and condition—perhaps the survival—of the human species in the world

For this, of course the responsibility lies on the progress of technology, the application of organized human knowledge to the processes of manufacture, distribution, transport, and the dissemination of ideas It is this which has revolutionized human life in a matter of generations, it is this which has confronted at last the widely separated branches of the human family in almost intimate contiguity after long eons of separation It is this which has organized the possibility of mass annihilation by the evolution of the sophisticated guidance systems of modern rocketry and the immensely destructive powers of modern fission and fusion

It is therefore this, and the intriguing possibility that science may afford even more challenging prospects of agreement and co-operation—a way out, as it were, of the ideological impasse through agreement on technological essentials—which can lend significance and hope to a meeting between scientists

Perhaps our most essential human characteristic is the desire to conquer our environment by conscious and co-operative effort In one sense this is what science, and in another sense this is what politics, is about But if this is so it is as well that politicans and scientists should think a little carefully about the human qualities which it is necessary to establish and encourage if the human species is to survive in what is essentially a new environment I do not think it is unscientific to do this If we are to pursue the attempt to control our environment we must surely make a serious and co-operative effort to determine the line of our own evolution, and by this I mean to include for this purpose the selection and development of the instincts and habits of mind and behaviour likely to lead to human survival I do not think this is simply a question of genetics, if it were it would be unlikely to yield quickly to scientific persuasion But as Prof P B Medawar has pointed out, human inheritance is not simply a question of genes and chromosomes, the dissemination of ideas and habits by communication in time and space themselves constitute a parallel system of genetics to the natural system, and I do not think that either scientists or politicians can exaggerate the survival value to the human species of such human characteristics as compassion, law-abidingness, rationality, patience, and what is called honesty and integrity It may be, as I think one of the American papers has recently pointed out, that in the past the so-called military virtues of courage and combativeness, the war-like instincts which are quite certainly part of our natural make-up have been essential for survival of any group whether in their contests with natural environment or in inter-tribal struggles No doubt, too, these instincts will have their properly controlled part to play in the future, since there is no reason to fear that the future will hold out any less scope for the spirit of adventure than the past But it is worth emphasizing that when what is at stake is the survival of the species, the civilized and peaceful instincts which are no less part of our natural inheritance will have a predominant part to play and will need whatever encouragement the politician and the scientist can have to offer them

When I said that there was no fully scientifically developed theory of politics, I had in mind primarily the so-called ideologies But I had also partly in mind political institutions For it is clear enough that these largely antedate the technological revolution, and derive their size and shape from factors which are only partly contemporary—the national sentiments, the geography, the economics, and the military strength of bygone generations Such as they are, they are clearly imperfectly adjusted to the actual requirements of the present age, and these very requirements are themselves altering so rapidly that to adjust political institutions perfectly to them as they are—in a state of flux and transition—would be a task almost beyond the wit of men Though this task must be attempted, it is evident that the period of adjustment will be difficult, prolonged, and continuous—another and pragmatic argument in favour of those peaceful qualities to which I have referred

Nevertheless, it is, I think, worth while to pause for a moment and survey the extent to which the imperfection of human political institutions may have an influence on the subject-matters of special interest to the supporters of the Pugwash Conferences, and on the extent to which such discussions may themselves point a way to the kind of institution which we ought to be developing

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* Substance of the opening address on September 3 to the Tenth Pugwash Conference held in London

Pugwash discussions have in the past largely concentrated on the technical problems of disarmament and on the equally important issues connected directly with the physical sciences and world affairs I regard them as extremely valuable, nor do I wish to complain, since I regard the assumption as essential to their fruitfulness—that they have been based very largely on the assumption of the continuance of existing political institutions and of some sort of *modus vivendi* between existing political systems or, as they are called, ways of life

I do not myself, however, believe that these assumptions are necessarily correct So long as negotiations for disarmament are based on what is fundamentally the negative approach of the reduction of actual arms, and the prohibition of their manufacture or development, I do not myself believe that they will enjoy complete success What I believe is required for complete disarmament is a recognition by the opposing powers not simply of the dangers of the continuance of the present arms race, but of a prior need for co-operation in projects as exciting and as expensive as the struggle to maintain the uneasy balance of terror which constitutes at present our somewhat precarious guarantee of peace In other words, I would like to see the Communist and the Western World agree to spend a proportion of their national income not less than they are now spending on arms and defence on ascertainable projects of a different order—internal and external—education, building, research, civil engineering, agriculture, and health The inspection and verification of these programmes could not present the difficulties to national security envisaged by the present disarmament negotiations, and if they were expensive enough and actually undertaken, preferably in co-operation, they would offer a fairly definite guarantee that disarmament would proceed

At the same time I am not myself prepared to proceed on the assumption that our present pre-scientific political institutions are necessarily to be regarded as immutable for all time or changeable only under the pressures, sometimes logical, and sometimes fortuitous, of events

I hope I shall not be regarded as one of those who regard war as an ineluctable biological necessity But I wish that more politicians and more scientists would examine the hypothesis to which I myself incline, that it is the invariable *sequela* of wholly independent and separate sovereignties, in close physical association with one another, in which case the creation of an effective world political organization for the human race which will respect human liberty, and afford a reasonable measure of national independence, but none the less impose limitations on both, becomes a prime necessity for the ultimate success of meetings such as the Pugwash Conference

Nor am I prepared to accept as presenting a stark and permanent dilemma the apparent antagonism presented by the alternative systems of socialism and free enterprise Both systems derive their principal economic and political ideas from traditions and writers whose point of view and even whose technical language largely antedate Darwin, and whatever of permanent value there may be in either, the greater part of the human race is at present living in conditions to which neither is self-evidently completely relevant

However this may be, I do not regard either the West or Communism as having reached a state of perfection which entitles either to adopt a purely negative, patronizing or destructive attitude towards the other The mere pressure on both of problems like population and food production will cause each to modify his practice, and, one hopes, not too long afterwards, to alter his political theories In the meantime aggressive or even moral talk unaccompanied by achievement in elevating the national and moral condition of mankind is a barrier to further progress which ought to be based on the interchange of technical, cultural and moral ideas by discussion, and on co-operation on objectives which can offer advantages to each What the world wants at the present time is less political polemics but deeper study and insight, more co-operation and more discussion, and greater concentration on practical and technical problems

The need for a central political authority, of which I have spoken, seems to me to be emphasized by two practical considerations The first is the nature of the nuclear weapon, and the second the magnitude and nature of the claim on resources likely to be made if human life outside the highly industrialized nations (or even including them) is to be substantially improved at an acceptable rate

Apart from pressing issues of testing, verification, and inspection which I do not propose to discuss, the core of the problem of nuclear weapons lies in the fact that they are not very secret, not very difficult to make, and that if nuclear energy for peaceful purposes is developed to anything like the extent we expect and hope, the quantity of plutonium (quite apart from enriched uranium) which will be available in the world will be substantial and widely diffused The so-called problem of the *n*th power will, therefore, always be with us whether or no the large powers conclude an effective disarmament agreement If, therefore, such an agreement is to achieve its purpose, it is plain that some international mechanism armed with effective powers must be created to contain this threat

The second argument which I use is related to the undoubted need of the so-called under-developed countries for aid I have made no attempt to calculate what this need amounts to since it is pretty obvious to anyone with even a nodding acquaintance with the communities and nations concerned that they could absorb without difficulty all the capital resources now being spent on armaments and a great deal more without their needs being met At present there seems to me to be neither rhyme nor reason for much that is being done, and I personally have no doubt that much of the resources at present being devoted by East and West to the provision of aid is either wasted, or even, as in the case of the provision of modern armaments, actually detrimental

Yet the dilemma presented is a genuine one New nations prize their independence more than any other asset It follows that it is difficult to assume any other means for identifying projects worthy of priority than the wishes of the Governments concerned—which are quite frankly not always very sensible—and there are perhaps few methods open to a developing country to secure favourable treatment other than a rather squalid attempt to offer, in return for aid, some degree of qualified political support, or the reverse, in the struggles for superiority between East and West

The need for an objective organization with adequate resources capable of selecting types of projects suitable for assistance, with a due regard to priority, and administering them without being drawn into the major ideological struggle would seem to be overwhelming in the interest of East and West alike and, if the proper means were discovered of carrying it out, a fertile field for co-operation would have been opened for cultivation

There is also clearly an established opportunity for international co-operation in the purely scientific field By 'international co-operation' I do not necessarily mean world co-operation in every part of the field, because I myself envisage much more complex and sophisticated patterns on which different groups of nations of different sizes and requirements co-operate for different purposes related to their special needs and responsibilities Still less by 'co-operation' do I necessarily mean 'centralization', for my own experience has led me to believe that certain types of international scientific co-operation can be achieved between nations supporting national programmes of scientific education and research of individual excellence

But I would identify the following fields in which international co-operation on some scale and with some kind of organization is becoming a practical necessity

The first of these in the field of the earth sciences—such as oceanography, geography, the study of the Earth itself, its mantle, its core, its magnetic field, the ionized belts beyond its atmosphere and so on, where quite apart from the expense involved the observations necessarily presuppose a world-wide chain of observers and observations Clearly, optical and radio astronomy are cognate fields in which parallel considerations apply

The second is where the mere cost of the apparatus necessary to carry out first-class research is such and its life as a research tool so short-lived that it is beyond the power of any nation and perhaps of any single group of nations to provide a complete range of the facilities required I would myself list here high-energy physics, nuclear physics, and, almost certainly, space research But as each science and discipline develops there are probably many other fields in which the same conditions will develop

The third is the whole range of studies connected with health Experience with the U K Medical Research Council has convinced me that in all the more radical aspects of medical research a single nation with, *ex hypothesi*, a limited geographical habitat is often too small a unit of study for positive results It is not simply that certain diseases are prone to attack all humanity wherever they live but also that new light on general problems can often be known only by study of local peculiarities I cite only the effect on the study of human genetics of the discovery of the relationship between sickle cell anæmia and resistance to malaria, or how the very suggestive facts recently come to light about the geographical distribution of a rare form of infantile cancer may throw a light on the general nature of cancer itself

But, of course, the whole problem of international co-operation comes to a head again on the subject of aid, with which I have already dealt in a slightly different connexion. For the greater part, human immunity to disease and want does not require any great advance in scientific knowledge so much as the application of known techniques and adequate resources, such as water, transport, fertilizers, power, engineering and building construction of all kinds I have already indicated that I do not myself believe that the use of aid by the two Power Blocs as a means of pursuing the ideological struggle is not really in the interests of either, or of the recipients of the aid I therefore identify as my last and most significant field for international technological co-operation the application of known technology for the development of human happiness and culture throughout the planet by means which will not by themselves secure to any Power Bloc a political advantage over the other

HAZARDS OF THE ROAD

By DAVID NAPLEY

Chairman of the Executive Council of the British Academy of Forensic Sciences

THE third annual scientific meeting of the British Academy of Forensic Sciences, held at the Law Society's Hall during July 28–29, was called to consider "The Hazards of the Road", a subject to which, having regard to the increasing public danger deriving from motor traffic, the sciences appear to have devoted disproportionately little research Following the presidential address by Sir Thomas Lund, secretary of the Law Society (in which he outlined the results of a comparative study which he had conducted into the practice and value of expert testimony throughout the world, and the merits of a panel of experts available to assist the judges), the meeting considered its subject, under four main headings the driver, the pedestrian, the vehicle and the road No doubt remained at the conclusion that the fitness, ability and skill of the driver transcended in importance every other single factor

As Prof H J Eysenck, of the Institute of Psychiatry (University of London), in the opening session estimated, while 10 per cent of traffic accidents are caused by mechanical and technical faults, something like 90 per cent are due to human factors Research work (much of it carried out in Finland, Austria and the United States), he said, in the form of psychological tests on those involved in accidents, for comparison with the results of such tests on those not so involved, tended to show personality traits such as aggressiveness, carelessness, impulsiveness and the like which are not present to the same degree in those persons who have been free from accidents

While noting this fact, the Law Section of the Academy had already observed how relatively little attention is paid to the physical and mental fitness of a person to drive before he or she is issued with a driving licence That this should be so is, perhaps, conditioned by the climate of public opinion exemplified by a speaker from the floor, who claimed "that, the driving licence, being a fundamental human right, the State has no right to deprive a man of such a licence unless it is absolutely certain that he cannot drive" This view was at direct variance with that of the Law Section as later expressed by Dr Ian Milne, H M Coroner for the County of London, who declared that the holding of a licence to drive a motor-vehicle is not a right but a privilege, for which the citizen must accept the responsibility of satisfying the authorities of his fitness, both mental and physical, to control the vehicle To take only two examples of the inadequacies of the present system: a driving licence need not be completed in the handwriting of the applicant Yet, is it not a significant factor in determining the qualifications to drive that the applicant is quite illiterate ? While, as Dr Milne remarked, a test of literacy is, perhaps, not always a test of intelligence, it is some guide. It would certainly afford no defence to a charge of disregarding traffic signs, it may be added, that a defendant had been unable to read them In the second place, driving licences are now issued for three-yearly periods, if one accepts the premise that in general applicants for licences truthfully and accurately answer the limited questions required of them (and there is clearly a predisposition to answer the questions in such manner as is most likely to induce the granting of a licence) there is no protection for the road-using public should some physical or mental disability supervene between the issue of the licence and the renewal at the end of the three-year period

If then a case is made out for more rigid control in relation to the granting of licences, what are the limits within which this control should be exercised ? Accident proneness is a concept, continued Prof Eysenck, which has haunted psychology for a long time There is, he asserted, much truth in the proposition that while most people tend to be relatively accident-free the great majority of accidents happen to a rather small sample of the total population at risk, nor, it appeared, was advancing age a significant cause of accidents Reaction-time is by no means the potent factor in the causation of accidents which popular opinion appears to believe The evidence is fairly strong that simple reaction-times in most situations are useless in identifying accident-prone drivers Older people tend to make up for their longer reaction times by driving more slowly, and Dr K G Norman, of the London Passenger Transport Board, while questioning the existence of accident-proneness at all, added that although as a man grows older, into middle age or further, his reaction-time is slowed, his eyesight is not quite what it used to be and his hearing is impaired, he nevertheless becomes a better driver Conversely, people with very fast reaction-times may tend to run greater risks and rely too much on their quick reflexes

A further series of tests have revealed that over large groups of drivers the relationship between ability and accident proneness is quite small, ability apparently making no difference to driving safety once an intelligence-quotient level of eighty or thereabouts has been reached Below eighty, however, there is a steep rise in the number of accidents encountered, and it does not seem to matter much which test is used to establish the mental level From this fact Prof Eysenck deduced that considerable caution should be exercised in allowing the dullest 20 per cent of the population to drive vehicles at all Of still greater importance, in his view, were tests of personality traits This work tended to show that on the whole the more emotional, more neurotic person tends to over-act to stress, to decline in performance and to take longer to recover his previous level of performance There is thus good reason to suspect that emotional people are contributing more than their share to the group of accident-prone drivers To what extent, therefore, should factors of this nature be involved in determining a citizen's right to be granted a licence to drive a motor-vehicle?

The application of greater stringency in the testing of applicants for driving licences would not, as Dr Francis Camps, secretary-general of the Academy, pointed out, be directed primarily to laws excluding from the roads those who were suffering or had suffered bodily or mental disability On the contrary, there are among those at present excluded, or who in the popular conception ought to be excluded, a number who would, as a result of properly conducted tests and investigations, be found to be within the safety limits, and thus eligible for a licence The current application form reads "Do you suffer from epilepsy ?" For all practical purposes an affirmative answer to-day debars the applicant from receiving a driving licence In the opinion, however, of Sir Francis Walshe, who presided over the first scientific session of the meeting, based on his own experience over a great many years of epileptics, the number of accidents which is due to epileptic attacks at the wheel seem to be extraordinarily small He appeared to lean against the automatic deprivation of driving licences in respect of all epileptics, especially, as one example, those who throughout their lives had fits only during sleep While in the view of Dr Walter Somerville, coronary artery disease and all heart diseases carrying a risk of sudden loss of consciousness should disqualify persons from driving public-service vehicles, long-distance lorries and taxis, he did not consider it feasible to ban persons from driving who are subject to occasional anginal attacks or who have recovered from a coronary thrombosis In his opinion, the application form for a driving licence should screen out those who know they are subject to fainting attacks, especially those with Stokes-Adams attacks, fainting due to vaso-vagal attacks, aortic stenosis, sensitive carotid sinus and other disease The difficulty, in this connexion, which the Law Section of the British Academy of Forensic Sciences had endeavoured to anticipate, is to be found in the words "those who know they are subject to fainting attacks, etc " The procedure, it was believed, should be sufficiently comprehensive to include in addition, and so far as practicable, those who might be subject to fainting attacks but who, until subjected to some compulsory tests, were unaware of that fact The difficulties, however, of designing such tests were not to be underestimated There must, moreover, be considerable support for the view expressed by Prof A L Banks that it would be invidious to draw a distinction between those persons who drive public-service vehicles and the like and the ordinary individual If, due to physical or mental disabilities, a driver is a menace on the road it seems reasonable to remove him from the road whatever the type of vehicle he may drive and however often he performs that function

Between 1949 (when the Road Research Laboratory first prepared comprehensive records) and 1961, casualties to pedestrians have been increasing as traffic increased but not in direct proportion On the other hand, casualties to non-pedestrians, that is drivers, motor-cyclists, cyclists and passengers, have been increasing more or less in direct proportion to the amount of traffic Since the number of motor-vehicles coming on to our roads tends to increase rather than diminish, the time may well come when all persons requiring a licence to take a potentially lethal weapon on to the roads may be required to subject themselves as a preliminary to tests, although much research remains to be done in this field before such tests are brought within the orbit of practical reality For the present, Dr Milne, in presenting to the meeting the recommendations of the Academy in this regard, suggested that the application form should be so worded as to make the questions regarding health difficult for the applicant to lie about without sound medical knowledge It should then be possible to divide the applicants into three classes those who were fit to drive on the face of their answers, those who were unfit to drive, and those who should be further assessed. The applicant would be expected to provide evidence from his own medical advisers, if such existed, and, additionally or in substitution therefore, would be required to attend before a duly constituted medical tribunal Dr Milne submitted to the meeting a suggested form of application, of which limited space prevents reproduction here but which will be published in *Medicine, Science and the Law*, the official journal of the Academy

If the emphasis in the granting of driving licences is to be moved from one of right to one of mere privilege should there not, as a corollary, be a substantial increase in the responsibility resting on Government authorities, both central and local, to ensure the maximum degree of safety in the construction of our roads, and on vehicle manufacturers in the construction of the vehicles? In a lively and informative contribution to the meeting, Mr J J Leeming, the county surveyor for Dorset, disputed the proposition that no matter what problems the road conditions may present to the driver it remains his responsibility to ensure his ability to solve them or take the consequences In reporting the results of his investigations he asserted that dressing curves on which the surface had polish, and applying no other treatment than the dressing, caused accidents to fall by 88 per cent It was, he said, his experience that it is not the places which look dangerous which have the accidents If the danger is obvious the motorist will take care, it is when the danger is hidden that he will fall into the trap He instanced an actual case in which two men having had enough, though not too much, to drink, disregarded a halt sign and drove into the intersection of cross-roads where they came into collision with a lorry and were killed It could be said that this was an accident caused by taking drink, but in fact a great number of other people who had taken no drink had done exactly the same thing at exactly the same cross-roads Following the death of these two men a roundabout was built at the cross-roads in question, since when there has been no accidents for two years despite a very heavy accident record theretofore

Dr John Bull, of the Road Injuries Research Group, Birmingham Accident Hospital, stressed the importance of accident design in the diminution of the number of road accidents He included within the term 'design' such terms as improved tyres, high hysteresis rubber for tyre construction and automatic releases from locking of wheels so as to continue control during skidding He also underlined the dangers to pedestrians of such things as car fins put on as a styling feature, mascots and projecting parts in general

Would the public interest be better served were a statutory obligation placed on both road authorities and manufacturers of motor-vehicles so that a claim to damages might lie, where an accident could be shown to have occurred due to some feature of the road or the vehicle which, while not such as might fairly be described as a defect in construction of the road or the vehicle, was nevertheless a contributing cause in the ultimate damage or injury which reasonable foresight, applied against the background of the facts and technical knowledge, might have avoided? Thus, where a collision occurs at the intersection of cross-roads, which, it may be reasonably inferred, would have been avoided had the appropriate authorities inserted a roundabout within the intersection, is it just that the responsibility to pay damages for the collision should fall solely on the motorists to the exclusion of the highway authority? Again, should not the manufacturers be held responsible, if not wholly at least in part, for damage resulting from injuries caused or aggravated by the presence of unnecessary or dangerously positioned bars, handles and ornamental gadgets?

It is in regard to the ascertainment of the causes of accidents, the causes of death and the placing of responsibility both criminally and civilly that the forensic sciences have their greatest application to the subject of the symposium One questions, however, whether the contribution which the law can make towards ameliorating the hazards of the road is likely to prove decisive Certainly there is room for new thinking about the subject Should there not be more prosecutions for bad driving rather than limiting the prosecutions, as tends to be the case to-day, to those instances in which an accident involving injury or damage follows? In cross-road collisions one party is seldom wholly to blame, should the police continue to choose one of two drivers and prosecute him to the exclusion of the other? Should not both parties be brought before the court, and their relative responsibility assessed and suitably punished? Too many defendants, particularly in cross-road collisions, leave the court believing that, while they may have been wrong they were not wholly wrong, and that it is unjust that the other party to the collision should have suffered no penalty whatsoever Ought not some clearer distinction be drawn between those persons who are guilty of dangerous or reckless driving, those who are guilty of careless driving and those whose driving, while falling short of deliberate recklessness or carelessness, involves a reduction in the standards of driving required to ensure public safety? At present a person driving dangerously, but whose offence has been committed without deliberation on his part, is, since a specific intent is not a necessary ingredient of dangerous driving, convicted of the same offence as the man who wantonly or recklessly drives his motor-vehicle in a dangerous manner Should not a new offence be created of driving a motor-vehicle in a manner inconsistent with the safety of the public and the offences of dangerous or reckless driving amended so as to require proof of a deliberate intention to drive in a dangerous or reckless fashion?

The last two sessions of the proceedings at which questions of this nature were canvassed were each held under the chairmanship of a High Court judge, namely, Sir Frederick Lawton and Sir Roger Omerod. The range of the discussions was extensive and was designed to illuminate the current methods and procedure for investigating the causes and responsibility for road accidents. These discussions will be fully reported, as will the remainder of the proceedings, in *Medicine, Science and the Law*.

If, at the conclusion of the meeting, one was left with the impression that more questions had been raised than problems solved, this served only to underline how much remains to be done in the field of road-accident research. That stimulus was given to such research there can be no doubt, and there may still be many, both within as well as outside the ranks of the forensic sciences, who may yet have cause to thank those participating in this scientific meeting for the knowledge which was imparted, the thought that was provoked, and the lives and freedom from injury which such greater knowledge will have preserved.

SATELLITE OBSERVATIONS OF THE ENERGETIC PARTICLE FLUX PRODUCED BY THE HIGH-ALTITUDE NUCLEAR EXPLOSION OF JULY 9, 1962

By A. C. DURNEY, PROF. H. ELLIOT, DR. R. J. HYNDS and DR. J. J. QUENBY
Imperial College of Science and Technology, London

THIS is a short account of some results obtained from a preliminary analysis of data on the energetic particle flux produced above the atmosphere by the recent high-altitude nuclear explosion in the vicinity of Johnston Island (latitude 16.7° N., longitude 169.5° W.). According to press reports, this explosion occurred at 0900h 15s U.T. on July 9, 1962, at an altitude of about 320 km and was in the megaton range. These results were obtained by means of a Geiger counter carried in the joint U.S./U.K. satellite *Ariel*. *Ariel* was launched from Cape Canaveral on April 26, 1962, into an orbit with inclination 54°. On July 9, 1962, apogee was 1,209 km and perigee 393 km. The Geiger counter is an Anton 302 which, together with a heavy-particle Čerenkov detector, makes up the cosmic ray package in this satellite. The counter is shielded by approximately 2 gm/cm^2 of assorted material, including 1 mm of lead, and responds by direct penetration to protons with energy $\geqslant 43$ MeV and to electrons with energy $\geqslant 4.7$ MeV. It also responds with an efficiency $\ll 1$ to γ-rays and to bremsstrahlung from electrons below the penetration energy. For technical reasons the circuitry associated with the counter was designed so that it would not record counting-rates in excess of ~ 45 counts/sec.

Information is available in the literature[1] on the results of three high-altitude explosions carried out in 1958 under the code name of 'Project Argus'. On the basis of these results it was to be expected that the July 9 explosion would result in the trapping in the geomagnetic field of energetic electrons from fission products together with protons and electrons from neutron decay, and that this trapped particle flux would probably still be detectable after a period of months. This expectation has been borne out.

Fig. 1 is a chronological plot of the intensity recorded by the Geiger counter for the period 0800 U.T.—2200 U.T. on July 9, 1962. (The apparent Geiger counter-rate is plotted. For rates above 300 there is an appreciable correction for the counter deadtime.) Points of special interest have been numbered and these numbers are used in the discussion in the text and in Fig. 2. This latter figure shows the track of the satellite for the same period as Fig. 1. Apogee occurs at a latitude of about 7° N. between longitudes 94° E. and 58° W. for the period under consideration, while perigee occurs near latitude 6° S. at longitudes between 96° W. and 108° E. The positions of apogee and perigee and north and south apices are indicated in Fig. 1. It can be seen from Fig. 1 that up to 0900 U.T. the Geiger counter shows the normal latitude effect for cosmic rays as *Ariel* moves from its northern apex down past the equator to its southern apex. The small peak (1) corresponds to passage through the lower edge of the inner radiation belt. At 0900h 15s U.T. (the time of the explosion) *Ariel* was at an altitude of 819 km in position latitude 52° S. and longitude 163° E., at a distance of 7400 km from Johnston Island. An initial burst of particles (2), which saturated the counter, occurred within 20 ± 10 sec of the explosion and lasted for 2 min. Three different explanations could be advanced for this prompt burst of particles: (i) they are β-particles from the decay of fission products, (ii) they are electrons resulting from neutron decay, (iii) they are outer radiation belt electrons the mirror point distribution of which is perturbed by the hydromagnetic disturbance resulting from the explosion.

Explanation (i) requires that the heavy fission fragments should be situated close to the magnetic line of force passing through the satellite at this time. Simple calculation shows that the minimum distance the fission fragments would have had to travel in the maximum permissible time of 30 sec is about 5,600 km, and this leads to velocities of 180 km/sec for the fission products. Such velocities are improbably high and therefore make this explanation correspondingly unlikely. Furthermore, the fission fragments would have to be neutral in order to move freely through the geomagnetic field. Explanation (ii) has also to be rejected because the flux of neutrons required appears to be higher than that which could be supplied by the explosion, which is assumed to produce a total of $\sim 10^{26}$ neutrons. We therefore believe that (iii) is the most likely explanation, that is to say, a magnetic field disturbance propagated outwards from the site of the explosion, with a velocity of the order 10^7 cm/sec, leads to a breakdown

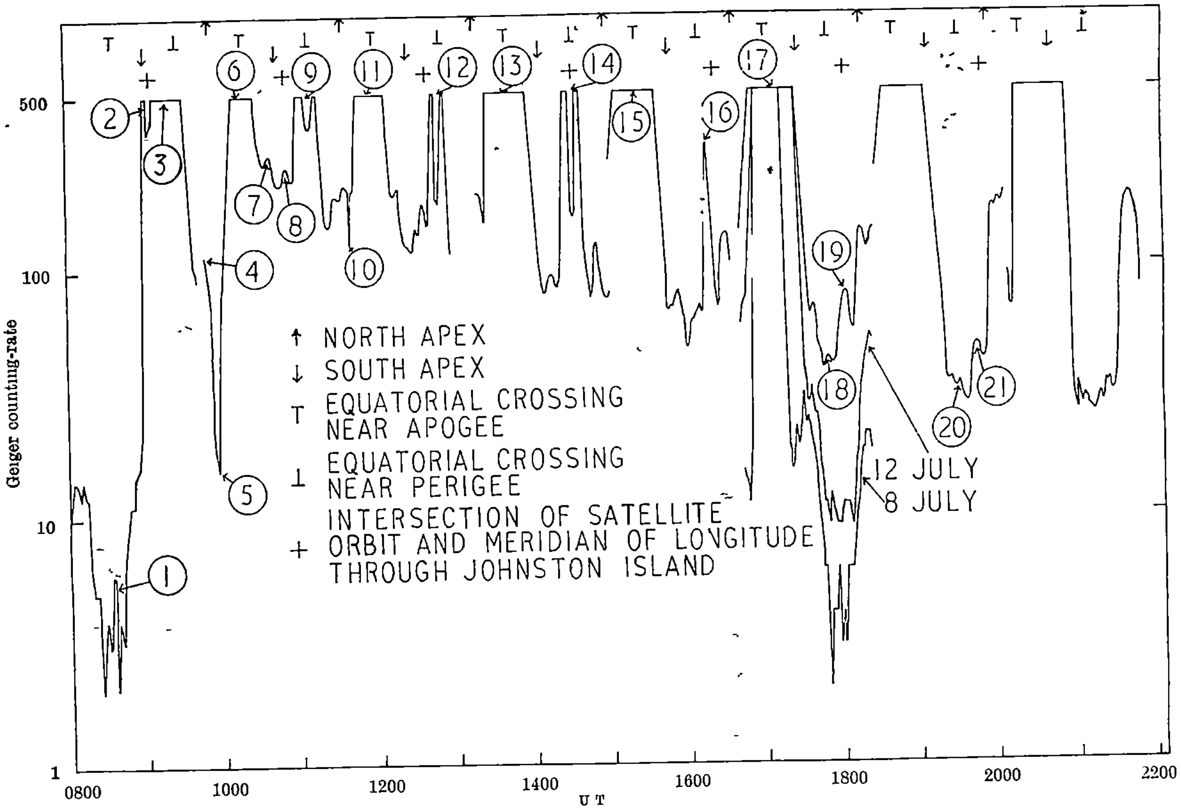

Fig. 1. Plot of *Ariel* Geiger counter-rate against time on July 9, 1962. The Geiger-rate is scaled by 8 and is taken over an interval of 92 sec. Corresponding data are shown for one orbit on July 8 and July 12, 1962

of the Alfvén adiabatic invariant of motion of the trapped particles and to a lowering of their mirror points with a consequent increase in intensity at the altitude of the satellite.

At 0908 U.T. the counter again saturates (3) as the satellite encounters a high flux of fission product β-particles drifting eastwards over the Pacific from the site of the explosion. As the satellite crosses the north Atlantic the intensity falls again, but at (4) it is still much above the pre-explosion background, indicating that there are additional trapped particles on the magnetic field lines which intersect the satellite at this time. These field lines go out as far as 4 Earth radii in the equatorial plane. The magnetic line of force passing through the point of explosion, on the other hand, attains a maximum altitude of ~800 km in the equatorial plane, and the radius of curvature of the β-particle trajectories being not more than a few kilometres, we would expect them to be rather closely confined to the magnetic shell containing this line of force. It is not immediately obvious, therefore, how the electrons find their way on to magnetic shells extending as far away as 4 Earth radii. There would appear to be two possibilities: either the electrons are neutron decay electrons, or else they are neutral fission fragments endowed with sufficient kinetic energy to enable them to travel on ballistic trajectories and so reach the appropriate lines of force, where they decay. In order to do this they do not, of course, need to reach heights of 4 Earth radii. It is sufficient that their trajectories carry them to high latitudes. At the present time we are unable to distinguish with certainty between these two possibilities, but the former is perhaps the more likely.

Returning to Fig. 1, it is seen that a minimum value (5), corresponding to normal pre-explosion intensity, is reached at 0956h. The eastward drift velocity for electrons with energy ≥4.7 MeV is such that electrons injected into the geomagnetic field at sufficient height to clear the atmosphere will occupy a shell completely encircling the Earth in a time of the order 20 min. Thus this shell will be complete before 0956, and it is necessary, therefore, to account for the apparent gap observed at this time and position. It can be shown that this gap arises because of the geomagnetic anomaly in the South Atlantic. These particles which we might expect to be observed by *Ariel* when it is in this position have their conjugate mirror points below sea-level in the South Atlantic, and have therefore already been absorbed west of the satellite's magnetic meridian. Thus, in a sense the satellite is at this time in the shadow of the geomagnetic anomaly and cannot observe the shell of additional electrons. When the satellite is in a corresponding position on its next orbit, a similar but much smaller dip in intensity (10) occurs at 1138h 30s. On this occasion, however, the intensity does not fall to the level prevailing prior to the explosion, as it did on the previous orbit. This behaviour appears to be consistent with the change in mirror point altitudes to be expected from the rather different magnetic field strength at the appropriate conjugate points.

After passing through this position of minimum intensity (5) the counting-rate rises again to saturation-level (6) as the satellite nears apogee in the equatorial region. In this position it is sufficiently far into the natural inner radiation belt for saturation to occur even in the absence of additional particles

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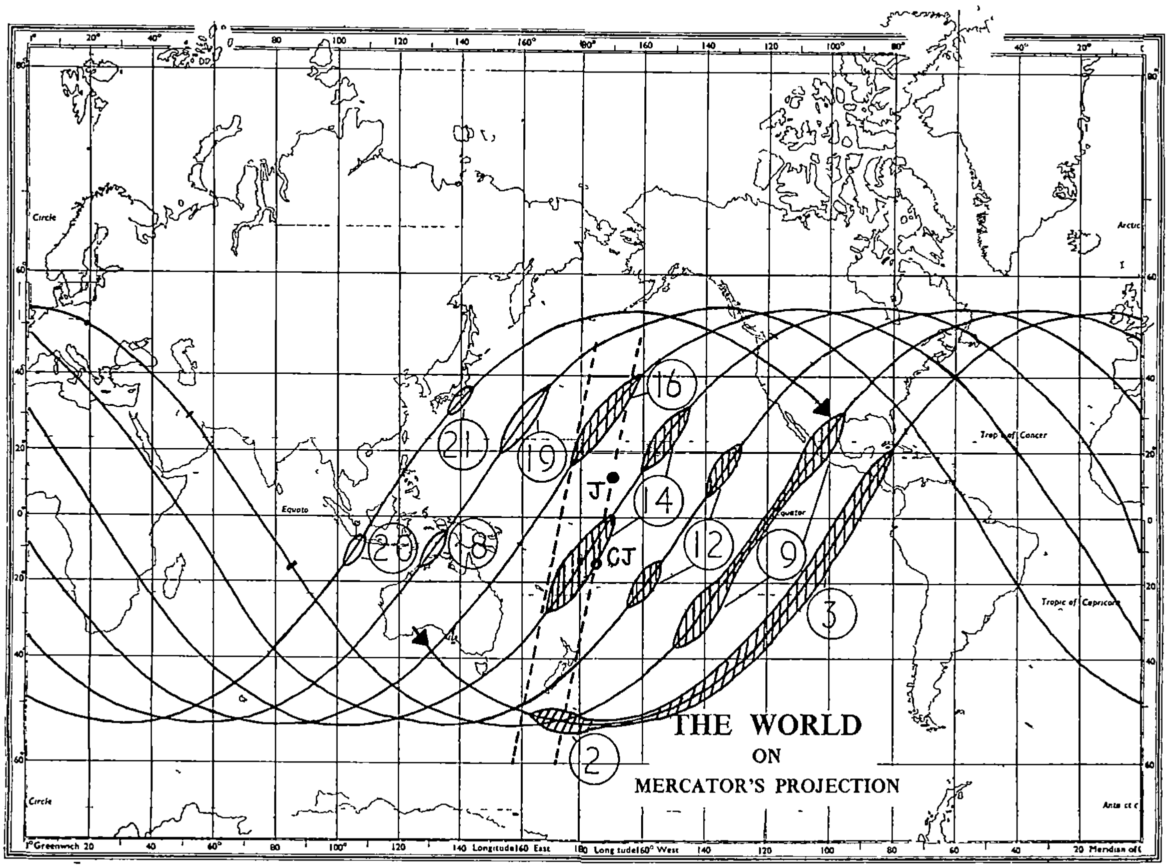

Fig 2 Geographical position of the low-altitude Johnston Island shell on July 9, 1962 Shaded areas indicate positions where the Geiger counter was near saturation Unshaded areas indicate relatively small intensity peaks Numbered positions are referred to in the text Position of Johnston Island (*J*) and its magnetic conjugate (*C J*) are indicated together with the magnetic meridian through Johnston Island (broken line)

from the explosion The Geiger counter, therefore, gives us no information about the additional particle flux in this region However, the behaviour of the Čerenkov counter reveals the presence of a high intensity of additional particles in this region

The twin peaks (7) and (8) correspond to the motion of the satellite, at high southerly latitudes, across the magnetic lines of force connecting with the heart of the outer radiation belt, indicating that the trapped particle intensity is greatly enhanced out to at least 5 or 6 Earth radii

The twin intensity peaks (9) occur on three successive equatorial crossings, and their geographical positions are shown approximately in Fig 2 These peaks correspond to the passage of the satellite through the magnetic shell containing the point of explosion over Johnston Island, and indicate the presence of fission product electrons drifting eastward on the surface of this shell From the distribution of geomagnetic field strength we know that particles on this shell must plunge into the atmosphere either over, or just to the east of, the South American sub-continent Consequently, this shell cannot extend completely around the Earth and the lifetime of particles on it is therefore only a matter of minutes A vestige of this particle shell can still be detected the following day, and in order to account for this we must conclude that it has been continuously fed by the decay of fission products remaining in the neighbourhood of the line of force connecting Johnston Island and its conjugate point It would seem likely that these fission products were charged and trapped in the magnetic field. The drift velocity for heavy ions of this kind is very low and is in a westerly direction, so that the western edge of the electron shell would be expected to creep westward only very slowly That there is a rather clearly defined western boundary to this shell can be seen from Fig 2 The regions of enhanced intensity to the west of Johnston Island, which are shown without hatching, represent much lower peak intensities than those to east of the magnetic meridian shown by the dotted line

Returning to Fig 1 once more, (15) represents the passage of the satellite through the inner radiation belt at apogee, while the peak labelled (16) lies in the western edge of the low-altitude Pacific shell which stretches from the longitude of Johnston Island to the Atlantic, and (17) is the inner radiation belt again For this particular orbit directly comparable data are shown for July 8, 1962, the day preceding the explosion, and also for July 12, 1962 Comparison of these three traces gives some indication of the rate of decay of the additional trapped electrons resulting from the explosion In this connexion it should be emphasized that the decay is not exponential, and consequently it is not possible to predict the probable life-time of the trapped particles A non-exponential decay is to be expected since the life-time of the trapped electrons observed at any particular point is a function of energy and pitch angle

For particles trapped in the geomagnetic field, the intensity at any point on a particular magnetic shell should depend only on the scalar magnitude of the

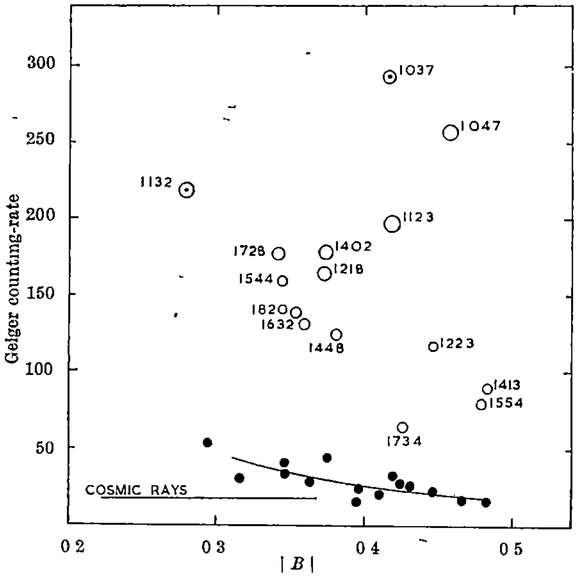

Fig 3 Plot of Geiger counter-rate against scalar magnitude of the magnetic field in gauss, | B |, for the magnetic shell intersecting the geomagnetic equatorial plane at 3 87 Earth radii O, July 9, 1962, observations, ●, April 30, 1962, observations Observation times are given in U T next to the July 9 points The Geiger-rate is scaled by 8 and is taken over a 92-sec interval

field at that point Fig 3 shows the distribution of intensity as a function of field strength on the magnetic shell which intersects the equatorial plane at approximately 3 9 Earth radii The open circles represent the counting-rates on July 9, 1962, and the black circles the corresponding rates for April 30, 1962 The background cosmic-ray rate is also shown It will be seen that for all times on July 9, 1962, the particle intensity is above normal, as represented by the April 30, 1962, data, and that it varies monotonically with $|B|$, indicating that we are observing genuinely trapped particles at this time The points at 10 37, 10 47, and 11 23 lie above the curve and probably contain a contribution from short-lived particles which disappear within 2 5 h of the explosion

This account is based on an analysis of only a part of the total data available, but, in view of current interest in the geophysical phenomena associated with this event, it seemed worth while to produce a preliminary report even though some of the interpretation is rather tentative Continued examination of these artificially trapped electrons during the next year or so will provide information on the trapping times for particles in the radiation belt This information is crucial in understanding the origin of the trapped radiation, but unfortunately it is unlikely to be forthcoming from *Ariel*, which has operated only intermittently since July 13, 1962, as a result of deterioration of the solar cells presumed to be due to the effects of this additional radiation—a result which was not foreseen

Our conclusions may be summarized as follows

(1) There is evidence for the redistribution of the mirror points of the naturally occurring electrons in the outer radiation belt This redistribution was probably the result of a hydromagnetic disturbance propagated outwards from the site of the explosion

(2) Following the explosion, an additional population of electrons remained trapped in the geomagnetic field These electrons occupied the region of the natural radiation belts out to a distance of at least 5 or 6 Earth radii and at large distances were most likely the product of neutron decay

(3) A low-altitude shell of fission product decay electrons was formed over the Pacific Ocean, stretching westward from Johnston Island to the neighbourhood of the Atlantic coast of the American continent This shell lasted for more than a day and was continually supplied with electrons by the decay of charged fission fragments supported in the Earth's magnetic field in the neighbourhood of Johnston Island

(4) A considerable part of the additional radiation had disappeared by July 12, 1962, as shown in Fig 1, but the decay is not exponential and consequently the life-time of the trapped radiation cannot be predicted until the decay law is established

We are indebted to the N A S A for making this joint U S /U K project possible, and to all those members of the N A S A team who were concerned in the preparation and launching of *Ariel* We wish particularly to thank the two project managers, Mr R C Baumann and Mr M O Robins

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[1] *J Geophys Res*, **64**, No 8 (1959)

CONVECTION CURRENTS IN THE EARTH'S MANTLE

By Prof S K RUNCORN

King's College, Newcastle upon Tyne, and Jet Propulsion Laboratory,
California Institute of Technology, Pasadena, California

In a recent attempt to provide an explanation of continental drift[1], I postulated that convection currents were occurring in the Earth's mantle This supposes the mantle to have zero strength under stresses persisting over millions of years and to have a Newtonian viscosity I then applied Chandrasekhar's[2] theory of convection in spherical shells under a radial gravitational field, in which he showed that instability in the marginal state occurs through a convection pattern described by a spherical harmonic, the degree of which depends on the ratio (η) of the radii of the inner to the outer spherical boundaries Vening Meinesz[3] had argued that spherical harmonic analysis of the present topography of the globe reflects the convection pattern in the Earth's mantle, as the continents would tend to lie above the descending currents He showed that the present positions of continents and oceans implied that the convection currents in the mantle had strong harmonics of third, fourth, and fifth degree Chandrasekhar's calculation showed that, as η for the Earth's mantle is at present 0 55, these harmonics are those through which the thermally induced instability would be expected to occur

Therefore, I argued that if the accretion theory of the origin of the Earth was accepted and it was supposed that the Earth's iron core had only separated gradually through the Earth's life, as originally suggested by Urey[4], and its radius was still increasing, a change in the number of convection cells in the mantle might recently have occurred. This increase in the degree of the convection was taken to be the mechanism for the dispersal of the continents since the Palæozoic. Thus the surprisingly recent time—compared with the life of the Earth—at which continental drift, as understood by geologists and palæomagnetic workers, occurred is given a rational explanation.

It has recently been possible to obtain new evidence for this process. The ratio η of core radius to the Earth's radius, at which the Rayleigh number of a given harmonic becomes less than that of the harmonic of next lower degree and therefore may be expected to develop in place of the latter, can be calculated from Chandrasekhar's theory; but the values depend on the boundary conditions used. It is clear that for the Earth these are a free inner boundary and a rigid outer one, for shearing stresses at the core-mantle boundary must vanish and there can be no doubt of the strength of the rigid crust of the Earth. If these boundary conditions are used one can derive[5], from Chandrasekhar's work, that the ratio of the radii of core to Earth at which the second harmonic replaces the first harmonic as the mode through which instability occurs is 0·06; for the transition from second to third the value of η is 0·36; and for third to fourth 0·49. During these transitions the continents will be under considerable stress as the convection-pattern changes to its new form. The last such transition from fourth harmonic to the fifth is observed in the geological column as continental drift.

The question now arises whether there is any record in the Earth's crust of the earlier transitions; these mode changes would be expected to cause world-wide orogeny, involving extensive recrystallization of crustal material. Radioactive age determinations have been made on igneous and metamorphic rocks which have been formed in the deeper parts of the crust as the result of orogenic forces. It is therefore of great interest that the ages obtained for such rocks have been found to be grouped in peaks of about ± 100 m.y. around the dates 200 my., 1,000 m.y., 1,800 m.y., and 2,600 m.y. ago. The most recent peak covers much of the geological record since the middle Palæozoic, and we have seen that this is associated with continental drift and the transition from a fourth to fifth degree convection pattern in the mantle. It is natural to identify the 1,000-m.y. peak with the transition of the third to fourth degree convection pattern, the 1,800-m.y. peak with the transition from the second to third degree, and the 2,600-m.y. peak with the transition from the first to second. Gastil[6] has reviewed the radioactive age dates and has shown that the peaks are present whether the ages are determined by the rubidium–strontium method, the lead method, or the potassium–argon method. American rocks give a peak at 1,300 m.y., which is thought to result from later metamorphism. He also shows that in spite of the poor coverage in some continents, the peaks appear to represent world-wide events. Davis and Tilton[7] had also come to a similar conclusion concerning the reality of these events: they are not simply due to deficiencies of sampling. It must, however, be emphasized that the peaks are broad (of the order of 200 m.y.) and further proofs that they are truly world-wide are much needed[8].

In this way the dates at which the core attained these critical values is determined as shown in Fig. 1. Before this new information was available, I had assumed that the core started its growth 4,600 m.y. ago—at the time of the Earth's origin[1]. Fig. 1 shows that it must have started its growth a little over 3,000 m.y. ago. But this, of course, is in fact entirely reasonable: one must allow time for the Earth to heat up to temperatures at which the creep processes necessary for convection will become important. As these depend on a factor exp (E/kT), where E is an activation energy, k Boltzmann's constant, and T the temperature, it may be supposed that the creep process rather suddenly became important and then convection commenced. The source of the heat is the uniform distribution of radioactivity. Thereafter, it may be supposed that the convection just carries off the heat generated by radioactivity within the Earth and by the release of potential energy due to the settling of iron into the core.

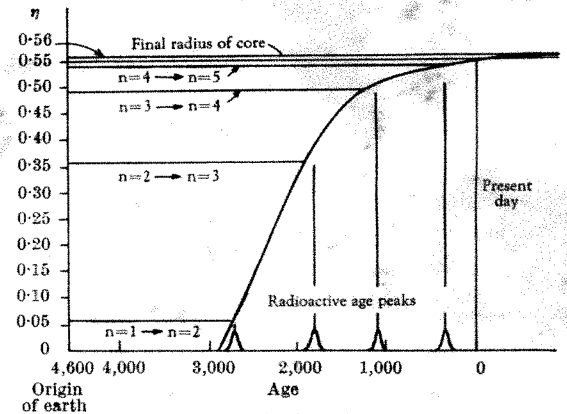

Fig. 1. Growth of radius of Earth's core

The law of growth of the core found by the above argument fits well with the simple model suggested previously by me[1], that is, that the rate of movement of iron into the core is proportional to the surface area of the core and to the mass of iron remaining in the mantle. Initially, the radius will grow linearly with time as equal increases in radius represent increments of mass proportional to the surface area of the core. Eventually, the curve will become asymptotic to the final radius (g) of the core when all the iron has left the mantle. It is therefore worth fitting by least squares the theoretical equation previously derived on this basis[1] :

$$\log_e \frac{(r/g-1)}{(r/g)^2+(r/g)+1} + 2\sqrt{3}\,\tan^{-1}(-2\,r/g-1)/\sqrt{3} = Kt + c$$

where K and c are constants and r the radius of the core at time t. The fitted curve is shown in Fig. 1. The value of g is found to be 0·56: thus about 6 per cent of the free iron still remains in the mantle.

[1] Runcorn, S. K., *Nature*, **193**, 311 (1962).

[2] Chandrasekhar, S., *Hydrodynamic and Hydromagnetic Stability* (Oxford, 1961).

[3] Vening Meinesz, F. A., *Proc. Kon. Ned. Akad. Weten.*, **55**, 527 (1952).

[4] Urey, H., *The Planets* (Oxford Univ. Press, 1952).

[5] Runcorn, S. K., *Continental Drift*, Chap. 1 (Academic Press, 1962).

[6] Gastil, G., *Amer. J. Sci.*, **258**, 1 (1960).

[7] Davis, G. L., and Tilton, G. R., *Researches in Geochemistry*, edit. by Abelson, P. H., 190 (John Wiley, New York, 1959).

[8] *Carnegie Inst. Washington Year Book*, **59**, 208 (1960).

A NEW SEISMIC RESEARCH GROUP FOR EDINBURGH

By P. L. WILLMORE
Royal Observatory, Edinburgh

THE formation of the Seismic Research Group in Edinburgh is a symptom of a deep-seated revolution in seismology—occasioned fundamentally by the impact of electronics on an old-established science. The developing pressures have been noticeable for the last decade; but their growth had been delayed, more than in most sciences, by the sensitivity, economy and reliability of the traditional electromagnetic seismographs. The photographic-drum seismogram produced by these instruments is so compact and inexpensive, and it contains so much information, that it still remains the best type of record for certain purposes. However, the data content is not readily convertible into a form suitable for electronic manipulation, and this fact is now imposing grave limitations on the utilization of such records.

A less-fundamental but more-potent demand for technical advance arose from the recognition that seismic observations offered the best prospects for the detection of underground nuclear explosions. The inadequacies of seismological technique began to emerge at the Geneva Conference of Experts in 1958, when the first proposals for a world-wide detection system were drawn up, and were further demonstrated when the analysis of underground tests in the United States in the autumn of 1958 revealed a number of unsatisfactory features in the proposed detection system.

The American reaction to this situation was to set up the Panel on Seismic Improvement which, in 1959, produced its report entitled "The Need for Fundamental Research in Seismology". Acting on the conclusions of this report, the United States expanded its already substantial effort by an order of magnitude, and now has a research programme which is prodigious by the standards of a decade ago.

In Britain, expansion had to start at a much lower level, because very little effort had been going into observational seismology beforehand. However, the Atomic Energy Authority set up a vigorous research group under the leadership of Dr. H. I. S. Thirlaway, the Meteorological Office established its Gassiot Fellowship as a step in the expansion of its own

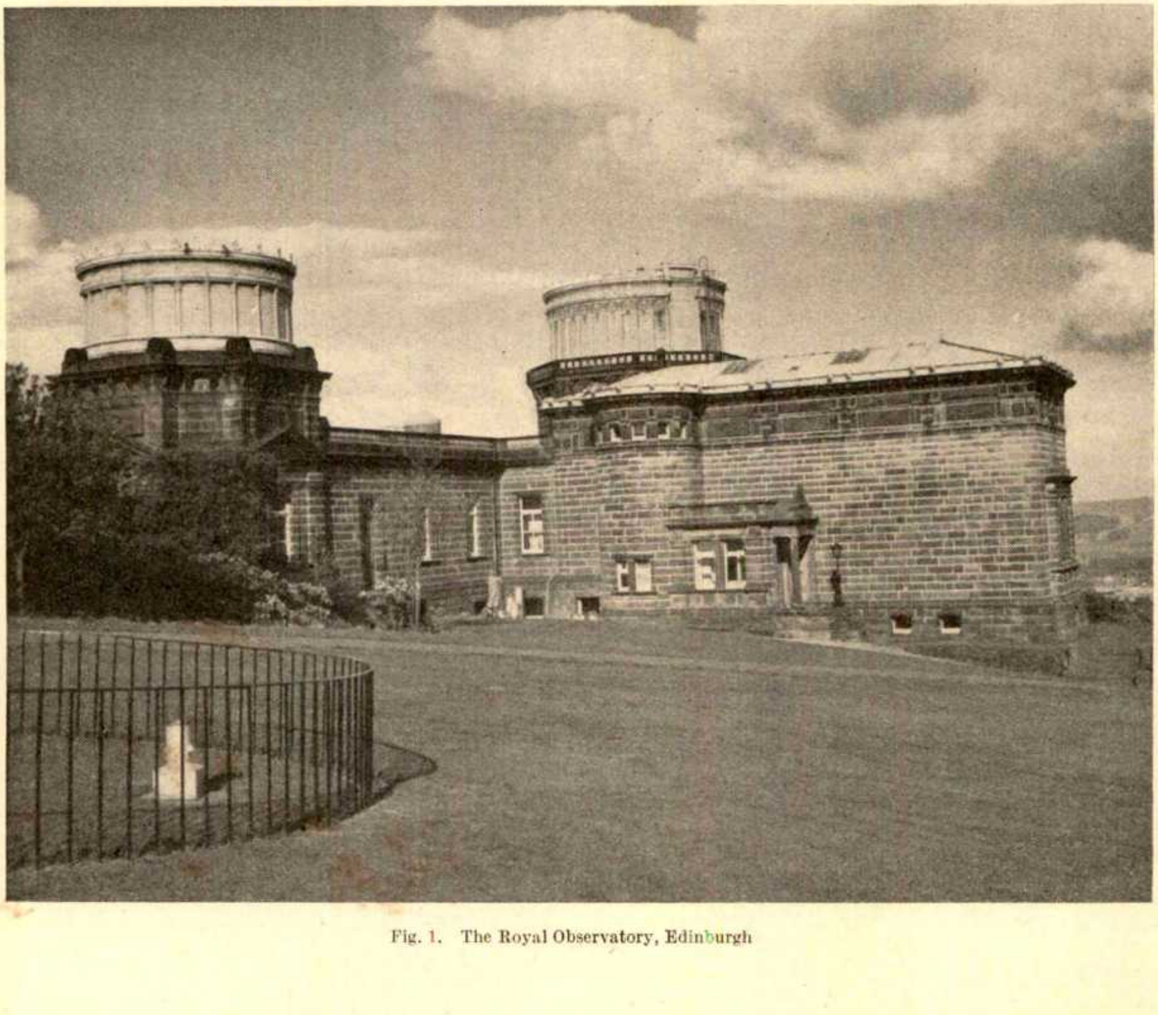

Fig. 1. The Royal Observatory, Edinburgh

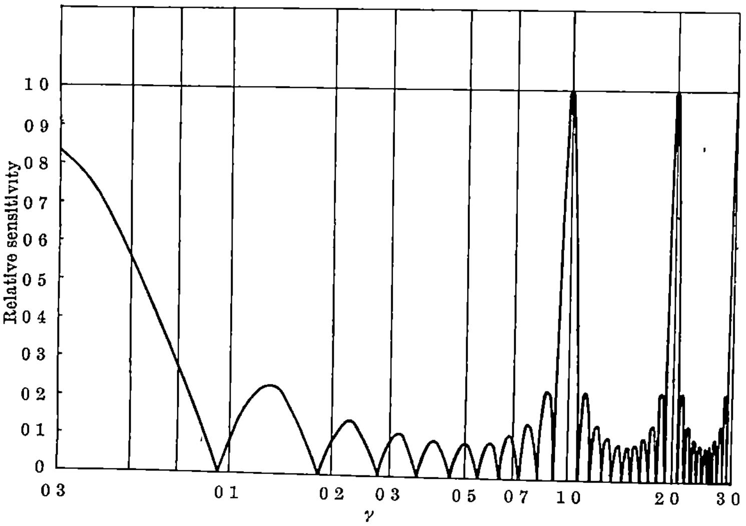

Fig 2 Output of a linear array γ is a parameter which depends on the velocity and frequency of the incoming wave (*By courtesy of the Atomic Energy Authority*)

effort in seismology, and the Advisory Council on Scientific Policy recommended that two university research groups should be set up These groups were to be at Cambridge and Edinburgh respectively

At the beginning, it was expected that the Edinburgh group would be a small one, but as plans advanced and field tests by the Atomic Energy Authority became available, the advantages of Scotland as a recording base began to emerge A modern seismograph can detect the vibration of a car moving a mile away, or of a train at 5–10 miles By such standards the whole of south-east England is intolerably noisy, and even secluded rural sites experience something like 100 times the amplitudes of high-frequency vibration which can be found in parts of Scotland The difference in noise-level does not apply to the movements of about 5-sec period which are generated by waves in the oceans, but probably reappears for periods of the order of minutes, which correspond to the tilts generated by the movement of heavy vehicles

As a consequence of the difference of noise-level, it was decided that the major recording centres of the Atomic Energy Authority and of the Meteorological Office should both be set up in the vicinity of Eskdalemuir, in the Southern Uplands of Scotland At this stage, the other advantages of Edinburgh as a centre of training and research came to be more fully appreciated and plans for development there were enlarged accordingly The seismic project is being housed in the Royal Observatory (Fig 1), which is related to the University through the fact that its director, the Astronomer Royal for Scotland, is also the University's professor of astronomy The numerous facilities available on the Observatory side include mechanical and electronics workshops, a library and a time service, and the support of senior research staff who are already applying advanced techniques in the fields of electronic instrumentation and data processing The University provides the academic opportunities with which it is hoped to attract students and research fellows to the project

The staff of the Seismic Group at present includes the senior seismologist, two experimental officers

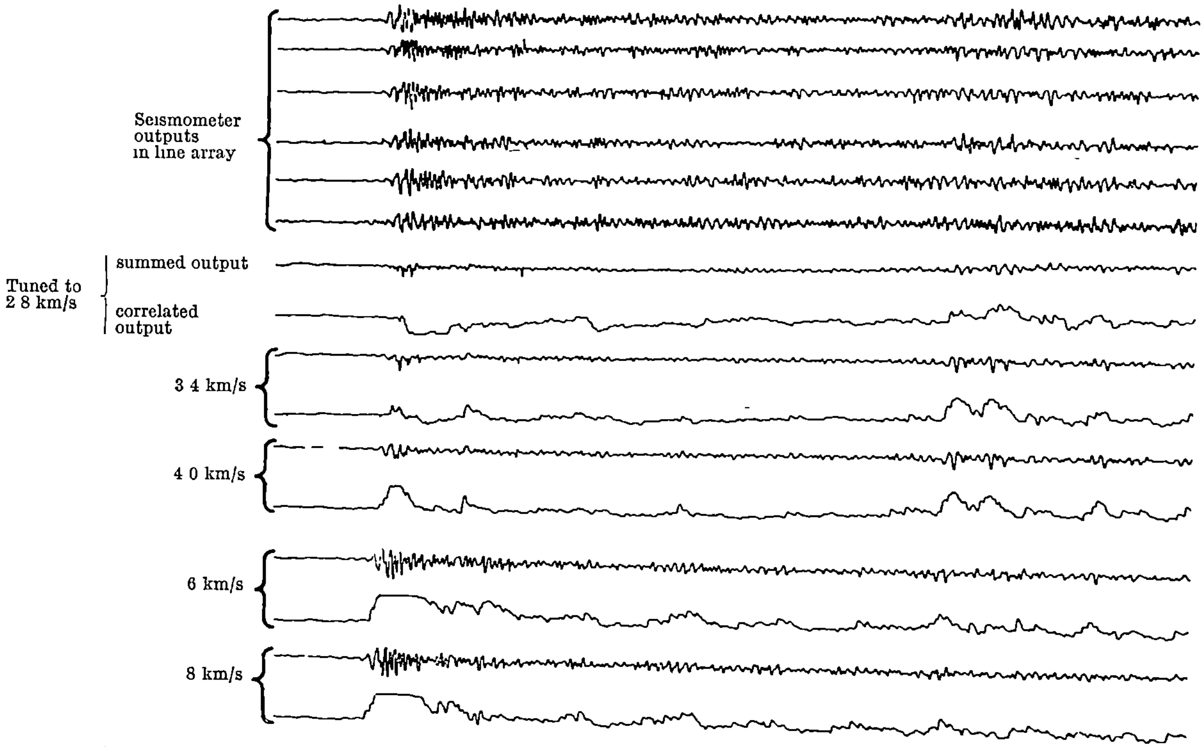

Fig 3 Outputs from six channels of an array, with phased sum and cross-correlator output for an explosion seismogram (*By courtesy of the Atomic Energy Authority*)

and a Department of Scientific and Industrial Research Fellow, who between them are responsible for field-work, fixed-station recording and data processing. The most urgent outstanding staff requirement is to strengthen the group on the theoretical side, and a second research fellowship is available for this purpose.

The first objective of the Research Group will be to exploit the possibilities of large seismic arrays for teleseismic studies. Several of these (including one at Eskdalemuir) are in service or under construction by the Atomic Energy Authority, each consisting of two lines of seismometers, 10 km or more in length, and having 11 seismometers in each line. The individual outputs are recorded side by side on magnetic tape.

The Authority analyses the data in a way which has been highly developed in radio astronomy, but which is comparatively new to seismology. First, the outputs from each line are added together, phase delays being introduced such that outputs corresponding to a set of waves travelling across the array in a given velocity and direction are added in phase, whereas the outputs from other types of event are distributed in time. As a result of this procedure, the ratio of the signal to the noise which is uncorrelated from one element to another is reduced in proportion to the square root of the number of elements. Disturbances which propagate coherently across the array, but at a velocity different from that which has been set up for playback, may be rejected more or less strongly than this (Fig. 2).

Next the summed outputs from the two lines are multiplied together, and the product smoothed so as to yield the cross-correlation of the two sets of data. As a result, signal components common to both lines are squared in the output, and therefore smooth to a positive value. Other signals, and random noise, yield products of fluctuating sign, the effects of which are reduced by the smoothing process. The results of this procedure are illustrated in Fig. 3, which shows a few of the outputs from one line of a cross, together with summed and correlated outputs for various lags. The correlator peaks for S waves are clearly visible in the velocity range of 3.4–4 km/sec, even though these waves are hard to see on the individual channels. The P-wave correlation at 6–8 km/sec saturates the playback system.

In addition to its preparations for utilizing data from the Atomic Energy Authority arrays, the Edinburgh Research Group is building up a set of portable seismographs capable of detecting earth movements in the range of periods from 0.1 to 100 sec. The outputs will be recorded on magnetic tape, in a form similar to that used in the fixed arrays. When the equipment becomes operational, it should be possible to set it up either for conventional studies of explosions and local earthquakes, or as an array for the study of teleseisms. In the latter case, the use of self-contained field stations removes the limitations on the scale of the array, and hence on the range of wave-lengths which can be handled. On the other hand, it will not generally be possible to lay out a set of detectors in exact conformity with an optimum geometrical pattern, and we shall also lose the convenience of having the outputs recorded side by side on a single tape. The theoretical and instrumental problems which arise from this situation will provide a challenging field of investigation.

The third problem of interest to the group is to improve the performance of detecting elements, for it is clear that progress in many fields of seismology has been limited by the lack of records having adequate band-width and dynamic range, adequate freedom from cross-coupling and the effects of parasitic oscillations, and adequate long-term stability. During the past two years seismometers have been much improved, and in Great Britain we now have two types in production, having periods adjustable up to 3 sec and 20 sec respectively. In each of these the minimum frequencies of parasitic oscillations have been pushed up to more than 100 c.p.s., and excellent temperature stability has been achieved. With the aid of appropriate electronic circuitry, there is now a good prospect of recording up to the limit of Earth noise, for all periods up to and including that of the fundamental period of the Earth's free oscillations.

The final problem of data acquisition is to set up the instruments in the right places. Although Scotland is the most favourable part of the United Kingdom for this purpose, there will be many types of problem for which it is not the best place in the world. For this reason we feel that our emphasis on mobility offers advantages over any approach which relies solely on the output of fixed stations.

OBITUARIES

Prof. R. Ruggles Gates, F.R.S.

REGINALD RUGGLES GATES, professor emeritus in the University of London, who died in London on August 12 at the age of eighty, held the chair of botany at King's College from 1921 until 1942. During this period, the Department grew considerably and attracted many research students, especially from overseas. He was made a Fellow of the Royal Society in 1931 and of King's College, London. He was a supporter and at various times an officer of many societies, especially the Eugenics Society, the Royal Anthropological Institute, the Linnean Society and the Royal Microscopical Society, of which he was an honorary member.

Gates was born on May 1, 1882, in Middletown, Nova Scotia, and educated at Mt. Alison University, McGill University and the University of Chicago. He also worked at the Woods Hole Marine Biological Station and at the Missouri Botanical Gardens. In London, he was lecturer in biology at St. Thomas's Hospital for two years from 1912, also lecturing on cytology at Bedford College and on heredity in relation to cytology at Oxford. During 1915–16 he was associate professor of zoology in the University of California, during 1917–18 he was instructor in aerial gunnery to the Royal Air Force.

Gates had wide scientific interests, and he was a prolific writer. Much of his botanical work was descriptive, dealing with the cytology of various plants and with the microspecies of *Œnothera* (evening primrose). The latter interest grew out of his early work on the mutants of *Œnothera lamarckiana*, discovered by Hugo de Vries, of whom he was an enthusiastic adherent. This led to his first book, *The Mutation Factor in Evolution*, published in 1915 He had strong and lasting views on the significance of mutations, not least those of large effect, in evolution, a view expressed in his *Mutations and Evolution* (1921)

The most important early work of Gates was the discovery (independently made by Miss Lutz) that the *gigas* mutant was tetraploid, with twice the chromosome number of the normal species He also found triploid and trisomic mutants and the dominant *rubricalyx*, but the tetraploid was of especial interest since it had arisen directly from the normal diploid, differed from it in certain small characters and was isolated from it reproductively, hybrids between diploid and tetraploid being infertile This first discovered instance of autotetraploidy was a great stimulus to further research His later work was on the evolution of the wild species of *Œnothera*, differing from one another only slightly, true breeding but easily crossed artificially, and perhaps rather impermanent in nature In it he came to accept the views of Renner, Darlington and Cleland, though grudgingly and preserving some of his own earlier misconceptions The disagreements which were involved at least served to stimulate useful research

Early in his scientific life, Gates developed a keen interest in human genetics and evolution and anthropology His books, *Heredity and Eugenics* (1923) and *Heredity in Man* (1929), were good introductions to their subjects and undoubtedly stimulated many people to gain an interest Later, he published an immense two-volume compilation, *Human Genetics* (1946), followed by *Human Ancestry* (1947) and *Pedigrees of Negro Families* (1949) He was particularly interested in primitive peoples, collecting data on blood groups and other characteristics, more recently he investigated the genetics of skin pigmentation in negroids

Gates was an inveterate traveller, whether collecting *Œnothera* species in North America or traversing Africa in the pursuit of anthropological data Travelling, indeed, was his sole recreation and one he never relinquished, in his seventies he visited Cuba, Mexico, Japan, Africa, Australia and India in furtherance of his studies of primitive peoples *A Botanist in the Amazon Valley* (1927) records observations which were often recounted to appreciative undergraduate classes

In outlook, Gates was primarily an observer, describer and systematist He made experiments rather blindly, for he had little capacity for conceiving scientific hypotheses and for devising experimental tests His early work, which was his best, was observational He had a strong faith in what the microscope would reveal to his eye, scorning theoretical principles which controverted these observed 'facts' His research students were closely supervised and expected daily to demonstrate a good cytological preparation, they were well trained in microscopical techniques, though conservatively

As a colleague he was difficult to get to know at all intimately, for he seemed unable to relax and be at ease Though generally mild-mannered, he could be most stubborn and would rarely admit to an error or retract a view previously expressed Through scientific controversy, not always on the right side, and his abundant writings, he certainly stimulated scientific thought and research

His first wife was the late Dr Marie Stopes, the well-known botanist and pioneer advocate of birth control and sex education, this marriage was annulled In 1930 he married Miss Jenny Williams, the singer, and in 1955 he married Mrs Laura Greer (*née* Nowotny) of Texas

D G CATCHESIDE

Prof. F. P. Dwyer

PROF F P DWYER, head of the Biological Inorganic Chemistry Unit in the Australian National University, died suddenly at his home in Canberra on June 22, from a coronary occlusion, at the age of fifty-one years

Prof Dwyer was born at Nelson Plains, New South Wales, and was educated at the Marist Brothers' College, Maitland, and the University of Sydney, where he graduated in 1932 From 1934 until 1945 he was senior lecturer and head of the Inorganic Chemistry Department at the Sydney Technical College, and during 1945–46 he was senior lecturer in inorganic chemistry in the University of Sydney He was a visiting professor at Northwestern University during 1953–54, and in 1956 he was appointed to a new chair in inorganic chemistry in Pennsylvania State University, but he resigned from this appointment in 1957 without taking up the post to become reader and head of the Biological Inorganic Chemistry Unit in the John Curtin School of Medical Research of the Australian National University For his outstanding contribution to inorganic chemistry he was elected, in 1960, to one of the first personal professorships created in the Australian National University

The versatility of Prof Dwyer and the breadth of his interests in research are shown by more than 150 publications spread over the following topics X-ray analysis, micro-analytical reagents and procedures, isomerism of the triazines and diazoamino compounds and their metal derivatives, the chemistry of platinum, palladium, rhodium, iridium, ruthenium and osmium, the redox potentials of simple compounds and complexes of the platinum metals, the optical activity and kinetics of racemization and substitution of Group 8 metal complexes, the diastereoisomeric effect and the principle of configurational activity, the stereochemistry of multidentate chelates, electron transfer reactions, the effects of metal complexes in biological systems and stereospecific influences in octahedral complexes

For his work on diazoamino compounds, their metallic salts and metallic hydroxide lakes, he was awarded the degree of D Sc of the University of Sydney, the Royal Australian Chemical Institute's Rennie Medal (1940) and the Smith Medal (1945) In 1953 he was awarded the University of Melbourne's David Syme Medal and Prize for distinguished work in natural science

In later years, as his international reputation grew, he held the George Fisher Baker non-resident lectureship in Cornell University (1954) and the first Chemical Society lectureship instituted by the Chemical Society of London for the purpose of encouraging the study of chemistry in Australian universities Shortly before he died he had given a series of lectures at the invitation of the American Chemical Society. He is survived by his widow, two sons and a daughter.

Prof Dwyer's colleagues will remember him for his outstanding ability, his personal charm, friendliness and modesty His students will remember not only these qualities, but also his unfailing cheerfulness, sense of humour, encouragement and enthusiasm in the face of their many problems and errors Scientifically, he is a great loss and he leaves many sad friends

A SARGESON

NEWS and VIEWS

Oceanographical Branch, Hydrographic Department, Admiralty:

Commander F W Hunt, M.B.E

COMMANDER F W HUNT, lately superintendent of the Oceanographical Branch of the Admiralty under the Hydrographer, has been appointed in command of H M Surveying Ship *Cook* operating in the Western Pacific Commander Hunt received his initial training in the *Conway*, entering the Royal Navy in 1939, and specializing in hydrographic surveying in 1942 He was appointed a member of the Order of the British Empire in 1945 for his hydrographic surveying work in connexion with the opening up of the ports in the Adriatic during the advance of the Eighth Army Since the War he has served in a succession of surveying ships on most stations including the R R S *John Biscoe* in the Falkland Islands Dependencies In 1955 he assumed command of the East Coast of England Survey and during 1957–60 served as hydrographer of the Royal New Zealand Navy, being the last Royal Navy officer to hold this post

Commander J Paton

COMMANDER J PATON has been appointed to succeed Commander F W Hunt as superintendent of the Oceanographical Branch of the Admiralty Commander Paton specialized in hydrographic survey work in 1947, after serving as a junior officer in the fleet for five years Since then he has served at sea in surveying ships at home and when abroad, mainly in Pacific Ocean waters For the past sixteen months he has been in command of H M S *Cook*, working in the Fiji Islands and Gilbert and Ellice Islands, and during this period undertook deep-sea surveys in connexion with the laying of the Pacific section of the projected Commonwealth cable

The Royal Armament Research and Development Establishment, Fort Halstead :

Dr D. H Black, C M G

DR D H BLACK is retiring from the directorship of the Royal Armament Research and Development Establishment, Fort Halstead, Kent He was born in New Zealand in 1899 and received his early education there, obtaining first-class honours in physics at Canterbury, and then went to work under Lord Rutherford in the Cavendish Laboratory, Cambridge, in 1923, gaining his Ph D in 1925 He spent some fourteen years in industrial research with Standard Telephones and Cables, ultimately as head of the Valve Laboratory, before joining the Government service in September 1939 when he was appointed superintendent of the then Air Defence Experimental Establishment In 1943 he was seconded to the Admiralty for special liaison developments for radar and communications He returned in 1945 as superintendent in charge of research at the now re-named Radar Research and Development Establishment, and in 1947 he moved to Ministry of Supply Headquarters, becoming director of electronics research and development in 1949 In 1953, Dr Black was appointed head of the U K Ministry of Supply mission in Australia During his three years in this post, the interests of the Ministry in Australia expanded considerably with the development of the Guided Weapon and Atomic trials at Woomera and Maralinga For his work in this post Dr Black was appointed C M G On returning to England in 1956, Dr Black was appointed director-general of electronics research and development at the Ministry of Supply Headquarters He assumed the appointment of the then Armaments Research and Development Establishment in 1958

Mr E. W Chivers

MR E W CHIVERS has been appointed to succeed Dr Black Mr Chivers, who has been principal superintendent of the Electronics Division of the R A R D E , graduated in physics (special) with honours in 1927 at Queen Mary College, London After a short period of postgraduate research he entered Government service in 1928 and went to the Research Department, Woolwich, where he was engaged on acoustical research in connexion with sound ranging In 1951 he was transferred to the Searchlight Department at the Air Defence Experimental Establishment, Biggin Hill His work there was concerned with physical problems associated with searchlights and their use, later extending to problems of searchlight design and automatic control In 1940 he was transferred with the remainder of the Biggin Hill establishment to Christchurch to join the Army Radar Group which had been transferred from Bawdsey There he became leader of the team responsible for the application of radar to the control of searchlights, the introduction of which into the Services made such a great improvement in searchlight operation He continued in radar work when the Establishment (by this time the Radar Research and Development Establishment) was transferred to Malvern and was concerned with a number of Army radar projects throughout the War In 1953, when the Radar Research and Development Establishment and the Telecommunications Research Establishment were combined as the Radar Research Establishment, he was promoted to deputy chief scientific officer and became head of the division responsible for ground radar for both the Army and the Royal Air Force In 1954, he was appointed director of atomic weapons (Development and Production) at the Ministry of Supply Headquarters

Mathematics at Dublin: Prof H Halberstam

PROF HEINI HALBERSTAM, who succeeds the late T S Broderick as Erasmus Smith professor of mathematics in the University of Dublin, is one of the group of distinguished mathematicians who were students at University College, London, immediately after the end of the Second World War He was on the mathematical staff of the University of Exeter from 1949 until 1957, with leave of absence to spend most of the session 1955–6 as a visiting lecturer at Brown University, Providence, R I, and the rest as a visiting lecturer at the University of California, Berkeley California Since 1957, Halberstam has been reader in mathematics at Royal Holloway College in the University of London He has been playing an increasingly prominent part in all aspects of the mathematical life of the University, where his presence will be sadly missed. As organizer of the seventh British Mathematical Colloquium in Exeter in 1955, and as a vigorous secretary of the London Mathematical Society since 1959, he has played a much appreciated part in the mathematical life of the whole country In 1961, for example, he undertook the organization of the London Mathematical Society's highly successful first 'instructional course' on functional analysis Halberstam is well known for his work in the theory of numbers, particularly on statistical properties of arithmetic functions and on various topics in additive number theory Apart from some present work connected with finite fields, he is collaborating with Prof K F Roth on a book about general arithmetic sequences In Royal Holloway College he has stimulated much work by colleagues and research students, he can be expected to build up a notable school in Dublin

The Grassland Research Institute

THE annual report for 1960–61 of the Grassland Research Institute, Hurley (7*s* 6*d*), records results of research on a wide field of scientific and practical interests The work is essentially concerned with the grass crop, and the broad object is to determine the right food for cattle and sheep and the type of sward which will most benefit the underlying soil The Department of Herbage Agronomy has continued the intensive study of introduced grasses and pure swards have been established to follow seasonal growth and reaction to management Further work has been concerned with methods of incorporating autumn- and winter-growing grasses into seed mixtures New chemical herbicides have made possible complete sward replacement by the combination of sprays, re-seeding and top dressing all done as one sequential operation without the plough or cultivator Investigations have been initiated in the Plant Physiology Department to elucidate the relative importance of leaf area and carbohydrate reserve on re-growth of pastures following defoliation It was found that some ecotypes of tall fescue from North Africa made better growth during winter at Hurley compared with indigenous grasses, and growth analysis revealed that this was in part due to a greater leaf-area ratio and in part to greater photosynthetic efficiency Preliminary results from a study on flowering of white clover indicated that low temperatures induced flowering which was augmented by long days although low temperature was not essential for flowering

Among the various experiments carried out by the Ley Agronomy Department may be mentioned the attempt to grow wheat after killing leys with herbicides and without disturbing the soil deeper than required to sow the seed In general, grain yield was the same from ploughed land as from the herbicide treatment Results from the Department of Microbiology suggest that there is a correlation between soil fertility and certain soil micro-organisms but absolute proof is lacking Ryegrass and cocksfoot have been successfully cultivated in an atmosphere of carbon dioxide labelled with carbon-14 to obtain uniformly labelled material for decomposition experiments on the soil Digestibility trials with six grasses were performed by the Department of Biochemistry and Animal Nutrition which confirmed that herbage digestibility depended both on species and stage of growth A new method for analysis of organic acids in silage was developed The Grass/Animal Ecology Department analysed the grazing system in so far as it affects milk supply, lambing percentage, stocking-rate and season, all of which influence the growth-rate of lambs The Animal Agronomy Department has been concerned with assessment of animal production (beef cattle and sheep) under different management conditions and on differing sward types

The Society for Folk Life Studies

THE second annual conference of the Society for Folk Life Studies was held in the University of Reading during September 7–9 It was attended by seventy people from all parts of the British Isles as well as overseas The meeting began with a lecture on "The Study of Folk Life from a Swedish Standpoint" by Prof Sigurd Erixon of Stockholm, one of the world's foremost authorities on folk-life studies The remainder of the conference was devoted to a consideration of folk-life studies in relation to other disciplines, and among the speakers were Dr R B Wood-Jones of the University of Manchester, the well-known architectural historian, Mr Basil Megaw, director of the School of Scottish Studies, University of Edinburgh, Dr Ronald Buchanan, a geographer from the Queen's University of Belfast, and Mr David Murison, editor of the *Scottish National Dictionary* In addition there was an excursion to the Cotswolds, where the domestic architecture of that region was studied, while the University of Reading provided a reception for delegates at its Museum of English Rural Life

The first volume of *Folk Life*, the Society's new journal, should be ready early in 1963 It will be a well-illustrated publication of some two hundred pages, edited by Stewart Sanderson of the University of Leeds The next conference of the Society (1963) will be held in the University of Leeds Membership of the Society is open to all those interested in the traditional life of Great Britain and Ireland, and the annual subscription is two guineas Members will receive *Folk Life* free of charge and they will be able to attend all the meetings organized by the Society Further information can be obtained from the secretary, J G Jenkins, Welsh Folk Museum, St Fagans, nr Cardiff

Index Biologicus

FOR many years biologists have felt the need of at least as comprehensive a cover of their literature as has been achieved in chemistry and physics With the setting up of the Department of Scientific and Industrial Research Library at Boston Spa a realistic appraisal of the problem became possible The Council of Management of Biological and Medical Abstracts, Ltd , with encouragement from a number of British biological societies and financial support from the Pergamon Press, carried out in 1961 an investigation as to the size, nature and cost of an *Index Biologicus* It was found that the number of journals which would contain material of biological information, excepting purely applied material and clinical papers already covered in *Index Medicus* would exceed 5,500 and total no less than 200,000 titles per annum If these were classified in an author and subject index the total number of entries to give a reasonable coverage would be 1,000,000 and require at least 10,000 pages per annum Such a mass of material would have to be handled with the maximum of automation It was estimated that the time from the appearance of an article to its publication in the

Index Biologicus would take six weeks and the annual cost would be £72,000 The Council and its publisher are very conscious of the lack of such a rapid, but by no means ideal, collation of the biological literature, but feel that if the cost has to be borne entirely by the subscriber it would be uneconomic and regret their inability to undertake the publication of the *Index Biologicus*

Oak Ridge National Laboratory Publications

THE brochure, *ORNL*—3300 (Volume 1), consisting of special lists by title of publications, reports issued and papers delivered at scientific meetings which originated from Oak Ridge National Laboratory during 1961, covers biology, chemistry, mathematics, physics, metallurgy and instrumentation Articles for which reprints are obtainable on request are indicated by the letter *R* as part of the index number and in requesting reprints only the index number need be quoted Papers listed in the section "Reports Issued" are available for use at the Atomic Energy Commission Depository Libraries or copies may be purchased The brochure concludes with a list of nine theses and an author index

ABC of the Universe

A BOOKLET recently issued under this title by the British Broadcasting Corporation consists of a set of seven broadcast talks by well-known astronomers (Pp 39 London. British Broadcasting Corporation, 1962 2*s* 6*d*) The series is designed to present a view of how the universe appears in terms understandable by the layman To this end the publication appears most lucid and informative, and at the price it is one of the best in the whole field of popular astronomical literature Dr Lyttleton conducts us through the solar system while Dr Reddish examines the life, birth and death of a star This leads naturally to a discussion of the galaxy from the point of view of optical astronomy by Dr Willstrop and from the point of view of radioastronomy by Prof Hanbury Brown The observational approach to cosmology is discussed in the final three lectures by Dr Baldwin, Prof Ryle and Sir Bernard Lovell Dr Baldwin discusses the expansion of the universe while Prof Ryle discusses the limits which are imposed on our observing capabilities He points out why radioastronomy can take us further into space than optical astronomy Sir Bernard Lovell finally links the various theories about the universe with the present state of our knowledge and demonstrates the exciting possibilities of future researches The B B C has done astronomers a service by the publication of this well-balanced account of present-day views at such a modest price

The International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in *Bulletin of Zoological Nomenclature* (19, Part 5, published on September 10, 1962) (1) Validation of *Ictonyx* Kaup, 1835, and *striatus* (*Bradypus*) Perry, 1810 (Mammalia) *Z N* (*S*) 758 (2) Designation of a neotype for *Rana fasciata* Burchell, 1824 (Amphibia) *Z N* (*S*) 1253 (3) Validation of *elegans* (*Arizona*) Kennicott, 1859 (Reptilia) *Z N* (*S*) 1454 (4) Designation of a type-species for *Eucypris* Vávra, 1891 (Crustacea, Ostracoda) *Z N* (*S*) 1462 (5) Validation of *rostrata* (*Aelia*) Boheman, 1852 (Insecta, Hemiptera) *Z N* (*S*) 1490 (6) Validation of *Tetrastichus* Haliday, 1844 (Insecta, Hymenoptera) *Z N* (*S*) 1503 (7) Designation of a type-species for *Dicellomus* Hall, 1871 (Brachiopoda) *Z N* (*S*) 1504 (8) Validation of *Lingulops* Hall, 1872 (Brachiopoda) *Z N* (*S*) 1505 (9) Designation of a type-species for *Orbiculoidea* d'Orbigny, 1847 (Brachiopoda) *Z N* (*S*) 1506 (10) Suppression of *piperita* (*Cypraea*) Gray, 1825 (Gastropoda) *Z N* (*S*) 1510 (11) Validation of *Jovellania* Bayle, 1879 (Cephalopoda) *Z N* (*S*) 1511 (12) Suppression of three specific names of Spanish Palæozoic Crinoidea *Z N* (*S*) 1513 (13) Validation of *Vanikoro* Quoy & Gaimard, 1832 (Gastropoda) *Z N* (*S*) 1524 (14) Validation of Phasmidae (Insecta, Orthoptera) *Z N* (*S*) 1167 Any zoologist who wishes to comment on any of the above cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road London, S W 7, before March 10, 1963

Pest Control

SOME of the results of the work carried out by the Infestation Control Laboratory of the Ministry of Agriculture, Fisheries and Food are shown in a publication, the second of its kind, issued by the Ministry of Agriculture, Fisheries and Food (*Infestation Control a Service to Agriculture and Food Storage* H M S O 6*s* 6*d*) Outlined first is the scientific work done by the Ministry on the control of pests of stored products and harmful mammals and birds during a period of sixteen years up to 1958 The second part covers the subsequent period to 1961 Since 1958, additional facilities have been acquired by the Ministry to meet the growing need for work on the scientific aspects of the control of land pests and birds One of the most difficult and urgent problems being investigated is the large increase of the numbers of coypus in East Anglia Following a description of the organization of the Laboratory, the three main sections of the booklet, insect pests of stored products, rats, mice and coypus, and land pests and harmful birds, give useful information on the habits of the pests, together with the latest available information about their control

Birds in Suffolk

1961 was noteworthy in Suffolk for the addition of two new species to the county list (*Transactions of the Suffolk Naturalists Society*, 12, Part 2, 1962) In April, a Bonelli's warbler was trapped and ringed at Walberswick and, on September 1, a buff-breasted sandpiper was seen at Minsmere Although examples of this American wader had previously occurred on Breydon, they had all been claimed for Norfolk Thanks to the intensive watching that now takes place in all likely areas on the coast, as well as the increase in mist-netting and trapping, it will be surprising if one or more species that are new to Suffolk are not recorded annually for some time to come Rarities from Minsmere included a gull-billed tern in June, followed by a Caspian tern and three roseate terns in July and August, a long-tailed skua, icterine warbler and pectoral sandpiper in August, and a yellow-browed warbler in October The long-tailed skua, which was seen at close range, was the first known record for Britain during this century Minsmere was also visited, for the second winter running, by a kite Another, or more probably the same, bird was seen at Tunstall and Snape a fortnight later An immature purple heron was found injured beneath electric pylons at Herringfleet on September 9, and an ortolan, the first definite record for many years, was identified at Walberswick on September 1

Requirements for Poliomyelitis Vaccine

OVER the past four years, a number of World Health Organization Study Groups have met to consider requirements for biological substances which could be recommended for international adoption Recommended requirements for inactivated poliomyelitis vaccine were published three years ago, but, since that time, live poliomyelitis vaccine, administered orally, has come into use for mass immunization programmes in many countries It has therefore become necessary to formulate requirements for the oral vaccine The recommendations of a recent report are restricted to the preparation of oral poliomyelitis vaccine in monkey kidney cultures, since, so-far, only vaccines prepared by this method have been extensively used in man (*Technical Report Series* No 237 *Requirements for Biological Substances* 7. *Requirements for Poliomyelitis Vaccine* (*Oral*) Pp 29 Geneva World Health Organization, London H M S O, 1962 1 Swiss franc, 1*s* 9*d*, 0 30 dollars) In drafting the requirements, the Study Group took into consideration the regulations and requirements already proposed by a number of national authorities, as well as data submitted by experts in the field, including the authors of three oral vaccines which have been tested in large-scale immunization campaigns in man

Ramsay Memorial Fellowships

THE following Ramsay Memorial fellowships in chemistry for the year 1962–63 have been awarded A Canadian fellowship to Dr R C. Thompson, University of Manchester, an Indian Fellowship to Dr R S Thakur, University of Cambridge, a Netherlands Fellowship to Mr A Schuyff, Imperial College of Science and Technology, London, a New Zealand Fellowship to Mr D T Dixon, University of Reading, a Spanish Fellowship to Dr D Vazquez Martinez, University of Cambridge, a United States Fellowship to Mr Jack H Freed, University of Cambridge

The following fellowships have been renewed for the same year to Dr H Suginome (Japanese Fellowship), University of Cambridge, Mr B. A Grigor (New Zealand Fellowship), University of Leicester, Dr F Alonso Matthias (Spanish Fellowship), University of Cambridge

University News : London

PROF R A L BLACK, Chamber of Mines professor of mining engineering in the University of the Witwatersrand, has been appointed to the chair of mining at the Imperial College of Science and Technology as from October 1, 1963

Dr J F Kirkaldy, reader in geology at Queen Mary College, has been appointed to the chair of geology tenable at that College Dr R L Bell, lecturer in metallurgy in the Imperial College of Science and Technology, has been appointed to the readership in science of materials tenable at Queen Mary College Dr P M Cohn, lecturer in the University of Manchester, has been appointed to the readership in pure mathematics tenable at Queen Mary College

The following titles have been conferred *Professor*, on Dr A M Cook (pharmaceutics), in respect of his post at the School of Pharmacy, on Dr B Dawes (zoology-parasitology), in respect of his post at King's College *Reader*, on Mr B B. Boycott (zoology), in respect of his post at University College, on Dr R E Burge (biophysics), in respect of his post at King's College, on Mr W H Gosney (mechanical engineering), in respect of his post at King's College

Announcements

THE Medical Research Council Clinical Chemotherapy Research Unit at the Western Infirmary, Glasgow, has been reconstituted as the Atheroma Research Unit under the direction of Dr. B. Bronte-Stewart The Unit will be primarily concerned with research on occlusive vascular disease

A MEETING on "Scattering of Elastic Waves", arranged by the Materials and Testing Group of the Institute of Physics and the Physical Society, will be held on October 12 Further information can be obtained from the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S W 1

THE annual Pittsburgh diffraction conference will be held at the Mellon Institute, Pittsburgh, during November 7–9 Subjects under discussion will include instrumentation and techniques, structures, metals and alloys, defect lattices and/or radiation damage, X-ray and electron diffraction microscopy Further information can be obtained from W H Robinson, Carnegie Institute of Technology, Pittsburgh 13, Pennsylvania

THE autumn meeting of the British Society for Parasitology will be held at the Zoological Society of London on October 19 Subjects under discussion will include biochemical techniques, ionizing radiations and radioisotopes, *in vitro* cultivation and serological techniques, electron microscopy Further information can be obtained from Dr L P Joyner. Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, New Haw, Weybridge Surrey

THE thirty-sixth annual conference of Aslib will be held in Blackpool during October 2–4 Subjects under discussion will include sharing skills, technical aid to developing countries, the foreign language barrier in science and technology, monitoring current technical information with the British technology index, linking research and design, relations between a central information unit and sectional units, a multi-purpose, computer-integrated system for centralized information dissemination, storage and retrieval Further information can be obtained from Aslib, 3 Belgrave Square, London, S W 1

A CONFERENCE on "The Epilepsies and Society", arranged by the Scottish Epilepsy Association, will be held at Dunblane Hydro during November 9–11 Subjects to be discussed will include the challenge of the epilepsies in the welfare state, the scope and limitations of present-day treatment of the epilepsies, the management of the epilepsies in Germany—vocational guidance, settlement in employment, problems within the family, the social integration of persons with epilepsy, the employment of persons with epilepsy Further information can be obtained from Mrs Christina G Kilgour, Scottish Epilepsy Association, 24 St Vincent Place, Glasgow, C 1

THE NUFFIELD FOUNDATION

THE seventeenth annual report of the Nuffield Foundation, covering the year ended March 31, 1962*, records allocations during the year totalling £1,823,996, of which £26,600 was for biological research and £219,150 for other scientific research. Repayable grants totalling £43,850 included interest-free loans of up to £25,000 repayable within 10 years to the University of Glasgow for rebuilding the freshwater biology research station on Loch Lomond and of up to £25,000 for 5 years to the Society for the Promotion of Nature Reserves to establish a loan bank so that when a site or area of scientific interest comes on the market they can buy it without delay. The sites which this Society and the County Trusts have been able to acquire are becoming more and more valuable to scientists, and biological and physiographical work of outstanding importance, both amateur and professional, is being carried out in these reserves. A further, final grant of £7,500 over 3 years has been made to King's College, University of London, for Prof. J. F. Danielli's studies of the cell cortex, cell movement and transport. A two-year programme of research on the chemistry and biochemistry of pteridines at the Royal College of Science and Technology, Glasgow, is being supported with a grant of £3,850 over two years, while a special grant of £3,000 was made to Armagh Observatory to complete and furnish the library, in the expectation that future needs of the Observatory will be met by the Government of Northern Ireland. A grant of £10,000 was made to Birkbeck College, University of London, to cover installation and initial working of a computer for mechanical translating.

Grants totalling nearly £650,000 in medicine included £200,000 over 10 years to the Institute of Child Health to enable it to develop its department of growth and development to become the basic science of the department of pediatrics, on the understanding that at the end of 10 years it would have been absorbed into the University of London, and £55,000 over 5 years, also to the Institute of Child Health, to establish a Nuffield Neonatal Research Unit at the Hammersmith Hospital, £200,000 to the University of Edinburgh towards the establishment of a tissue and organ transplantation unit, to be called the Nuffield Unit for transplantation surgery, on the understanding that the Department of Health for Scotland will provide about £100,000 over 5 years, up to £25,000 over 5 years to the Unit of Biometry, University of Oxford, for a programme of operational research methods applied to the improvement of health services, £15,000 to the University of Birmingham to enable Prof. Ian Bush to build up his department of physiology and create a research group in chemical physiology, a concluding grant of £7,050 over 3 years to the University of Leeds for extension of the work in the Departments of Diagnostic Radiology and Medical Physics on developing techniques of X-ray image detection by television methods, and a grant of £50,000 to the British Medical Association towards the conversion of a wing for the library: the reconstructed library will be known as the Nuffield Library of the Association.

In social research and experiment a capital grant of £50,000 was provided for the National Institute for Social Work Training towards the cost of putting the buildings in good order, this, with the grant of £250,000 over 10 years previously promised, assures the Institute of continued existence for 10 years, within which period help is expected to be forthcoming from public funds and other sources. A grant of £22,500 was offered towards the cost of an inquiry being conducted by the University College of Swansea into the lower Swansea Valley as a basis for subsequent development. £56,000 over 4 years to the London School of Economics and Political Science and the School of Oriental and African Studies is for a scheme of field research in south-east Asia and the borderlands of southern China into modern social economic, political and cultural changes in the region, a third survey of London life and labour, to be conducted by the Centre for Urban Studies, University College, London, and the London School of Economics and Political Science is being supported by a grant of £30,000 over 3 years, and an investigation into the functions and development problems of town centres by King's College, Newcastle upon Tyne, by a grant of £10,500 over 3 years. Of special significance at the present moment is the grant of £23,000 over 2 years to Political and Economic Planning for a study of management attitudes in industry, and the ways in which these attitudes may retard growth of the economy and the development of the export trade.

Among grants for the Commonwealth Overseas, besides those for the newly independent territories, those of up to £13,000 for 3 years on a tapering basis to the Government Central Pedagogical Institute, Allahabad, in support of a diploma course in teaching English (on condition that the Government took over the full cost at the end of the period) and of £3,200 to the Universities of New South Wales and Sydney for a joint research project into crystal-structure analysis using X-ray data from a rotating-anode source at low temperature may be mentioned. No change is reported in the types of awards in medicine and dentistry, but a considerable extension of the programme for scholarships in tropical medicine is to take effect in 1962–63. One medical research fellowship, three medical fellowships, three fellowships and three assistant fellowships, three Heinz fellowships, one dental research fellowship, two dental fellowships and two dental scholarships were awarded. There was an unusually promising group of applicants for biological scholarships and bursaries and one scholarship and four bursaries were awarded. Under the scheme for the Dominions and Colonies ten awards were made in medicine, 13 in the natural sciences (including one Indian fellowship in engineering), and 11 in the humanities and social sciences: there were also 10 Canadian travel grants. In addition to scholarships to three African farmers from the Federation of Rhodesia and Nyasaland, eight scholarships were awarded to farmers from Australia,

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* The Nuffield Foundation. Seventeenth Report for the year ended 31 March, 1962. Pp. xiv+159. London: The Nuffield Foundation, 1962 (see also p. 1235 of this issue).

Canada, Rhodesia and New Zealand The scheme of awards for Indian civil servants is to be continued, and recommendations for three fellowships have been accepted for 1962 The report notes that some 50 recipients of the Foundation's fellowships now hold chairs or appointments of similar status in universities overseas Under the Royal Society and Nuffield Foundation Commonwealth Bursaries scheme, to which the Foundation contributes £5,000 a year, 18 bursaries were awarded in 1961

THE NATIONAL CHEMICAL LABORATORY

WHEN the work of the National Chemical Laboratory was last laid open to public view, in October 1960, a good deal of what could then be shown represented the early stages of a thorough-going revision of its objectives Open days were again held this year between September 25 and 28, and provided an opportunity for visitors to the Laboratory to see what progress has been made in the interval On this occasion there was perhaps more scope for the Laboratory to show by the manner of development of its programme that its contribution to the totality of pure and applied chemical research in Great Britain is distinctive and is of the character appropriate to a national laboratory Unquestionably, open days, as a means of bringing the efforts of the Laboratory directly into public notice, are indispensable the large influx of visitors from other Government departments, from industrial laboratories and from universities, colleges and schools makes this quite evident In all, some 129 exhibits were displayed in 55 separate laboratories But to arrange for the movement of large numbers of people through many small laboratories where space is at a premium was a difficult exercise

Research on metallic corrosion is one of the Laboratory's oldest interests, and over the years noteworthy contributions have been made to knowledge of corrosion mechanism and corrosion inhibition A substantial element of the present work is concerned with the microbiological factors that come into play when metals are buried in soil or clay, or exposed to mud or saline and polluted waters Several exhibits illustrated the manner in which field studies are used to supplement controlled laboratory experiments Thus, information is secured which is of the greatest practical value to civil engineers who must devise adequate protection for buried metal structures, pipes or cables A series of sites representative of different soil conditions has been chosen where metals can be buried and left undisturbed over periods of time The soils are examined for resistivity, redox potential, *p*H, uptake of hydrogen, sulphate-reducing bacteria population, etc , and the results are compared with the aggressiveness of the soil as shown by the corrosion of the test metals It has been revealed by laboratory experiments that when the corrosion of iron and steel in waterlogged clay is kept going by bacterial activity, various strains of the bacteria differ in their power to stimulate corrosion, probably through differences in enzyme make-up With many strains, corrosion stimulation is proportional to the hydrogenase activity of the bacteria, but one salt-water strain from California causes rapid corrosion although apparently deficient in this enzyme In collaboration with the Port of London Authority a study is being made of the aggressiveness of Thames water and mud towards unprotected mild steel at different sites along the estuary between Richmond and Southend In these experiments information is being sought on the role (if any) of water-borne pollution

Whether subject to bacterial stimulation or not, corrosion is the resultant of chemical and electrochemical factors, and several exhibits illustrated how these factors are being identified and studied The rate at which oxygen or other reactant is transported by diffusion or convection to a metal surface exposed in aqueous solutions often controls the corrosion process It is not easy to design experiments for defining the hydrodynamic factors precisely, a system amenable to analysis involves the conditions of flow over a metal disk rotating at an accurately controlled speed The adsorption of certain anions on a mild-steel surface is believed to be important in initiating the inhibition of corrosion Methods for measuring the amount of adsorbed phosphate (tagged with a radioactive atom) were shown Adsorption was found to vary with the electrode potential of the steel Electrode potentials of mild steel in aerated solutions of a wide range of electrolytes have shown that all anions stimulate corrosion at low concentrations and all tend to become inhibitive at high concentrations, the concentration at which this change occurs being characteristic of each anion

Primary processes occurring on metal surfaces are studied in the Director's laboratory using the twin techniques of field-emission and field-ionization microscopy Exhibits illustrating both these techniques were presented They allow magnification of more than a million diameters, and the high resolution of the field-ion microscope renders single atoms visible, thus, both techniques allow processes to be examined in atomic detail Condensation of metal vapour on clean metal surfaces in ultra-high vacuum is studied by field-emission microscopy and the field-ion microscope is used to study chemisorption and reaction of gases on metal surfaces

In the Inorganic Group an increasing amount of attention is being devoted to the rare earths Separation processes utilizing ion-exchange chromatography and reduction by sodium amalgam were demonstrated, the latter method being used for the separation of the more readily reducible elements samarium, europium and ytterbium In a search for uses for the rare earths a section has been formed to study the solid-state chemistry of some of the rare earth compounds, particularly the chalcogenides The preparation of rare earth selenides from the anhydrous chlorides and hydrogen selenide was shown together with some preliminary work on phase relationships Equipment for studying adsorption, catalytic activity, electrical and thermal conductivity was on view together with induction-heated furnaces for the preparation and pre-treatment of specimens It is hoped that this work will not only have its applied value but also that it will lead to a fuller appreciation of the characteristics of solids, especially the nature of internal and surface bonding

The Extraction of Metals Group showed the progress it has made in developing a rotary-disk contactor for the solvent extraction of solutes from slurries. A 5-stage unit utilizing 4-ft diameter disks with a processing capacity of 1–2 tons/day was exhibited. This is an element of a full-scale plant. Solvent extraction in the presence of mineral solids can be rendered troublesome by the chemical adsorption of extractants on the solid surface. Measurements of the specific adsorption of amine-type extractants on different minerals have been undertaken. Several exhibits were concerned with the study of processes for the extraction of beryllium from mineral concentrates containing from 2 per cent to $>$ 10 per cent beryllium oxide as beryl. Sufficient understanding of the reactions involved has led to the establishment of conditions under which beryl can be rendered water- or acid-soluble by heating the concentrate with sodium silicofluoride or with calcium carbonate. The variety of products formed by the devitrification of the glasses obtained on melting and quenching beryl-containing materials (on which the subsequent chemical processing depends) was illustrated by results obtained from differential thermal analysis, X-ray diffraction, hot-stage microscopy and cooling-curve studies. Solvent extraction methods have been newly worked out for beryllium. Recovery from sulphate leach liquors by solvent extraction was demonstrated in a continuous counter-current unit, while the possibility of selectively leaching beryllium from suitably sulphated products with aqueous ethanol formed the subject of a further exhibit.

A most interesting new research project was an investigation into high-temperature reactions undergone by solid inorganic compounds introduced as fine particles into flames. Two flame sources were exhibited, one an oxy-acetylene torch and the other a plasma jet through which particles of simple refractory compounds or minerals could be blown continuously. Projected experimental work includes a study of the reconstitution of minerals, carbothermic reduction of minerals to metals and the chemistry of the condensation processes after complete atomization of minerals in flames of suitable temperature. A marked interest in solution equilibria was indicated by a number of exhibits dealing with the use of ultracentrifugation, potentiometric titration, ion-exchange and cryoscopic techniques for the study of the hydrolytic polymers present in solutions of silica, gallium and aluminium salts and spectrophotometric techniques for the study of complex cyanides. Developments in ion-exchange processes were illustrated by the demonstration of a method for the separation of copper, cobalt and nickel by selective elution from a cation-exchange resin with organic solvent-mineral acid mixtures and by anion-exchange resins, prepared by the amination of polyvinylchloride, which show little tendency to absorb silica from acid solution.

For visitors interested in organic chemistry the New Materials Group displayed exhibits illustrating recent progress in four distinct fields of study. These are the organo-metallic chemistry of boron and aluminium with nitrogen, a quest for organic polymers resistant to heat and oxidation, charge-transfer and semi-conduction phenomena, and ion- and electron-exchange polymers. It will be recalled that the original discovery of ion-exchange resins (now so vastly important in chemical technology) was made at the Laboratory some thirty years ago. New compounds (borazynes) based on the $-B=N-$ monomer unit were shown. These are related to borazoles in the same way as *cyclo*-octatetraene to benzene. The chloro compound $[t\text{-BuNBCl}]_4$ is an effective anti-knock, and serves as a starting point for the preparation of other derivatives, for example, the remarkably stable tetra-azide. Work on the preparation of dichloroborazoles and their application to the synthesis of polymers containing the borazole structure has been continued. An achievement worthy of notice is the discovery of tetrameric alazynes, analogous to the tetrameric borazynes above mentioned. A typical example is $[C_6H_5NAlC_6H_5]_4$. X-ray crystallographic data are consistent with two molecular structures—a cubic form or an 8-membered ring of alternate aluminium and nitrogen atoms. The latter would be the first example of π-bonding between aluminium and nitrogen. New technological possibilities exist in a class of organic polymers—the polypyromellitimides, formed from pyromellitic dianhydride and aromatic diamines—remarkable for their thermal and chemical stability. In addition to this synthetic work, the exhibits of the Group illustrated the progress that has been made with physico-chemical studies of ion-exchange resins and membranes and in the characterization of polymers, particularly by the use of gel-filtration. Other exhibits included demonstrations of the use of polarography, electron spin resonance and nuclear magnetic resonance to elucidate the structure and behaviour of charge-transfer complexes and of ion-exchange resins.

The world's common stock of precise physico-chemical (and especially thermochemical) knowledge is the necessary basis both for considering chemical reactions in the research laboratory and for designing processes on the manufacturing scale. The Chemical Thermodynamics Group has been set the task of surveying published data on the thermodynamic properties of substances, identifying deficiencies and undertaking new measurements where these are most obviously needed. Work started earlier on phenols is now being supplemented by work on aliphatic oxygen-containing organic compounds. A programme on inorganic compounds has been initiated which embraces phosphorus compounds and the thermodynamics of non-stoichiometric metallic oxides. The measuring equipment exhibited covers a full range of properties: heat of combustion, low-temperature heat capacity, vapour heat capacity, latent heat of vaporization, vapour pressure, critical temperature pressure and volume, and gas imperfections. The precision of these measurements is the highest attainable. Work on infra-red absorption and Raman spectra—including new work on the observation and identification of frequencies in the far infra-red—was also to be seen, since the computation of thermodynamic properties by the methods of statistical mechanics from vibrational assignments is being developed *pari passu* with their direct measurement.

New exhibits illustrated the measurement of the free-energy of formation of an iron–phosphorus alloy by the study of the vapour pressure of phosphorus above the alloy and free energy measurements on non-stoichiometric oxides by means of solid electrolyte cells. The use of shock tubes for the investigation of high-temperature reactions of inorganic gases could be seen in the Aero-Physics Laboratory of the National Physical Laboratory.

Precise measurements are only meaningful if made on specimens of high purity. Although raw materials can sometimes be bought and purified, considerable ingenuity often has to be exercised in the synthesis of specimens free from troublesome and persistent impurities. The chemical purification of *n*-pentanol and the synthesis of *m*-ethylphenol illustrated some facets of this work. Purification techniques now being used include fractional distillation and freezing, zone melting and sublimation. Purity control is by gas chromatography, mass spectrometry, differential thermal analysis, cryoscopy and high-resolution nuclear magnetic resonance spectroscopy. The above-mentioned techniques are also used to prepare and certify pure materials as 'Standard Samples'. This work was illustrated by the purification of fatty acids for the synthesis of higher paraffins and by the synthesis of compounds in the indane series. An apparatus for ampouling pure gaseous and liquid samples was on display. National Chemical Laboratory Standard Samples supplied by this Group are purchased by industry at the rate of about 400 per year for the calibration of mass and other spectrometers which are in use for the analysis of chemical plant streams.

E. A. Coulson

BROOM'S BARN EXPERIMENTAL STATION

By Dr. R. HULL

On July 27, the Minister of Agriculture, Fisheries and Food, the Right Hon. Christopher Soames, formally opened Broom's Barn Experimental Station, Higham, Bury St. Edmunds, Suffolk, in the presence of about 250 guests. The Right Hon. the Earl of Radnor, chairman of the Lawes Agricultural Trust Committee, welcomed the Minister and invited him to open the Station, and Sir Peter Greenwell, chairman of the Sugar Beet Research and Education Committee, thanked the Minister.

Broom's Barn farm was acquired in 1959 by the Lawes Agricultural Trust Committee, the governing body of Rothamsted Experimental Station, and has been developed as a research station to study sugar beet growing. The land, new farm buildings and laboratories have been paid for by grants amounting to nearly £250,000 from the Ministry of Agriculture's Sugar Beet Research and Education Fund, to which both sugar beet growers and the British Sugar Corporation, Ltd., subscribe.

Broom's Barn Experimental Station replaces the Rothamsted Field Station, Dunholme, near Lincoln, where research for the Sugar Beet Research and Education Committee has been carried out since 1947. Work on diseases of sugar beet, which began at Hackthorn in Lincolnshire in 1935 in association with the Midland Agricultural College, became a charge of Rothamsted Experimental Station after the Second World War and a field station was established in temporary buildings on a disused airfield at Dunholme. The work extended to include the manuring and pests of sugar beet, but was handicapped by lack of land and suitable buildings.

The ultimate aim of all the work is to improve the health and yields of both the root and seed crops. To this end, field experiments and observations, supported by the necessary glasshouse and laboratory work, are made to elucidate the biology of pests and pathogens, to assess the damage they cause, and to find how the losses can be lessened. The research workers co-operate closely with the field staffs of the sugar factories in field experiments, in recording the incidence of pests and diseases, in organizing a spray-warning scheme to advise growers when to spray the crops against aphids that threaten to spread yellows, and in the certification of mangold and sugar beet stecklings.

The work on manuring aims at ensuring that fertilizers are used most efficiently and economically. Each year many field experiments, performed in conjunction with the British Sugar Corporation, test responses to a range of fertilizers applied in different ways. Laboratory methods of forecasting these responses are investigated. This work will continue and be expanded at Broom's Barn, where it will also be possible to do long-term experiments on the effect of fertilizers, cultivations, crop rotations and agricultural chemicals (weed killers, insecticides and fungicides), and to study irrigation of sugar beet.

The Buildings

The new buildings consist of an administrative block with offices, library, meeting hall and mess room (3,740 sq. ft.), chemistry and biological laboratories (4,040 sq. ft.), field laboratories (3,700 sq. ft.), tarehouse (3,000 sq. ft.), glasshouses (2,830 sq. ft.), cloakrooms (790 sq. ft.), circulation areas (1,650 sq. ft.) and a service block (2,160 sq. ft.). There are five cottages for Staff and a house for the Head of the Station (Dr. R. Hull).

The buildings were designed by Cowper, Poole and Partners, Chartered Architects, to blend into the open Suffolk landscape. The choice of the traditional rural pantiles as roofing set limits on the pitch of roofs and width of buildings. The layout of the buildings and their interior design give flexibility and allow for expansion should this prove necessary. The service mains within the buildings are at high level and have a 'fishbone' layout, so that they are easily accessible and altered. The courts between buildings give sheltered outside working space—a boon on this windswept exposed site.

In the administrative building the offices occupy the south side, the library, mess room and cloakroom the north and the meeting hall lies between them. This airy hall, with underfloor heating, will seat more than 200 people. It will be used only occasionally for such large audiences, so the furnishings have been designed to divide it into parts suitable for small conferences, staff meetings or demonstrations.

The laboratory building provides a large chemistry and a biology laboratory with eight smaller laboratories on the south side. The north-facing, large laboratories are lit by the south-facing clerestory windows. The circulation area is cut off from the laboratory only by pillars, an arrangement which avoids tunnel-like corridors. There are a photographic studio and dark room, balance room, chemical dark room, inoculation room and medium room and main store.

The field laboratory is the same basic construction as the main laboratory, but has a cheaper finish.

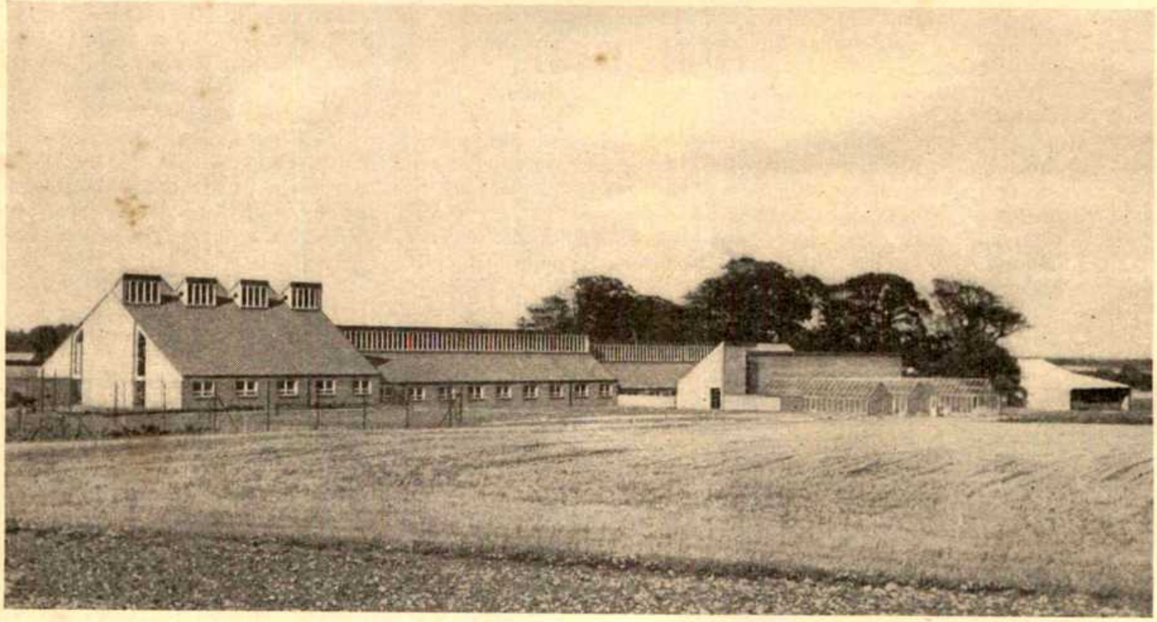

Fig. 1. View of Broom's Barn Experimental Station from the south-west. From left to right are the administrative building, laboratories, field laboratories, and tarehouse. In front of the latter is the headerhouse with four compartmented glasshouses. On the right, among the trees, are the farm buildings

It contains a room for preparing and storing potting-soil, a soil-sample store, an area for drying, treating and storing seed, a general reception area and a laboratory.

The adjoining tarehouse has the sample reception and storage platform at high level and the machinery at low level. The two washers use recirculated water from a settling pond situated near the farm buildings. One of the spray washers serves an automatic line in which the roots are not handled after being tipped into the washer hopper; from the other, the roots are delivered to baskets on roller conveyors so they can be examined, topped, etc., after washing. Both lines feed a multiple saw, and the dross is delivered into a lorry or heap outside. The brei from the saws goes for test of sugar content and juice purity into the adjacent tarehouse laboratory, which is part of the field laboratory.

The Farm

The farm lies 230–280 ft. above sea-level and consists of 190 acres in a rectangle approximately 1,200 yd. by 770 yd. The soil is glacial deposit over chalk. On the north side of the farm it is light and sandy (Moulton and adjoining series) grading through medium loam with flints (Newmarket and Ashley series) to a heavy loam (Stretham series) as the land rises to the south.

The old farm dirt tracks have been ploughed and a concrete road laid to give access to the farm buildings and to all fields. There is a concrete beet-loading platform at the south end of the farm. All but one of the eleven fields into which the farm has been divided are roughly rectangular and most boundaries are parallel to the sides of 5-chain grid squares, marked by permanent sockets around the farm boundary. Soil samples have been taken from each square chain, analysed for available phosphorus, potassium, *p*H and organic matter and stored for future reference.

A 5-in. irrigation main runs the length of the farm, and temporary surface mains can be taken from hydrants to irrigate any part. The water is pumped electrically from a 400 ft. borehole near the farm buildings, which yields more than 12,000 gallons per hour, enough to irrigate 2 acres at a time, and also supplies the buildings. The pumping mechanism is arranged so that experimental watering can be done from the irrigation main at a delivery rate much lower than that needed for farm crop irrigation.

The farm buildings are surrounded by a concrete apron. The old timber Broom's Barn is used for storage, and a new general purpose building (2,250 sq. yd.) is used for machinery, workshop and storage. A new covered cattle yard (6,750 sq. ft.) has a centrally placed self-fed silage silo for feeding 80 bullocks in winter. The pumphouse, tarehouse settling pond and sewage plant are near the farm buildings.

The current farming system aims to get the land into good condition for experiments. The basic crop rotation is sugar beet, three cereals and temporary ley, but each field in turn carries a silage crop for cutting in June, after which it is fallowed. This will help to free the land of a heavy weed infestation.

At present, the sugar beet crop provides the site for annual experiments on the control of downy mildew (*Peronospora schachtii*), chemical weed control, the use of insecticides as seed dressings, soil applications and sprays for controlling aphids and virus yellows, testing and selecting yellows-tolerant varieties and the effect of nitrogen and sowing date on yield. Experiments on the cereal crops determine nitrogen responses. When the land is clean and in good condition, long-term experiments will be started. The experiments will be designed to investigate sugar beet as a crop of the rotation and determine how treatments applied to it affect following crops and how treatments applied to previous crops affect the sugar beet. A subject of immediate importance for investigation is the sugar percentage and juice purity of beet, both of which have decreased as the yield of beets and sugar have increased in recent years.

HYDRAULICS RESEARCH STATION, WALLINGFORD

"WERE water a perfectly non-viscous inelastic fluid, whose particles, when in motion, always followed sensibly parallel paths, hydraulics would be one of the most exact of the sciences. But water satisfies none of these conditions, and the result is that in the majority of cases brought before the engineer, motions and forces of such complexity are introduced as baffle all attempts at a rigorous solution."

These words, written in 1908 by the late Prof. A. H. Gibson, might still serve as a *raison d'être* for the science of experimental hydraulics and for the large research stations at (for example) Delft, Vicksburg, Grenoble and Wallingford. Such stations are largely concerned with problems in free-surface hydraulics, but when one expresses this academic phrase in terms of water-supply, flood prevention, and sea defences, its impact on everyday life becomes apparent. One needs only to think of drought at any time or place, the catastrophic floods at Lynmouth, or the *seiches* of the North Sea in 1953.

So much for the reality of the problems demanding experimental study. But the cost of experimentation on the full scale is generally too high in money and, perhaps, in lives. Thus, most of this laboratory work is scaled down in space and time, so that the accuracy of the results depends on the degree of dynamical similarity achieved.

*Hydraulics Research 1961** embodies the annual report of the Hydraulics Research Board and the report of the Director of Hydraulics Research, and in 90 pages summarizes the work of the Hydraulics Research Station, Wallingford, for the year. There is a brief section on hydrological research, an activity to be developed considerably after the attachment to the Station of a special Hydrological Research Unit. This unit is expected to concern itself particularly with the problem of rainfall and run-off in natural catchments. Such a development, clearly of national importance, will be widely welcomed. Much of the hydraulic work undertaken is necessarily of an *ad hoc* nature, in the sponsored solution of diverse practical problems. Nevertheless, there is evidence of a fundamental approach, for example, to the question of dynamical similarity in sediment transport, and again in the study of vortices.

It is stated that about one-half of the sponsored schemes originate overseas, so that the Station has already attained a measure of international recognition: its work, in fact, may be compared with that of the Waterloopkundig Laboratorium at Delft. The principal study-groupings are: *hydraulic structures* (including interesting reports on critical-depth gauging-flumes, vortex formation at intakes, and hydro-pneumatic ship-fenders), *unidirectional channel flow* (including, for example, the measurement of sand transport with the aid of radioactive tracers), *estuarial studies*; *waves and sea defences* (including full-scale field studies of littoral drift, and laboratory studies of the forces exerted by waves and currents on a submarine pipe), and there are short sections on *instrumentation and mechanization* which might usefully be expanded in future years.

These casual references do little justice to the variety of the problems studied (in fact, no fewer than forty projects are summarized in the booklet), and the ingenuity with which they are tackled can only be appreciated after a careful reading.

There is a useful appendix listing the papers written during the year by members of the Station staff, which will enable readers to follow up particular topics in greater detail. R. B. WHITTINGTON

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* Department of Scientific and Industrial Research. Hydraulics Research 1961. The Report of the Hydraulics Research Board with the Report of the Director of Hydraulics Research. Pp. iv+96+8 plates. (London: H.M.S.O., 1962.) 7s. net.

NUTRITIONAL PREFERENCES AMONG THE NIGERIANS

THE West African Science Association was founded in 1953. Although its members were distributed throughout West Africa, they were predominantly resident in Ghana, where all its activities took place. It had been felt for some time that similar activities should take place in Nigeria, and, in May 1957, a circular was dispatched to as many scientists as possible resident in or near Ibadan. The replies received were mainly in favour of the formation of an Ibadan Branch of the West African Science Association though many persons preferred the formation of an independent association in Nigeria. A meeting was held on October 25, 1957, at which it was agreed that an organization to bring scientists together should be formed in Nigeria and should, if possible, be associated with the West African Science Association. This was later agreed, and the inaugural meeting of the Association was held in December 1958. The Proceedings of the Association have now been published, the first volume covering the first three years of its existence*.

It contains summaries of many important articles, among them being one by Dr. W. R. Stanton of the Ministry of Agriculture, Regional Research Station, Samaru, Zaria, dealing with future changes in preference among Nigerian peoples for locally grown foodstuffs.

Preference is divisible into two types—rational and irrational. The author suggests that the study of the 'irrational' causes of preferences is equally important to that of the study of 'rational' causes. These may be listed as follows:

Irrational factors (namely, those ethnobotanically or animistically developed or a result of the cultural development of the community): (1) religious dictates, (2) animistic taboos, (3) archaic survivals of the eco-biological evolution of society, (4) traditional methods of preparations and uses, (5) prestige and status value, (6) attractiveness (in the sense that attractiveness can be the result of conditioning).

Rational factors: (1) availability and price, (2) palatability alone and with other foods (palatability, however, is subject to conditioning), (3) ease of culture, harvesting, preparation, storage, handling and marketing; yielding ability, appropriate developmental time in relation to climate, soil and water, (4) use of the plant as a whole (gross as distinct from net utility).

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* Science Association of Nigeria. Proceedings, Volumes 1–3, 1958–1960. Edited by Dr. B. Hopkins. Pp. 78. (Ibadan: Science Association of Nigeria, 1962.) 5s. 6d.

Stanton cites examples of the operation of these various types of preference particularly within and between different types of cereal grown in Nigeria. In studying the variation within and between the food crop species in a country, correlation with the biological factors (a convenient index for which is the ecological zone) is incomplete and a useful stratification is provided by maps showing the cultural units of Nigeria such as those defined previously by Buchanan and Pugh. The author compares interaction with the ecological zone of the strongly developed 'sorghum' culture of the savannas of West Africa with the development of grain cultures in equivalent zones in East Africa.

He suggests that present observed preference should not be the sole means of determining the development policy for food crops (species and type), but that account should be taken of the trend of preference as shown in more developed countries, of the weakening of religious ties and tribal taboos and of the increasing awareness of the urban elements of the community of the 'expressed preference' in other countries.

Lack of accurate statistics on food crop production of less-developed territories is becoming of increasing concern and the need for more data prior to development planning has been stressed by the Food and Agriculture Organization. This lack of data applies to Nigeria. Nigeria is favourably placed at present in respect of potential productivity in relation to the present population. This is a general situation, but signs of over-population, at present levels of agriculture, are already apparent in parts of the Eastern Region and Katsina and Sokoto Provinces in the Northern Region. It is also shown that the mean rate of population increase was approximately 2 per cent per annum. This figure is higher than that of any other West of Equatorial African territory.

If such a rate of increase is maintained without a concomitant rate of increase of productivity (which postulate demands a higher rate of increase of productivity than at present), it would vitiate any predictions of changes in the consumption of food crops based on comparison with that of more developed countries, since opportunities to develop a balanced, highly productive, high plane of nutrition agriculture would be superseded.

Other partially developed countries differ in their attitude to the awareness of a close approach to saturation-levels of population, and Stanton contrasts the educational and developmental policies of India and Japan. The latter country is far in advance of others in the active control of population structure by education and birth control, and an assessment of the probable future attitude of a people to such measures is of overriding importance in predicting a country's development.

CHEMISTRY, TOXICOLOGY, PHARMACOLOGY AND MICROBIOLOGY OF A NEW ACID-STABLE PENICILLIN, RESISTANT TO PENICILLINASE *(BRL.1621*)*

By J. H. C. NAYLER, A. A. W. LONG (Chemistry), D. M. BROWN, P. ACRED (Toxicology and Pharmacology), G. N. ROLINSON, F. R. BATCHELOR, SHIRLEY STEVENS and R. SUTHERLAND (Microbiology)

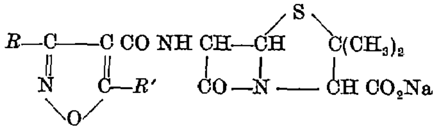
Beecham Research Laboratories, Ltd., Brockham Park, Betchworth, Surrey

THE use of methicillin, 2,6-dimethoxyphenyl penicillin, in the treatment of infections caused by penicillinase-producing strains of staphylococci is now well established in clinical practice. Its one disadvantage is that it must be given by injection since it is unstable in acid solution and poorly absorbed from the gastrointestinal tract. This article describes a new penicillin which does not suffer from this defect, and is, moreover, active at lower concentrations than methicillin. Clinical investigations are being reported concurrently elsewhere[1].

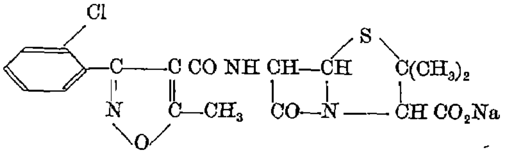
Chemistry

Acylation of 6-aminopenicillanic acid with reactive derivatives of several types of sterically hindered carboxylic acid gives penicillins which resist inactivation by penicillinase. These penicillins vary considerably in their chemical and antibacterial properties, and a particular class which combines resistance to both penicillinase and mineral acid with useful activity against most Gram-positive bacteria consists of 3,5-disubstituted 4-isoxazolyl penicillins (I). The preparation of two such penicillins, *BRL* 1400† ('Oxacillin') [I, $R = C_6H_5$, $R' = CH_3$] and its isomer, *BRL* 1454 [I, $R = CH_3$, $R' = C_6H_5$], has been described in detail[2]. Many other penicillins of type (I) have been prepared[3] and examined, and one of them, 3-o-chlorophenyl-5-methyl-4-isoxazolyl penicillin [II, *BRL* 1621], has been selected as the compound of choice.

BRL 1621 is available as the sodium salt monohydrate, which forms a colourless microcrystalline



(I)



(II) *BRL 1621**

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* 'Orbenin', Beecham Research Laboratories, Limited, registered trade mark.

† 'Prostaphlin', Bristol Laboratories, Inc., registered trade mark.

powder It is freely soluble in cold water to give a solution stable at 5° C for one week A 1 per cent solution in water has $[\alpha]_D^{20}$ + 163° and *p*H of 6 0–7 5 The half-life in solution in 50 per cent aqueous alcohol at *p*H 1 3 and 35° C as determined by a previously described method[4] is 160 min, which is approximately equal to that of *BRL* 1400 and penicillin V

Animal Studies, Toxicology and Pharmacology

Protection tests The protective effect of *BRL* 1621 against the penicillin-resistant *Staphylococcus pyogenes* Russell (type 80) was estimated using the method of Selbie and O'Grady[5] as modified by Brown and Acred[6] Groups of 10 mice (18–22 g) were infected by injecting into the muscle sheath of the thigh 0 2 ml of a 1/2 dilution of an overnight growth of *Staphylococcus pyogenes* Russell *BRL* 1621, methicillin and penicillin *G* were administered in doses of 200 and 50 mg/kg orally and subcutaneously, daily for three days, the first injection being given immediately following the infection An infected control group received no treatment The maximum thigh diameter of each mouse was measured with calipers on the first, second, third and sixth day after infection The results are expressed as the percentage protection calculated in the following manner

$$\frac{\text{mean daily thigh enlargement infected controls} - \text{mean daily thigh enlargement penicillin group}}{\text{mean daily thigh enlargement of infected controls}} \times \frac{100}{1}$$

Acute toxicities The acute oral, subcutaneous and intravenous toxicities were determined in male mice (18–22 g), 10 mice being used at each dose level The LD_{50} values (g/kg) for the various routes were respectively > 5 0, 3 0 and 1 2 g/kg

Prolonged tests (1) *Rats* *BRL* 1621 was administered orally and subcutaneously to groups of 12 male rats over a period of 12 weeks at doses of 500 and 100 mg/kg A control group of rats received 0 2 ml /100 g normal saline subcutaneously

During the test, daily weights and food intake were recorded Weekly observations were made of packed cell volume, white cell counts, and qualitative tests for proteins, sugars and occult blood were carried out on urine specimens Spectroscopic examination of the blood of the rats in all groups and hæmoglobin concentration was determined on the last week of the experiment

All rats were killed at the end of the test and post-mortem examinations made Weights of the spleens, livers, kidneys, adrenals and testes were recorded In addition to determination of organ weights at autopsy, the following tissues were removed for histological examination salivary gland, lymph node (mesenteric), thymus, thyroid, heart, lung, liver, kidney, adrenal, spleen, testes, bone marrow, stomach, duodenum, pancreas and small intestine The tissues at the site of injection were also removed No hæmatological, biochemical, histological or organ weight abnormalities were observed Macroscopically the only untoward effect was small areas of necrosis noted at the site of injection in the rats receiving *BRL* 1621 500 mg/kg subcutaneously These tissue reactions were only seen during the first 3 weeks of the test after which time they disappeared.

Table 1 PERCENTAGE PROTECTION BY *BRL* 1621, METHICILLIN AND PENICILLIN *G* IN MICE INFECTED WITH *Staph pyogenes* RUSSELL (PENICILLIN-RESISTANT)

Penicillin	Dose (mg/kg)	Per cent protection	
		Oral	Subcutaneous
BRL 1621	200	98 3	97 6
BRL 1621	50	58 6	83 9
Methicillin	200	Inactive	84 7
Methicillin	50	Inactive	40 2
Penicillin *G*	200	Inactive	Inactive
Penicillin *G*	50	Inactive	Inactive

(2) *Dogs* *BRL* 1621 was administered in doses of 2,000 and 500 mg/kg twice daily to groups of 3 dogs for a period of 4 weeks The following biochemical and hæmatological estimations were made at the beginning and end of the test ESR (mm/h), PCV (per cent), Hb G (per cent), reticulocytes (per cent), MCHC (per cent), total white cell count and differential count, blood urea (mg per cent), blood glucose (mg per cent) serum proteins—total, albumin, α_1-, α_2-, β- and γ-globulins, alkaline phosphatase KA units, SGPT units and presence of bilirubin At autopsy the following organs were weighed brain, heart, lungs, liver, spleen, pancreas, kidneys, thyroids, adrenals and gonads In addition to these, the histology included examination of stomach, ileum and colon, cervical and mesenteric lymph glands, salivary glands, skeletal muscle and bone marrow No hæmatological, biochemical or histological abnormalities were noted

Local toxic effects There were no observable tissue reactions to intradermal and intramuscular injection of 1 per cent solutions of the penicillins in groups (3 per group) of guinea pigs and rats 3 days after the injection The only observable reactions to administration of 10 per cent solutions were a hardening of the skin and erythema around the sites of the intradermal injections

Following the subconjunctival injection in rabbits of 0 5 ml of a 50 per cent solution, *BRL* 1621 penetrates into the aqueous humor but an opacity of the cornea develops which persists for at least 14 days with only a slight decrease in the intensity. It would appear, therefore, that *BRL* 1621 is contraindicated for ophthalmological use

General pharmacology The effect of *BRL* 1621 on the blood pressure and respiration was determined in the anæsthetized cat

Four cats were anæsthetized with a chloralose (1 per cent) urethane (5 per cent) mixture Doses of up to 100 mg/kg administered intravenously had no effect on the blood pressure but doses in excess of

Table 2 MINIMUM INHIBITORY CONCENTRATION OF *BRL*.1621 (Serial dilutions in agar, incubated at 37°C for 24 hours)

	M I C (µg/ml)	
	Penicillin *G*	*BRL* 1621
Staph aureus (6 strains)	0 02–0 05	0 1–0 25
Strep β-haemolytic (5 strains)	0 002–0 005	0 05–0 1
Strep viridans (4 strains)	0 002–0 05	0 1
Strep pneumoniae (5 strains)	0 01–0 02	0 1–0 5
Sarcina lutea	0 005	0 25
Strep faecalis	12 5	25
E coli	50	> 250
Proteus vulgaris	50	> 250
Salm typhi	2 5	> 250
Salm typhimurium	1 25	> 250
Ps pyocyanea	> 250	> 250
Shigella shigae	50	50
Aerobacter aerogenes	250	> 250

Table 3 ACTIVITY OF *BRL* 1621 AGAINST FRESH CLINICAL ISOLATES OF PENICILLIN-RESISTANT STAPHYLOCOCCI COMPARED WITH THAT OF METHICILLIN

	M I C (µg/ml)		
	Penicillin *G*	Methicillin	*BRL* 1621
Staph aureus strain *C*440	500	2 5	0 5
Staph aureus strain *C*465	500	2 5	0 5
Staph aureus strain *C*417	250	2 5	0 5
Staph aureus strain *C*444	250	1 25	0 5
Staph aureus strain *C*452	250	2 5	0 5
Staph aureus strain *C*443	250	1 25	0 25
Staph aureus strain *C*442	125	2 5	0 5
Staph aureus strain *C*427	25	1 25	0 25
Staph aureus strain *C*433	25	1 25	0 25
Staph aureus strain *C*420	5 0	1 25	0 25
Staph aureus strain *C*421	2 5	1 25	0 25
Staph aureus strain *C*438	0 1	2 5	0 5

Table 4. Activity of *BRL* 1621 compared with *BRL* 1400 using Staphylococci recently isolated clinically

	M.I.C. μg/ml 125 or greater	50	25	12·5	5·0	2·5	1·25	0·5	0·25	0·12	0·05	0·02	0·01 or less
Penicillin-sensitive strains (total 152)													
Penicillin *G*											19	63	70
BRL 1400								3	28	74	41	6	
BRL 1621							1		31	81	35	2	2
Penicillin-resistant strains (total 230)													
Penicillin *G*	81	20	31	49	14	14	3		2	16			
BRL 1400							36	74	87	20	12	1	
BRL 1621							2	66	116	40	5		1

this up to 400 mg/kg had slight hypotensive effects which usually did not exceed 10 mm mercury.

The responses to adrenaline (5·0 μg), acetylcholine (0·2–0·5 μg), histamine (0·5–5·0 μg), were unaffected by doses of 200 mg/kg of *BRL* 1621. Repeated doses of *BRL* 1621 at 5-min intervals increasing from 25, 50, 100, 200 and 400 mg/kg were given. Death occurred when the cat had received a total of 1·6 g/kg.

BRL 1621 penetrates poorly into the C.S.F. After a dose of 500 mg/kg intramuscularly in rabbits the concentration in the C.S.F. was no greater than 2·3 μg/ml 1 h after administration, while the corresponding serum concentration was 370 μg/ml.

BRL 1621 is eliminated from the body by excretion in high concentration both in the bile and urine of rats. After the oral administration of 100 mg/kg 18·7 per cent is recovered in the urine after 24 h, while after intramuscular administration 42·1 per cent is recovered. 10·2 per cent of the orally administered antibiotic can be accounted for in the bile.

Metabolism in animals. Paper chromatographic examination of the urine collected over 24 h from rats which had received 100 mg/kg *BRL* 1621 intramuscularly, indicated the presence, in small amounts, of two metabolites. Further investigation of these substances is in progress.

Microbiology

Antibacterial activity. Minimum inhibitory concentrations (M.I.C.) for bacteria were determined as described previously[7]. It will be seen from Table 2 that *BRL* 1621 is highly active against *Staph. aureus, Strep. pyogenes, Strep. viridans* and *Strep. pneumoniae*, but against *Strep. faecalis* and the Gram-negative bacilli the level of activity is very low. Penicillin-sensitive staphylococci were usually inhibited by 0·1–0·25 μg/ml. *Strep. pneumoniae, Strep. viridans* and β-*haemolytic Streptococci* were usually inhibited by 0·1 μg/ml.

Penicillin *BRL* 1621 is primarily of interest for its activity against penicillin-resistant staphylococci. Results in Table 3 show that *BRL* 1621 is appreciably more active than methicillin in this respect. Results for the activity of *BRL* 1621 against a larger number of hospital strains of *Staph. aureus* are shown in Table 4. The total of 382 cultures tested comprised 152 penicillin-sensitive (penicillin G M.I.C. < 0·1 μg/ml) and 230 penicillin-resistant (penicillin G M.I.C. 0·1 μg/ml or greater). Taking the penicillin-sensitive strains, only one required a concentration of *BRL* 1621 as high as 1·25 μg/ml to inhibit growth and approximately 95 per cent of the strains showed M.I.C. values in the range 0·05–0·25 μg/ml. Of the total of 230 penicillin-resistant strains only two required a concentration as high as 1·25 μg/ml for inhibition and 97 per cent of the strains showed M.I.C. values in the range 0·125–0·5 μg/ml. The largest single group of strains were those with an M.I.C. value of 0·25 μg/ml.

Comparative results are also included in Table 4 for *BRL* 1400 (oxacillin). It will be seen that *BRL* 1621 is slightly more active than *BRL* 1400. Of the total of 230 penicillin-resistant strains, only two showed an M.I.C. as high as 1·25 μg/ml with *BRL* 1621, whereas with *BRL* 1400 the corresponding number of strains was 36 (15 per cent).

Determination of M.I.C. values in solid media and in broth give similar results and M.I.C. values are not significantly influenced by inoculum size.

Effect of serum on activity. Activity of *BRL* 1621 is depressed by the presence of human serum to an extent somewhat greater than is the case with methicillin. Results are shown in Table 5 for 36 strains of staphylococci. In the absence of serum all were inhibited by 0·5 μg/ml *BRL* 1621 or less and most of the strains were inhibited by 0·25 μg /ml. In the presence of human serum, however, the majority of the strains required 1·25 μg/ml to prevent growth. It will be seen from Table 5 that *BRL* 1400 is also influenced by serum, the extent being similar to that with *BRL* 1621.

Table 5. Activity of *BRL* 1621 compared with *BRL* 1400 in Tests with and without Human Serum, using 36 Strains of Staphylococci recently isolated clinically

	Serial dilution in agar M.I.C. μg/ml and number of strains 5·0 and over	2·5	1·25	0·5	0·25	0·12	0·05	0·02 and below
Penicillin *G*	20		7		2		3	4
BRL 1621				8	21	6	1	
BRL 1621 +			23	10	3			
BRL 1400				13	17	3		
BRL 1400 +		3	28	5				

+ denotes agar containing 40 per cent human serum

Bactericidal activity. Viable counts in broth cultures containing *BRL* 1621 were carried out in a manner similar to that of Miles, Misra and Irwin[8]. Penicillin *BRL* 1621 is highly bactericidal to penicillin-resistant staphylococci at concentrations only slightly higher than the M.I.C. values. In bactericidal tests *BRL* 1621 is substantially more active than methicillin and also slightly more active than *BRL* 1400. At a concentration of 2·0 μg/ml *BRL* 1621, a kill of approximately 99·9 per cent is obtained in 7 h in broth cultures of penicillin-resistant staphylococci.

Activity of BRL 1621 by the paper disk method. Whatman antibiotic assay disks 6 mm diameter were used with two levels of *BRL* 1621, 2 μg and 5 μg per disk. Nutrient agar plates were inoculated with the test organism to result in dense but non-confluent growth and the dishes were left at room temperature for 3–4 h with the disks in place before incubating at 37° C overnight. For a range of staphylococci of varying penicillin *G* sensitivity the zone diameters with *BRL* 1621 showed a fairly good correlation with the M.I.C. values for the strains. Using a 2 μg disk the zone diameters ranged from 19–29 mm, depending on the strain, and with a 5 μg disk the range was 22–33 mm diameter, including the diameter of the disk. Very heavy inoculum, and

Table 6 Stability of *BRL* 1621 to Staphylococcal Penicillinase Compared with *BRL* 1400 and Methicillin

(1) At a concentration of 50 μg/ml

Time (min)	μg/ml remaining Penicillin *G*	Methicillin	*BRL* 1621	*BRL*.1400
0	50 (50)*	50	50	50
15	0 (35)	49	46	43
30	0 (17)	47	42	39
45	0 (6)	46	39	33
60	0 (0)	45	35	27
90	0 (0)	42	28	18

* Penicillinase at a dilution of 1/1,000

(2) At a concentration of 2,000 μg/ml

Time (min)	μg/ml remaining Penicillin *G*	Methicillin	*BRL* 1621	*BRL* 1400
0	2,000 (2,000)*	2,000	2,000	2,000
15	0 (1,600)	1,840	1,550	1,340
30	0 (1,400)	1,720	1,350	830
60	0 (820)	1,520	800	250

* Penicillinase at a dilution of 1/200

particularly the presence of blood in the agar, results in smaller zone diameters

Stability to penicillinase A penicillinase-producing strain of *Staph aureus* was grown as described previously[7], and the whole culture, including the cells, was used as enzyme The tests on penicillinase stability of *BRL* 1621, *BRL* 1400 and methicillin were carried out at two levels of substrate, 50 μg/ml and 2,000 μg/ml One volume of the enzyme was added to two volumes of phosphate buffer, *p*H 7, containing the penicillin The reaction mixture was incubated at 37° C and assays carried out at intervals of time Because of the extreme instability of penicillin *G* to penicillinase, a parallel test was carried out with penicillin *G* in which the penicillinase was present at a given dilution since with the penicillinase at the concentration used for the methicillin and isoxazole tests the penicillin *G* was destroyed almost instantaneously It will be seen from Table 6 that *BRL* 1621 is extremely stable to penicillinase although slightly less stable than methicillin, and that *BRL* 1621 is slightly more stable than *BRL* 1400 It should be noted that the penicillinase stability of *BRL* 1621, and also that of *BRL* 1400 and methicillin, is greatly influenced by the concentration of the compound present in the test With these compounds, the concentration of substrate is a limiting factor for penicillinase activity except at very high concentrations and the stability of these compounds is largely due to this effect of substrate concentration on rate of enzyme action Consequently, the lower the concentration of these penicillins the more stable they become to penicillinase

Penicillin *BRL* 1621, like methicillin, is a powerful inducer of penicillinase formation

Emergence of resistance to BRL 1621 By repeated sub-culture of staphylococci in the presence of *BRL* 1621, resistant strains can be selected out in a step-wise manner similar to that seen with penicillin *G*, methicillin and other penicillins Single step mutants of high resistance to *BRL* 1621 have not been encountered in laboratory experiments with staphylococci On the other hand, naturally occurring staphylococci resistant to methicillin[9] are also resistant to *BRL* 1621, although since *BRL* 1621 is a more active compound the M I C values with *BRL* 1621 against these resistant strains are lower than those for methicillin This means that although the M I C values for methicillin may be above the serum levels normally obtained, the same may not be the case with *BRL* 1621 For example, certain strains showing M.I C values of 12 5–25 μg/ml with methicillin were found to be still sensitive to 1·25 μg/ml *BRL* 1621 In general, the highly resistant strains which showed M I C values of 125 μg/ml and over with methicillin, also showed M I C values with *BRL* 1621 above the concentrations obtainable in the body but one strain showing an M I C value of 50 μg/ml. with methicillin was still sensitive to 1 25 μg/ml *BRL* 1621

Metabolite of BRL 1621 After administration of *BRL* 1621, either orally or intramuscularly, in normal human subjects a small proportion of the compound undergoes a metabolic change in the body to give rise to another penicillin This metabolite is present in the serum as well as in the urine, and in human beings approximately 10 per cent of the penicillin excreted is in the form of the metabolite A similar metabolite also occurs with *BRL* 1400

The metabolite of *BRL* 1621 has been isolated and found to have antibacterial activity very similar to *BRL* 1621 itself Moreover, using *Sarcina lutea* and also a penicillin-resistant staphylococcus as test organism, *BRL* 1621 and the metabolite both have the same ring-diameter concentration relationship in the cup-plate assays

In view of the very close similarity in the biological properties of the metabolite and *BRL* 1621 itself, and also in view of the small amount of the metabolite formed it seems quite certain that the latter does not in any way complicate the determinations of serum levels or the general evaluation of *BRL* 1621

We wish to acknowledge the guidance accorded by Mr F P Doyle, director of research, Brockham Park, in the preparation of the manuscript We also thank our many colleagues who assisted in the work discussed in this article

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COMPOSITION OF CIGARETTE SMOKE : SOME LOW-BOILING COMPONENTS

By Dr R. A W. JOHNSTONE, P M QUAN and Dr W CARRUTHERS
Medical Research Council Carcinogenic Substances Research Unit, University of Exeter

CIGARETTE smoke condensate contains a fraction, volatile in methanol vapour, from which dipentene has been isolated[1] The use of preparative scale gas chromatography has enabled us to isolate and identify from this fraction a number of other constituents (Table 1)

Benzene and toluene have been detected in, but not isolated from, the gaseous phase of cigarette smoke[2], and claims based on scanty evidence have been made for the presence of 2,5-dimethylfuran (ref 3), 1,3,5-trimethylbenzene and 1,2,4-trimethylbenzene (ref 4)

The methanol volatile fractions from Russian (flue-cured tobacco) and Argentinian cigarettes ('black' fermented tobacco) were compared quantitatively by analytical gas chromatography with the corresponding fraction from British cigarettes (flue-cured tobacco) As shown in Table 1, the results are broadly similar except for the notably low content of 2,5-dimethylfuran in the smoke from Argentinian cigarettes This is probably related to the low carbohydrate content of fermented tobacco

Table 1

Substance isolated	Yield, μg/cigarette British (av wt 1 15 g)	Russian (av wt 1 01 g)	Argentinian (av wt 0 99 g)
Benzene	27	48	12
2,5-Dimethylfuran	45	48	3 5
Toluene	46	164	65
Ethylbenzene	7	20	7
m-Xylene	30	48	20
o-Xylene Styrene	22	48	20
F 5 (*a*)	32	43	23
Mesitylene	9	11	8
ψ-Cumene	11	14	7
Dipentene	200	165	126
p-Isopropenyltoluene	11	—	—

The substances listed in Table 1 do not occur in tobacco, and their origins were investigated in a number of experiments carried out with modified cigarettes, types I–V Complete extraction of British cigarettes with petrol had little effect on the composition or amount of the methanol volatile fraction (Table 2) Further complete extraction with methanol caused marked reductions in the yields of dipentene and 2,5-dimethylfuran, but the amounts of the remaining components were only slightly affected The precursor of dipentene was in a chloroform-soluble fraction and that of 2,5-dimethylfuran in a water-soluble fraction of the methanol extract

Table 2

Substance	Cigarettes Ordinary Type I	Petrol extracted Type II	Methanol extracted Type III	Water-soluble part added Type IV	$CHCl_3$-soluble part added Type V
Benzene	27	23	20	22	39
2,5-Dimethylfuran	45	35	10	25	8
Toluene	46	57	42	54	67
Ethylbenzene	7	9	8	12	10
m-Xylene	30	31	12	15	28
o-Xylene Styrene	22	21	13	21	19
F 5 (*a*)	32	31	6 5	7	32
Mesitylene	9	11	1	3	10
ψ-Cumene	11	11	3	11	11
Dipentene	200	163	11	22	160
p-Isopropenyltoluene	11	11	—	—	3

Figures refer to yields in μg/cigarette

Dipentene could arise by the dimerization of isoprene which is present in cigarette smoke, but it has been found[5] that this reaction yields, among other products, almost equal amounts of dipentene and its isomer *m*-mentha-1,8-diene The latter could not be found in cigarette smoke

The presence of the precursor of 2 5-dimethylfuran in a water-soluble portion of the cigarette tobacco increases the probability that it is a carbohydrate

The remaining aromatic substances listed in Table 1 appear to arise from complex insoluble components of tobacco, as, for example, the lignins, proteins and higher carbohydrates Carotenes, known to yield *m*-xylene on pyrolysis[6], may be a source of some of the *m*-xylene in smoke

Preparation of methanol-volatile fraction of cigarette smoke Uncased British cigarettes containing a blend of flue-cured tobaccos were smoked mechanically with a 2-sec puff of 25 ml every 15 sec The mainstream smoke from 25,000 cigarettes was collected at – 70° and the condensate dissolved in ether–methanol (1 · 1) The solvents were distilled off, and further quantities of methanol were repeatedly distilled from the residue until no more material co-distilled The combined distillates were diluted with a large volume of water and the ether layer was washed several times with water and dried (calcium chloride) Distillation of the ether gave a pale yellow liquid (77 g), b p approximately 60–200°, which was filtered through a column of 250 g of alumina (neutral, Brockman grade I) Elution with petrol (b p 40°–45°) and removal of the solvent by distillation through a Vigreux column gave 16 9 g of a mobile yellow liquid (*M*) Sodium fusion tests for nitrogen, sulphur and halogen were negative Gas chromatographic and infra-red analyses showed that chromatography on alumina had removed constituents containing hydroxyl and carbonyl groups, but had not altered the amounts of the residual substances

Gas chromatography of the petrol eluate, M The liquid *M* was gas chromatographed at 150° with a nitrogen flow of 100 ml /min on a 6 ft × 1 in column of 30–60 mesh brickdust (*C*22, Johns–Manville) coated with 20 per cent by weight of a silicone elastomer (*E* 301, Imperial Chemical Industries, Nobel Division) Seven fractions, *F*'s 1–7, were collected by cooling the effluent to – 70°. These fractions, corresponding to peaks on the gas chromatogram, were examined in more detail by gas chromatography on a 15 ft × ¼ in column of 30–60 mesh brickdust coated with 20 per cent 'Apiezon *M*' grease, at 95° and an argon flow of 40 ml /min Fractions *F* 4 and *F* 5 were mixtures and were rechromatographed to obtain fractions richer in the individual components Identifications were based on gas chromatographic and infra-red data The results are summarized as follows

F 1 Benzene, confirmed by ultra-violet absorption

F 2 2,5-Dimethylfuran, with 2,4-dinitro-phenylhydrazine and sulphuric acid it gave acetonylacetone *bis*-2,4-dinitro-phenylhydrazone, m p and mixed m p 261–262°

F 3 Toluene, 2,4-dinitro-derivative m p and mixed m p 70°.

F 4 (*a*) Mixture of *m*-xylene and ethylbenzene Nitration of the mixture and crystallization from ethanol afforded 2,4,6-trinitro-*m*-xylene, m p and mixed m p 182–184°
(*b*) Mixture of *o*-xylene and styrene Bromination of the mixture and sublimation of the product at 50°/5 mm gave styrene dibromide, m p and mixed m p 71–73°

F 5 (*a*) Mixture of unsaturated aliphatic compounds, containing vinyl groups
(*b*) 1,3,5-Trimethylbenzene, confirmed by nitration to 1,3,5-trimethyl-2,4,6-trinitro-benzene, m p and mixed m p 236°
(*c*) 1,2,4-Trimethylbenzene characterized as the 3,5,6-trinitro-derivative, m p and mixed m p 185°

F 6 Dipentene, optically inactive, tetrabromide, m p and mixed m p 124° Dehydrogenation of the hydrocarbon with platinum on charcoal at 240° gave *p*-cymene in high yield but *m*-isopropyltoluene could not be detected, thus showing the absence of *m*-mentha-1,8-diene

F 7 *p*-Isopropenyltoluene With 2,4-dinitro-benzenesulphenyl chloride (ref 7) it gave 1-methyl-1-*p*-tolyl-2-(2,4-dinitrobenzenesulphenyl) ethylene as orange red needles, m p 115–117° (from ethanol) undepressed when mixed with the derivative of the authentic hydrocarbon (Found C, 58 2, H, 4 2 $C_{16}H_{14}N_2O_4S$ requires C, 58 2, H, 4 3 per cent)

Preparation of modified cigarettes British cigarettes (type I) containing flue-cured tobacco were continuously extracted with petrol (b p 60°–80°) to give type II cigarettes, evaporation of the petrol left a residue of 0.036 g/cigarette Further continuous extraction of type II cigarettes with methanol gave type III cigarettes, evaporation of the methanol yielded a residue of 0.36 g/cigarette Partition of this residue between water and chloroform yielded part *A* (0.31 g/cigarette) from the aqueous phase and part *B* (0.05 g/cigarette) from the chloroform phase To type III cigarettes 0.31 g/cigarette of *A* was added to give type IV cigarettes and addition of 0.05 g/cigarette of *B* to type III cigarettes gave type V cigarettes All five types of cigarette were re-humidified before use

We thank Dr J W Cook for his interest and encouragement in this investigation

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COMPARATIVE INVESTIGATIONS OF THE EURYHALINE CHARACTER OF *LITTORINA* AND THE POSSIBLE RELATIONSHIP TO INTERTIDAL ZONATION

By Dr P A MAYES

Royal Veterinary College, London, N.W 1

THE zonation of plants and animals in the intertidal region of the rocky shore is strikingly similar throughout the world[1] The factors determining zonation must be closely connected with the variation in tidal level[2,3], and it is reasonable to assume that species farthest up the shore and washed less by the sea have to withstand the greatest fluctuations in environmental conditions[4] The littoral flora and fauna are adapted both physiologically and anatomically to withstand this changing environment A more detailed investigation of the physiological characteristics of the intertidal species might indicate which of the many environmental factors are likely to influence their zonation

The euryhaline character of the Littorinidae has been noted previously[5–8] and the changes in weight of the British species in dilute sea-water and their relative ability to withstand various dilutions has been described in a previous communication[4] *L neritoides*, which inhabits the splash zone[6,10] where there is maximum fluctuation in environmental conditions, showed the greatest ability to withstand and adapt to change in salinity, whereas *L littoralis*, which is confined to the fronds of the seaweeds of the middle shore, showed the least tolerance to variations in salinity None of the species survived dilution of sea-water to 25 per cent of normal, demonstrating that an osmo-regulatory mechanism is not present in the Littorinids studied The present experiments were carried out to ascertain whether this group has developed a mechanism of salt loss or gain which would help to explain their euryhaline character Specimens were transferred from normal sea-water into sea-water of either decreased or increased salinity and after a suitable period of time they were transferred back into normal sea-water If there was neither loss nor gain in salt, their weight, which would increase in the hypotonic or decrease in the hypertonic medium, would return to its original value when the animals were replaced in normal sea-water As specimens in normal sea-water lose weight slowly, due presumably to absence of food, expenditure of energy and loss of fæces, the final weight attained after replacement in normal sea-water would be somewhat less than the original weight.

Littorinids were divided into batches of seven average-size specimens of the same species and were equilibrated in normal sea-water (salinity 35.5 gm /l) for 24 hr They were transferred into 50 per cent (v/v) sea-water or 200 per cent sea-water (normal sea-water plus 35.5 gm sodium chloride/l) and after several days were returned to normal sea-water. Each batch of specimens was weighed throughout the experimental run, allowing the animals to drain, drying the outside, and after retraction, absorbing excess fluid inside the shell with filter paper. The solutions were replaced every 12 hr with freshly aerated ones The live non-calcareous weight was calculated by subtracting the weight of the shell from the original weighings Results (Fig 1) are expressed as a percentage of the live weight at the end of the period of 24 hr equilibration in normal sea-water

On immersion in 50 per cent sea-water *L neritoides*, in contrast to the other species, remained loosely retracted within its shell Ultimately, it did emerge and show locomotion in this solution, but the time required for this adaptation was quite variable The other species were active, increased in weight and showed evidence of swelling of the tissues The tendency of the species to gain weight in this hypotonic sea-water was in the following order of increasing magnitude *L neritoides*, *L saxatilis*, *L littorea*, *L littoralis* (Fig 1) When immersed in 200 per cent sea-water all specimens retracted into their shells and lost weight Again, *L neritoides* showed the least change in weight and ultimately emerged from its shell in this medium

On transference back into normal sea-water, all species dropped weight if they had been in 50 per cent sea-water and gained weight if they had been in 200 per cent sea-water, but only *L saxatilis*, *L littorea* and *L littoralis* returned to a value which was slightly less than the original, irrespective of whether they had been in hypotonic or hypertonic solutions

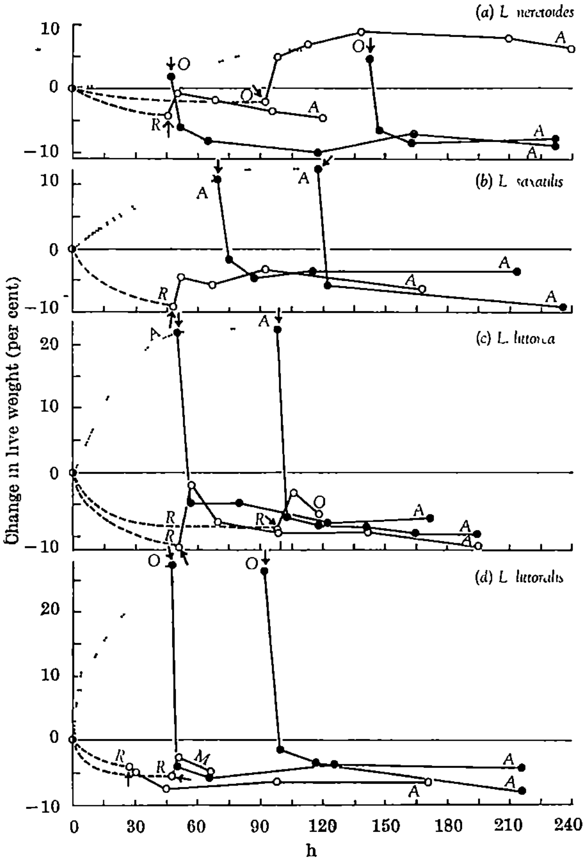

Fig 1 Changes in weight of *Littorina* when immersed in 50 per cent ( ) sea-water or 200 per cent (- - -) sea-water (after equilibration in normal sea-water for 24 hr) and the subsequent effect of transfer back into normal sea-water The arrows show when transference back into normal sea-water was made The shape of the curves in 50 per cent sea-water has been taken from a previous publication (ref 4) Each point is the mean of seven animals Live weight = total weight minus shell weight *A*, animals showing active locomotion, O, operculum open but animals partly retracted into shell, *R*, animals fully retracted into shell, *M*, moribund specimens

This is good evidence that no appreciable salt loss or gain occurs in these particular species On the other hand, *L neritoides*, when transferred from 50 per cent sea-water back into normal sea-water, showed a marked drop in weight below the original level, and when transferred from 200 per cent sea-water showed a marked increase in weight, well above the original This indicates that the small gain in weight which occurs when *L neritoides* is transferred into 50 per cent sea-water is due to loss of salt and a reduced uptake of water, and that the small loss in weight in 200 per cent sea-water is due to uptake of salts and a reduced loss of water When *L neritoides* was removed after 46 hr in 200 per cent sea-water, before the operculum had opened, and replaced in normal sea-water (Fig 1*a*), the small increase in weight was probably due to the protective effect of the operculum which in this species is most efficacious in excluding and controlling equilibration with the external medium The goodness of fit of the operculum of all four species in descending order of efficiency is: *L neritoides*, *L littorea*, *L saxatilis*, *L. littoralis*, which correlates with the time of survival in 25 per cent sea-water[4]

The euryhaline character of the Littorinidae is limited by the absence of an osmo-regulatory mechanism Within this limitation *L neritoides* appears to be better equipped for dealing with extremes in salinity than the other species because it is capable of loss or gain in salt which prevents excess swelling or shrinking of the tissues in hypotonic or hypertonic media respectively Whether this occurs as a result of the activity of a particular organ or is due to a permeability to salts of the general surface is not known The relative ability of the other species to withstand changes in salinity appears to depend on their respective capacities for tolerating hydration or dehydration and the consequent swelling or shrinking of their tissues

L neritoides and *L saxatilis* are sharply cut off at their upper limits of distribution, the former reaching a foot or so higher than the latter Colman[9] has posed the question as to what environmental factors determine such upper limits so finely The work recorded here does not seek to answer this question directly but demonstrates, in conjunction with previous work[4], the existence of physiological differences within the Littorinidae which could determine their zonation provided the appropriate conditions of salinity variation were operating in their environment

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RELATIONSHIPS BETWEEN THE GENETIC EFFECTS OF DEFICIENCIES FOR CHROMOSOMES, III AND V ON MEIOTIC PAIRING IN *TRITICUM AESTIVUM*

By C. KEMPANNA and RALPH RILEY

Plant Breeding Institute, Cambridge

THE chromosomes of the hexaploid common wheat of agriculture, *Triticum aestivum* ($2n = 6x = 42$), normally make 21 bivalents at meiosis, although it has been shown that euploid plants of standard varieties usually have about 5 per cent of pollen mother cells in which one or other bivalent is not formed[1]. However, apart from these slight deviations in some euploid cells, deficiencies for two different chromosomes cause pronounced disturbance of meiotic regularity

Nullisomic plants, with 40 chromosomes, deficient for the chromosome III pair, have considerable pairing failure at first metaphase of meiosis (Table 1)[2]. 0–19 of the 20 potential bivalents are not formed (Table 2), although most commonly only one or two bivalents fail. The chromosomes which do not participate in bivalent formation are left as univalents.

Table 1. MEAN PAIRING AT FIRST METAPHASE OF MEIOSIS IN *T. aestivum* EUPLOID, NULLISOMIC-III AND NULLISOMIC-V

Plant	Chrom No	Cell No	Univ	Biv	Triv	Quad	Chiasmata per cell
Euploid	42	30	0·20	20·90	—	—	43·60 ± 0·40
Nullisomic-V	40	23	0·26	18·91	0·35	0·22	37·65 ± 0·48
Nullisomic-III	40	30	8·56	15·72	—	—	23·90 ± 1·51
Nullisomic-III	40	30	5·00	17·50	—	—	27·60 ± 0·97

Chromosome III, therefore, carries one or more genes which are responsible for the maintenance of the normal bivalent pattern, and Sears[3] has shown that the relevant genetic activity is restricted to one arm of the chromosome. The modification of the sequence of meiosis in the absence of chromosome III could result either from the failure of homologous chromosomes to synapse (asynapsis) or from the failure of chiasma-formation after normal synapsis (desynapsis). The difficulties of making adequate prophase preparations, in wheat, preclude the formation of a firm choice between the two alternatives. Nevertheless, from the examination of prophase in nullisomic-III plants, in the present work, it appears likely that the abnormality is desynaptic in origin, since no failure of pairing was detected. The conclusion may be tentatively advanced, therefore, that the activity of chromosome III is responsible for the completion of regular chiasma-formation in wheat.

One or more genes on the long arm of chromosome V are responsible for the restriction of meiotic pairing to the formation of bivalents[4,5]. In the absence of this chromosome, from nullisomics and from haploids or hybrids, homoeologous chromosomes from the three component sub-genomes can pair and multivalents are common (Table 1). The meiotic affinity between corresponding chromosomes of the different sub-genomes is therefore normally suppressed by the activity of chromosome V in euploid individuals of wheat.

The evidence so far available suggests that the restriction of pairing to complete homologues, exercised by this chromosome, does not result from any alteration in the frequency or distribution of chiasmata. Consequently the mechanism probably functions through modifications of the processes which initiate synapsis and it may be said to enhance genetically the affinity of homologues relative to homoeologues.

In view of the relaxation of the genetic control of apparently different aspects of the meiotic process, which results from the absence of chromosomes III and V, it is of some interest to determine the outcome of the simultaneous deficiency for both. Material for this purpose has been developed in the present work in the variety Chinese Spring.

Plants with 41 chromosomes, monosomic for chromosome V, were pollinated with pollen from 40-chromosome plants nullisomic for chromosome III. Plants with 40 chromosomes were selected in the F_1 generation—these were the segregants to which chromosome V of the monosomic parent had not been transmitted. At meiosis the 40-chromosome F_1 plants had 19 bivalents and two univalents and they were, therefore, monosomic for chromosome III and chromosome V. No attempt was made to obtain the doubly nullisomic segregants in the F_2 generation because of the known low rate of transmission through the pollen of deficiency for chromosome V. Instead, the doubly monosomic F_1 individuals were pollinated with pollen from the perennial rye species, *Secale montanum* ($2n = 14$).

Potentially, plants with 26, 27 and 28 chromosomes were possible in the progeny of this cross, depending on the transmission of the two univalents. Those with 28 chromosomes would carry both III and V, and those with 26 chromosomes neither III nor V. The 27-chromosome plants could be deficient for either chromosome III or V, but not both. Among the eight *T. aestivum* × *S. montanum* hybrids grown one had 28, three had 27 and four had 26 chromosomes.

Meiosis was studied in pollen mother cells in Feulgen–orcein squashes of the hybrids, and the mean pairing at first metaphase is shown in Table 3. Hybrid 1—a euploid with 28 chromosomes—had a mean bivalent frequency of 1·08, and the range in bivalent frequency was 0–3 per cell. There were no multivalents. This pattern of behaviour coincides well with that commonly reported for euploid wheat-rye hybrids[6].

The three 27-chromosome hybrids fell into two categories. Hybrid 2 had a high level of pairing with a mean of only 12·40 univalents per cell, bivalents and trivalents were common and there were occasional quadrivalents. As many as 20 chromosomes were associated in some cells—in one such there were seven univalents, seven bivalents and two trivalents. The cell with the least pairing had two bivalents and a trivalent. This is the pattern of homoeologous pairing to be expected when chromosome V is deficient and it has been shown that the deficiency of no other wheat chromosome can produce a similar increase over the level in a eulpoid hybrid[7].

By contrast, in the other two 27-chromosome plants, hybrids 3 and 4, only one cell was recorded with any pairing and this merely had one bivalent. Apparently chromosome III was deficient from these hybrids, and it is clear, by comparison with the euploid hybrid, that the absence of this chromosome had led to some bivalent failure like that which occurs in nullisomic-III wheat plants. It should be indicated that, although Riley, Chapman and Kimber[7] recorded

Table 2. FREQUENCY OF CELLS WITH VARIOUS NUMBERS OF BIVALENTS AT FIRST METAPHASE OF MEIOSIS IN *T. aestivum* NULLISOMIC-III

No bivalents	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	Total
No cells	5	13	16	9	5	2	1	2	1	1	0	1	0	1	1	1	0	0	0	1	0	60

Table 3. MEAN PAIRING AT FIRST METAPHASE OF MEIOSIS IN *T. aestivum* × *S. montanum* HYBRIDS WITH OR WITHOUT CHROMOSOMES III AND V OF *T. aestivum*

Hybrid	Chrom III	Chrom V	Chrom No	Cells	Univ	Biv	Triv	Quad	Chiasmata per cell	Cells with only univalents
1	present	present	28	50	25·84	1·10	—	—	1·12 ± 0·01	19
2	present	absent	27	50	12·40	5·64	0·96	0·12	9·18 ± 0·22	0
3	absent	present	27	50	27·00	—	—	—	—	50
4	absent	present	27	50	26·96	0·02	—	—	0·02	49
5	absent	absent	26	50	17·08	3·66	0·48	0·04	6·02 ± 0·58	14
6	absent	absent	26	50	14·58	4·76	0·58	0·04	7·98 ± 0·55	8
7	absent	absent	26	50	16·25	4·25	0·42	—	6·25 ± 0·44	6
8	absent	absent	26	50	16·70	4·08	0·38	—	6·00 ± 0·39	4

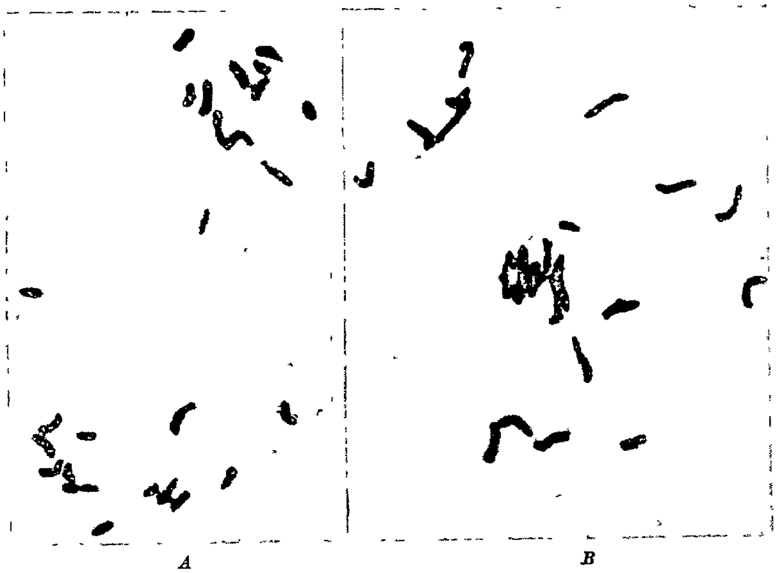

Fig. 1. First metaphase of meiosis in Feulgen and orcein stained squashes of pollen mother cells in a 26-chromosome hybrid from the cross *T. aestivum* Chinese Spring monosomic-III and -V ($2n=42-2$) × *S. montanum* ($2n=14$) (× 700). *A*, 26 univalents, arranged in two polar groups with one at the equator; *B*, 5 bivalents and 16 univalents.

a low level of pairing in *T. aestivum* × *S. cereale* hybrids deficient for chromosome III, the reduction of pairing was not so pronounced as in the present material. The difference in behaviour may relate to the use of a different rye species as a parent, or may merely be environmental determined.

It is clear that there were marked differences between the euploid and the two types of 27-chromosome hybrids. Moreover, the data for all three were different from those for the 26-chromosome plants simultaneously deficient for both chromosomes. Although there was high pairing in these (Fig. 1*B*) with bivalents, trivalents and quadrivalents—as in the *V*-deficient hybrid—the mean pairing was considerably lower (Table 3). This showed most clearly in the higher mean frequency of univalents and the low mean chiasma-frequency per cell. Thus the relaxation of the regulation normally exercised by both chromosome III and V over the course of meiosis might have been apparent in all cells.

However, the 26-chromosome hybrids displayed a pattern of pairing not shown by those with higher chromosome numbers. In all the doubly deficient plants there was a number of cells, which ranged from 8 to 28 per cent of the total, with no chromosomes paired (Figs. 1*A* and 2). This discontinuity in the distribution of cells with various numbers of chromosomes paired did not occur in the V-deficient hybrid, which the pairing of the III- and V-deficient hybrids otherwise most nearly resembled. If the cells with no pairing are excluded and the mean pairing recalculated for the remainder, the resulting means are much closer to those of the 27-chromosome, V-deficient, hybrid (Table 4). Slight differences remain, however, in that there was a higher univalent frequency and a smaller number of multivalents.

Table 4. MEAN PAIRING AT FIRST METAPHASE OF MEIOSIS IN *T. aestivum* × *S. montanum* HYBRIDS DEFICIENT FOR CHROMOSOMES III AND V OF *T. aestivum*, EXCLUDING THOSE CELLS WITH ONLY UNIVALENTS

Hybrid	Chrom. No.	Cells	Univ.	Biv.	Triv.	Quad.	Chiasmata per cell
5	26	36	13·61	5·08	0·66	0·06	8·36 ± 0·32
6	26	42	12·40	5·67	0·69	0·05	9·50 ± 0·29
7	26	44	14·86	4·85	0·47	—	7·14 ± 0·32
8	26	46	15·89	4·43	0·41	—	6·52 ± 0·32

The bimodality of the distribution of cells with various amounts of pairing demonstrates that in different cells the effects of both deficiencies were separately apparent. Those cells in which chiasma formation occurred had a pattern of pairing characteristic of chromosome V deficiency. By contrast the cells with no pairing at first metaphase matched the behaviour of the chromosome III-deficient hybrids. It is not apparent whether the slightly lower level of pairing in those cells in which there was chiasma formation implied that an influence of the deficiency of chromosome III occurred throughout and not only in the cells completely without pairing.

If the effect of deficiency for chromosome III is to cause desynapsis, chiasma-formation must have been completely inhibited in some cells in the III- and V-deficient hybrids. There was no similar discontinuity in the distribution of cells with various amounts of bivalent failure in III-nullisomic plants (Table 2). This suggests that the causal factors determining the failure of pairing, which result from the absence of chromosome III, were much closer to a threshold

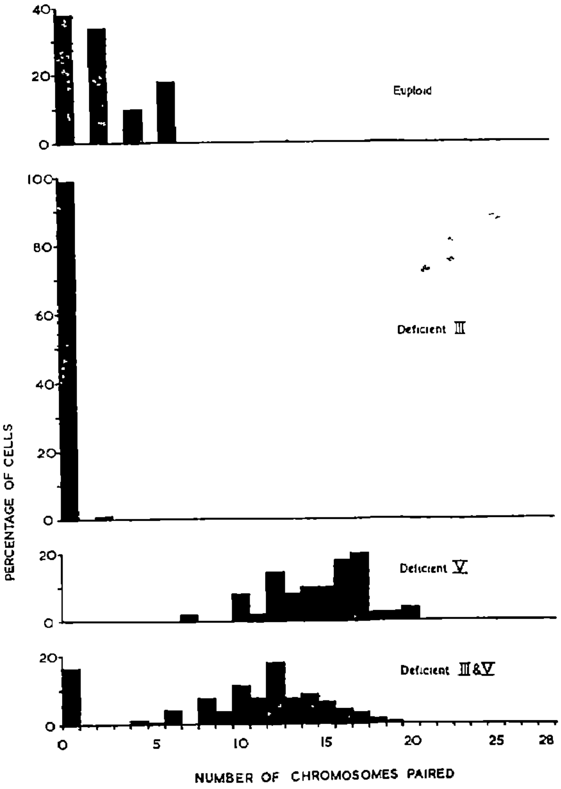

Fig. 2. A histogram showing the frequency distribution of cells with various numbers of chromosomes paired at first metaphase of meiosis in hybrids derived from the cross *T. aestivum* Chinese Spring monosomic-III and -V ($2n=42-2$) × *S. montanum* ($2n=14$). Euploid = 28-chromosome hybrid, chromosomes III and V present. Deficient III = 27-chromosome hybrids, chromosome III absent. Deficient V = 27-chromosome hybrid, chromosome V absent. Deficient III and V = 26-chromosome hybrids, chromosomes III and V absent.

value when chromosome V was simultaneously absent. Moreover, the modifications which followed an apparent switch of the determinants from one side of the threshold to the other were quite abrupt. Either the pairing was very little different from that in the simple V-deficient hybrid, or there was no pairing whatsoever. Further information on the nature of the differences between the two distinct meiotic patterns may well result from future work on environmental influences.

The choice between the possibilities of a desynaptic or an asynaptic effect of deficiency for chromosome III is not aided by the present results. If the effect were asynaptic the behaviour of the 26-chromosome hybrids would be ascribed to the complete absence of prophase pairing in some cells, if desynaptic to the complete absence of chiasma-formation in some cells. Nevertheless, there must be a functional relationship between the disturbances of the meiotic process caused by deficiencies of chromosomes III and V since only when V is deficient is there an abrupt discontinuing in the pattern of pairing. This leads to speculation concerning the possible evolutionary relationships of the systems controlled by the two chromosomes.

Modification of a function of chromosome V by mutation subsequent to its incorporation in wheat has been responsible for the diploid-like meiotic behaviour and disomic inheritance of the polyploid species[8]. It may be that the genetic influence of chromosome III, in maintaining bivalent formation, has also arisen as a result of a change in function, and that this change was in some way complementary to that on chromosome V. Both chromosomes were derived from the same diploid species, *Aegilops speltoides* ($2n = 14$), in the evolution of polyploid wheat, but although it has been possible to obtain evidence on the function of chromosome V in its original state this has not yet been done for chromosome III. Experiments with this object have now been commenced.

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EFFECT OF FEEDING X-RAY IRRADIATED COLIFORM BACILLI TO TUMOUR-BEARING MICE

By Dr. ARTHUR COMPTON
Wright-Fleming Institute, St. Mary's Hospital, London, W.2

THIS article follows earlier work[1] on the feeding to tumour-bearing mice of homologous and heterologous tissues, or tissue extracts, which had been irradiated with X-rays, with the view of testing the effect, if any, of such irradiated material on tumour growth[2]. The present communication is the record of two initial experiments, utilizing bacteria instead of animal tissues, to fix the supposedly beneficial radiation.

Experiment 1. 10 silver-grey mice, to all intents of an inbred strain, were grafted subcutaneously in the right groin with tumour Compton 22, a progressively growing adeno-carcinoma, which arose spontaneously some five years ago in a white female mouse of our laboratory stock[1]. Characteristic features of this tumour are that grafts grow to enormous dimensions, frequently without ulceration, and, when the latter does occur, it remains superficial, with a noteworthy absence of suppuration. The grafting was done with a wide-bore needle and a 1-ml. Record syringe, each mouse receiving approximately the same-sized, unusually large, implant—to ensure good tumour 'takes' and vigorous follow-up growths—of an average weight 24 mg, lodged carefully in the barrel of the needle prior to being injected by the syringe. The tumour is now in its seventy-fifth generation of transplantation.

Immediately after grafting, the 10 mice were separated at random into two lots of 5 mice each, in two separate mouse cages. One lot (treated series) was fed daily, during 16 days—the equivalent of some 10 months in the human being—with 15 ml. of an equal parts mixture of 24-h broth cultures of three different coliform bacilli, which, after mixing, were irradiated in an open Petri dish of 4 in. diameter for 1–2 min at a distance of 20 cm from the X-ray anode, the apparatus being otherwise run at 30 m.amp. and 50 kV (that is, a radiation dose of 15,000–30,000 r.). After the irradiation, the mixture was transferred to a 4-oz. feeding bottle with a further 20 ml. of tap-water, making 35 ml. in all, and the bottle stoppered with a rubber teat perforated by a short length of glass tubing, inverted and placed in position on the roof of the mouse cage. During the 16 days' feeding this was interrupted on two separate days as rest-periods, when tap-water alone was given. The other lot of 5 mice were kept as controls, receiving tap-water for drinking. Both lots were fed with the same solid food throughout, small fragments of commercial sticks of 'Diet 41' (Ranks).

Experiment 2. Nine silver-grey mice—5 of medium size and average weight 32–33 g, and 4 larger of 40 g average weight—were grafted subcutaneously into the right flank with tumour 22 Compton—now in its eighty-third generation of transplantation—using a wide-bore needle as before. The point of the needle was introduced into the right groin and pushed along a pinched-up fold of skin towards the axilla. Immediately after grafting, the lot of medium-sized mice (Nos. 1–5) was fed daily during 28 days—equivalent to about 1·5 years in the life of a human being—with 10 ml. of an irradiated 24-h nutrient-broth culture of *B. coli communior Alex II*, 825 *B*(2) (ref. 3), the remaining mice (Nos. 6–9) being kept as non-treated controls. The fact that the 4 control mice were initially larger, heavier animals than the 5 treated is viewed in no way as detracting from the experiment. It may be viewed even as all to the good, since graft tumours are known to grow as a rule better in older, larger animals[4].

The irradiation dosage used was as follows. During the first 12 days the broth culture was irradiated daily in an open Petri dish for 1 min at 20 cm from

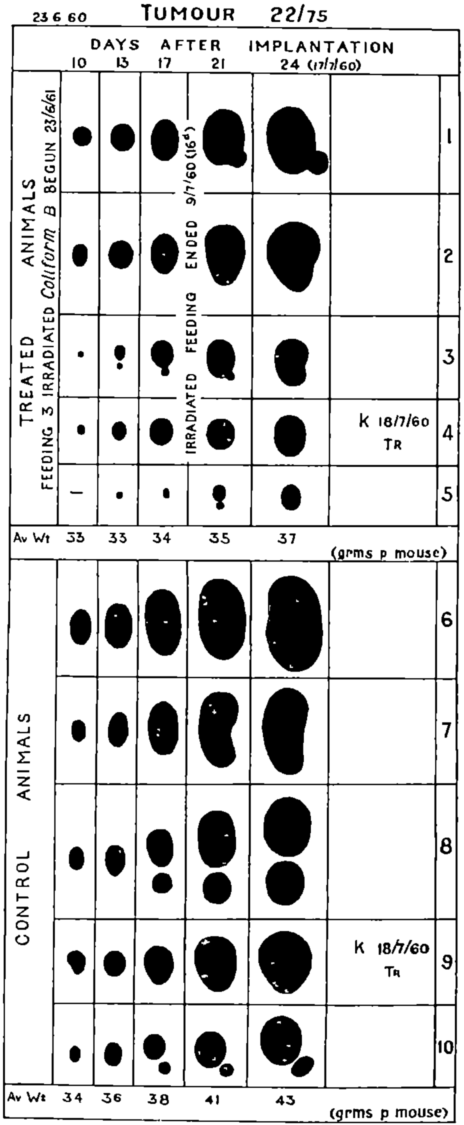

Fig 1

the anode, the apparatus otherwise being run as before at 30 m amp and 50 kV (15,000 r), then, during the subsequent 15 days, for 1 5–2 min at 15–20 cm (22,000–50,000 r)

The steps after the irradiation were about as before the 10 ml of irradiated liquid from the Petri dish, on transference to the feeding bottle, were diluted to 30–35 ml with tap-water

In brief summary, experiments 1 and 2 present the following contrasts (1) three coliforms were used in experiment 1, of which only one was used in the repeat experiment 2, (2) the daily irradiation dosage was about the same in both 15,000–30,000 r in experiment 1, and 15,000–50,000 r in experiment 2, (3) the irradiated coliform feeding was kept up for 16 days in experiment 1 and 27 days in experiment 2

The results of the two experiments are given graphically in Figs 1 and 3, where tumour growths are recorded in terms of (1) individual tumour silhouettes, as measured by calipers at various charting times, and (2) collective average weights of the animals, weighed at these charting times

Consider Fig 1, which records the results of experiment 1 The tumour silhouettes here indicate very different rates of tumour growth in the treated (Nos 1–5) and in the control (Nos 6–10) animals In the latter all 5 implants (100 per cent) show homogeneously progressive tumour growths But not so in the treated, where 3 tumours out of 5 (60 per cent) show early, comparative inhibition of tumour growth, with subsequent comparatively slower development

The defect of silhouette representation, as an evaluation criterion of tumour growth, is that it fails to reveal tumour dimensions in depth It reveals dimensions in one plane only, so that, on such representation, a tumour may occasionally be larger than it seems, and vice versa On the other hand, average body weights, the other evaluation criterion, are seen to parallel so closely the silhouette appearances that it is evident the latter can be relied on as providing fairly accurate measures of tumour progress, in spite of their limitation

But collective mean body weights, as measures of tumour development, are so instructive that they merit a brief consideration on their own At the start of the experiment (June 23, 1960) they were roughly identical in both the treated and control series, considering the 'at random' basis on which the animals, uniformly sized and healthy, were separated By the tenth day they are seen to have begun to diverge slightly, to go on diverging still more afterwards By the seventeenth day, the collective average weight of the control animals is 38 g per mouse, as against 34 g per mouse for the treated animals At the twenty-first day there is a maximum divergence of 6 g more per mouse in the controls than in the treated, which difference is still maintained at the next charting 3 days later

By plotting collective average body weights against the day of tumour growth for the treated and control animals, we get the two curves of Fig 2 Here the initial slowing of tumour growth, as reflected in body weights for the treated animals, and characterized by the initial flattening of their growth curve, is noteworthy, being in marked contrast with the steadier, upward slope of the curve for the control series

It thus becomes evident that these curves, in their contrasting shapes, provide not only factual evidence,

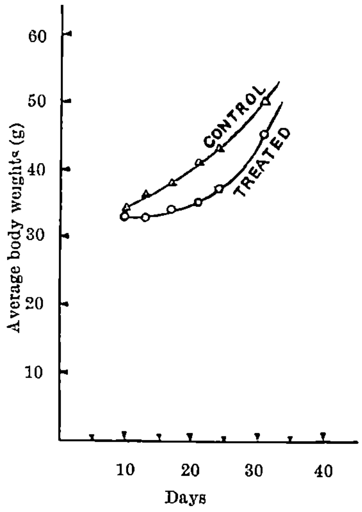

Fig 2

but also some measure, of the inhibiting influence on tumour growth of the treatment Actually, as previously pointed out[2], theoretical considerations confirm that there should occur, for the treated animals, unless the treatment is 100 per cent effective with no tumour growth occurring, such initial delay or lag in the time growth curve before it finally turns abruptly upwards

The slow rate of weight increase in the treated animals might be thought of as due perhaps to their drinking less and eating less—though proof of this is lacking If this were the explanation, one would expect the situation to change dramatically with the cessation of the treatment on the sixteenth day, to result in a very definite, sudden weight increase soon thereafter But no immediate increase of weight is seen to occur In fact, the rate of increase of weight, on suspension of the treatment, continues at the same initial rate throughout the following week, with no pronounced increase being noted until well beyond the twenty-fifth day, when the curve commences to rise rapidly to the normal ending characteristic of tumour growth in general Hence, from this *reductio ad absurdum* type of argument, it is reasonable to conclude that the early loss of weight and lessened tumour growth cannot be due to the animals merely drinking and eating less *per se*, but rather to the effect of the irradiation on the drink they received

Now, body weights are a function of (1) the rate of tumour growth, of which tumour silhouettes, as already stressed, give an only on the surface presentation; (2) the fitness, or otherwise, of the mice, in which connexion it is to be noted that the animals here appeared equally lively and fit throughout in both experiments, with no deaths in either It can, then, be assumed that the differences in body weights are not due to any lack of fitness of the treated mice in either series, but are, in the main, a simple expression of the more vigorous tumour growths in the control than in the treated animals—thus confirming the silhouette findings

Both criteria, silhouettes and body weights, are, then, mutually demonstrative of the restraint on tumour growth exerted on the treated animals by their feeding with the irradiated coliforms. It is to be remarked, in passing, that in this respect not every coliform is effective; 9 others were tested, but proved ineffective It may well be that only one of the three here used was the active radiation carrier

Why the tumours of mice 1 and 2, in the treated series (Fig 1), do not appear to have been favourably affected by the treatment cannot for the moment be explained, unless it be that these mice (1) for a day or so at the start of the treatment may have refused the irradiated feeding mixture, at a time when the grafts would normally be being established, when new blood vessels would be being formed, linking the graft in its new surroundings to the blood supply of the host, or (2) may fall into the category of one of the paradoxes of cancer, namely, that agents which at times seem to cure, at others seem to foster growth, to be in fact potential carcinogens X-rays are the classical example of this.

Fig 1 further records that two tumours, one in the treated series and one in the control series, those of mice 4 and 9, the former seemingly rather slow growing, were transplanted when 25 days old (July 18, 1960) each into 10 normal mice Both transplanted tumours gave rise to normal types of progressively growing tumours The slower-growing tumour in mouse 4 was thus seen to have reverted, on transplantation into normal animals, to its customary, rapidly progressive, growth behaviour. So then, however its growth may have been restrained and checked when undergoing treatment in the parent animal, no permanent, hereditary change had obviously thereby been imposed on it

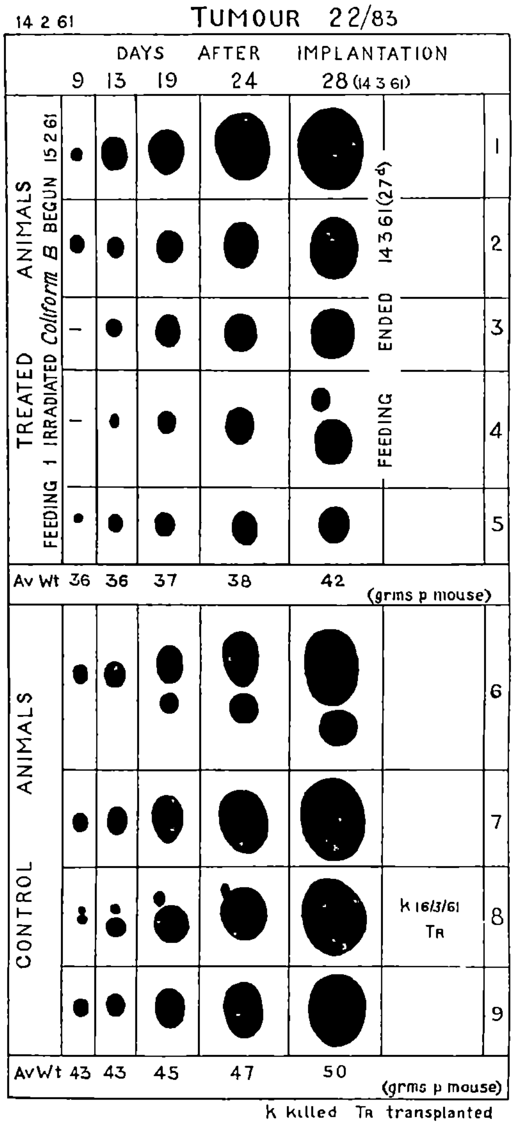

Fig 3

Turning now to Fig. 3, which records the result of experiment 2, the overall picture here is remarkably like that of the previous experiment · good, vigorous, progressively growing tumours in all (100 per cent) of the control mice (Nos 6–9), with restrained, clearly 'held-up', initial growths in 3, even 4, out of 5 (60–80 per cent) of the treated animals (Nos. 1–5) The treatment here was limited to feeding with only one type of irradiated coliform bacillus

Experiment 2 confirms experiment 1 in providing results which are, to all intents and purposes, replicas the one of the other, both showing · (*a*) a like initial 'hold-up' of tumour growth in about 60 per cent of the treated mice; and (*b*) a like 100 per cent of steadily progressive tumour growths in the controls

Such results call for prudence in their interpretation, pending repetition on a larger scale Some may be inclined to dismiss them as due to chance, others not That they are unlikely to be due to chance follows, I think, from the following, among other, considerations, which appear to me to justify publication now, in order that others can have an opportunity to take part in such further repeat work:

(1) That the mice used (a strain of silver-grey) were specially susceptible to graft cancer, the controls showing no resistance whatever

(2) That specially large tumour implants were purposely used, to enhance to the full the occurrence of good tumour-taking throughout

(3) That the mice destined for treatment and for controls were in no way selected, but were separated 'at random' after the transplantation in each experiment

(4) That the number of mice to be fed with irradiated bacteria was limited in both experiments, so that the quantity of material to be daily irradiated for the feeding might be handled with ease, and thus be satisfactorily dealt with, without haste

(5) That on the two occasions (Figs 1 and 3) when the work was done with irradiated effective, in contradistinction to irradiated non-effective, coliform bacilli, the same kind of result invariably occurred initial inhibition of tumour growth among a certain proportion of the treated animals, with good, steadily progressive, tumour growth among all the control animals

The preliminary findings thus recorded, with only a small number of animals in each experiment, can be viewed as capable of being improved on, as follows

(1) To discover other bacteria still more elective as fixers of the beneficent irradiation

(2) To perfect still further the irradiation dosage

(3) To try out other routes of administration than by the alimentary tract

(4) To isolate the hypothetical, active, inhibitory substance in a pure state, and thereby to make way for a study of its chemical constitution and possible synthesis

All four, in the hope of restraining still more effectively, even possibly reversing, cancer growth

I thank Prof C A Pannett, whose own work inspired the investigation, the Principal of the Wright-Fleming Institute, St Mary's Hospital Medical School, London, W 2, for facilities to conduct the research, and the Frederick F A Pearson Foundation, U S A , for financial support

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SELECTION FOR DIGESTIBILITY IN HERBAGE GRASSES

By Dr. J P. COOPER

Welsh Plant Breeding Station, Aberystwyth

AND

Dr J M A. TILLEY, W F RAYMOND and R A TERRY

Grassland Research Institute, Hurley

THE primary factor governing the nutritive value of herbage is its digestibility, which not only determines the proportion of the feed which can be utilized by the animal, but also greatly influences the amount eaten[1-3] The development of herbage varieties of high digestibility is thus an important objective for the plant breeder

It has long been known that the leaf blade is lower in fibre and higher in protein content than the stem[4], and as these fractions have been assumed to be closely correlated with digestibility, herbage breeding programmes have emphasized breeding for leafiness However, Minson *et al* [5] have shown that there is frequently only a small correlation between herbage digestibility and leaf/stem ratio, and that leafy cuts of different herbage species may also differ markedly in digestibility perennial ryegrass, for example, has consistently been more digestible than cocksfoot at the same stage of growth This demonstration of differences in digestibility between species suggested that it might be profitable to look for genetic variation within a species, which could then be used in a selection programme

The standard estimation of digestibility by feeding trials with sheep cannot, however, be used to screen breeding material at the individual genotype level, because of the large quantities of herbage required An *in vitro* laboratory method for estimating herbage digestibility, developed by Tilley *et al* [6], requires only small amounts of herbage (In this method 0 5 g of dried milled herbage is first digested for 48 h with strained rumen liquor at *p*H 6 5–7 0, and the residue further treated for 48 h with acid pepsin to remove digestible protein *In vitro* digestibility is calculated from the weight of 'indigestible' residue) Estimates of digestibility so obtained show a high correlation ($r = 0\ 95$) with the corresponding *in vivo* results, and the method appeared promising for the investigation of the digestibility of small samples of breeding material

Preliminary analyses of individual genotypes and families of ryegrass (*L perenne*) and cocksfoot (*D glomerata*) have given encouraging results The ryegrass genotypes, all from the variety *S* 23, were harvested in April 1961, the cocksfoot genotypes included material from Brage, *S* 37, *S* 143 and *S* 341, and were harvested in the aftermath in September 1961 All plants were completely vegetative Families derived from certain of these genotypes were sown in boxes in May 1961 and two primary harvests were taken one in July and the second in August, again only vegetative material being used (*N B* The cocksfoot families were sampled for analysis before the cocksfoot genotypes)

As shown in Table 1, considerable differences in the percentage digestibility of the dry matter were found between the individual genotypes Even within the single ryegrass variety (*S* 23) a range of 63–84 per cent was recorded, although most plants were more than 78 per cent, the cocksfoot digestibilities ranged from 53 to 68 per cent The lower digestibility levels of the cocksfoot may have been due in

Table 1 Genotype and Family Differences in Digestibility

Original populations	Mean	Range	No of single plants (genotypes)
Perennial ryegrass (*S* 23)	79 5	63 3–83 5	30
Cocksfoot*	60 7	52 5–67 5	35

Families	July cut			August cut			
	Mean	Range	*LSD* 5 per cent	Mean	Range	*LSD* 5 per cent	No of families
Perennial ryegrass (*S* 23)	82 4	79 9–85 9	3 2	76 0	70 7–79 9	3 6	15
Cocksfoot*	76 3	73 2–79 6	3 2	65 5	60 3–70 0	3 6	18

(*Includes Brage, *S* 37, *S* 143, *S* 341)

Table 2 Repeatability and Heritability Estimates

Repeatability	July × August cut
Perennial ryegrass	*r* = 0 44
Cocksfoot	*r* = 0 53

Heritability	July cut	August cut
Perennial ryegrass	− 0 29^{ns}	+ 0 17^{ns}
Cocksfoot	+ 0 52	+ 0 53

part to inherent differences in digestibility and in part to the different dates of harvesting[5]

Significant variation in digestibility was also found between the families within each species, in this case the ryegrass and cocksfoot were more comparable, having been cut on the same dates and at similar stages of growth The differences within each species were greater at the second cut, the extreme families differing by about 10 units of digestibility The cocksfoot families were all less digestible than the ryegrass, although the most digestible cocksfoot family almost reached the lower end of the ryegrass range

In order to assess the possibilities of selection for high digestibility, a knowledge of the repeatability and the heritability of this character is necessary (Table 2) The repeatability between the two successive cuts of the families proved to be reasonably high (0 44 for ryegrass and 0 53 for cocksfoot) The heritability estimates, derived from parent–progeny correlation, gave little information in the ryegrass families, as all the parents which had been used for crossing were of high digestibility, and the mid-parent values thus showed little variation (79–83 per cent) As a result, no significant correlation with progeny means could be obtained, in spite of the differences between the families In the cocksfoot families, on the other hand, the heritability was + 0 52 for the July cut and + 0 53 for the August cut, this indicated that more than half the variation in digestibility between the cocksfoot families was of genetic origin The stability of these family differences from year to year has now been confirmed by the analysis of individual seedlings from contrasting ryegrass and cocksfoot families in April 1962 These analyses have also revealed considerable plant-to-plant variation even within 'high' and 'low' families, and have thus made it possible to continue with a second cycle of selection

These results thus suggest that genetic variation in digestibility exists within herbage species and can be utilized by the breeder Some advance under selection can be expected in species already of high digestibility, for example, ryegrass, particularly when at a fairly mature stage at or after flowering The margin for improvement appears greater, however, in species at present of lower digestibility, for example, cocksfoot, and it may be possible to bring the digestibility of such species up to the ryegrass level However, even a small improvement in overall digestibility within a herbage species would be of practical value, because of the much greater resulting potential of that species for animal production[3]

The development of a laboratory technique for the analysis of digestibility at the individual genotype level has thus made possible the rapid screening of breeding material, and the selection of desirable genotypes which may allow the breeding of herbage varieties of improved nutritive value

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SOWING WINTER WHEAT ON LEYS DESTROYED WITH A HERBICIDE

By R. A. ARNOTT and C R. CLEMENT

Grassland Research Institute, Hurley

A PRELIMINARY investigation of the use of herbicides, instead of ploughing to kill the grass sward before growing wheat, was stimulated as a result of the success of sod-seeding herbage crops in New Zealand[1,2], in the United States[3], and in the United Kingdom[4,5] Investigations reported by Clement and Williams[6] showed that after 3 or 4 years under leys, the increase in soil organic matter and crumb stability is largely confined to the top 2 cm of soil This finding suggested the need to investigate the possibility of growing wheat after leys without disturbing the soil more than is required to sow the seed the effect of the compactness of soils under leys on the growth and yield of wheat could also be examined Another question arises concerning the release of nitrogen in the soil after the sward is destroyed by herbicides compared with that in soil ploughed and cultivated unpublished results of soil analyses from our previous experiments suggest that the mineralization of organic nitrogen occurs more slowly after destruction by herbicides

In the autumn of 1960, an experiment was started to compare the following treatments on a 4-year-old ryegrass/white clover ley (1) Ploughing and seed bed preparation in the normal way (2) Applying 10 lb of amino-triazole per acre (with ammonium thiocyanate activator) (3) Skimming the grass off just below the soil surface with a flail type forage harvester

Five rows of Capelle winter wheat were sown on six occasions The first was on September 29, 1960, the day the amino-triazole was applied The wheat was hand sown in rows seven inches apart on all three treatments This was done with the minimum

Table 1 YIELDS OF WINTER WHEAT
(Dry weight of grain in g /3-yd row)

Sowing date	Treatment mean of three replicates			
	Ploughed	Sprayed	Skimmed	*S.E.*
29 9 60	183	173	72	±15 5
30 9 60	142	145	87	± 6 5
3 10 60	120	166	95	± 8 8
10 10 60	203	199	90	± 8 9
8 11 60	217	211	63	±16 5
16 11.60	165	178	24	±13 2

disturbance of the soil on the mechanically cleared and sprayed treatments No fertilizer was applied to the wheat at any time

The herbicide killed the ley slowly but completely Residual toxicity reduced the number of established wheat plants only in the first and second sowings Skimming the soil surface reduced competition from the old sward until the following spring afterwards, the grass recovered and adversely affected the wheat Although the wheat on the sprayed treatment was shorter and lighter in colour than that on the ploughed treatment during winter and spring, there was no difference at harvest in either height or appearance

Table 1 shows that wheat grown after skimming the soil surface gave significantly lower grain yields for all sowing dates than that grown on the ploughed or the sprayed treatment Grain yields on the ploughed treatment did not differ significantly from those on the herbicide (sprayed) treatment The only significant difference between these two treatments occurred after the third sowing date (October 3 1960), that is, five days after spraying, when the sprayed gave the higher grain yield This experiment has shown that it is possible to establish winter wheat after leys killed by a herbicide, and to obtain yields similar to those after ploughing and conventional seedbed preparation There was no evidence that yields on unploughed land were limited either by soil compaction or reduced mineralization of nitrogen

We thank Mr. Lewis Jones for advice and assistance with herbicidal spraying and Dr William Davies for advice and facilities

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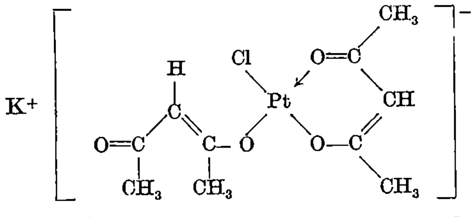
STRUCTURE OF A CARBON-BONDED ACETYLACETONE COMPLEX OF PLATINUM (II)

By Dr B N FIGGIS, Prof J LEWIS*, R. F. LONG, Dr. R. MASON, Prof R S NYHOLM, F R S, Dr P J PAULING and G B. ROBERTSON

Inorganic Crystallography Laboratory, Imperial College, S W 7, and Department of Chemistry, University College, W C 1

IN 1901, Werner described a group of compounds formed from the reaction of potassium chloroplatinite with acetylacetone[1], among these was the complex potassium *bis*acetylacetonato-chloro-platinate (II) (KPt(Acac)$_2$Cl) This compound was re-investigated by Grinberg and Chapurskii[2], who, on the basis of conductivity data, concluded that the structure (1) formulated by Werner was essentially correct The structure was based on a co-ordination number of four generally observed for platinum (II) complexes



(1)

Alternative formulations of the stereochemistry of KPt(Acac)$_2$Cl could be a five co-ordinate complex utilizing all oxygens of the acetylacetone residues and the chlorine in a way similar to the structure of *tris*-(*o*-diphenylarsinophenylarsine)iodo-platinum (II) tetraphenyl borate[3] or a four co-ordinate structure involving one of the acetylacetones bonding through the γ-carbon atom Such bonding has been previously observed in the case of some platinum (IV) complexes[4], and we can now report evidence for the existence of such bonding in complexes of platinum in the lower oxidation state

The crystals of KPt(Acac)$_2$Cl (from aqueous solution) are monoclinic a = 25 97 Å; b = 13 73 Å, c = 7 81 Å, β = 92 0° The space-group is I2/c, which is a non-standard setting of C2/c The X-ray analysis, using some 3,000 three-dimensional reflexions, was determined by Patterson and Fourier methods, the molecular geometry of the platinum ion shown in Fig 1 corresponding to the usual residual factor R = 0 10 after two cycles of least-squares refinement of atomic co-ordinates and anisotropic Debye factors

The standard deviations of the bond-lengths of Fig 1 are 0 015 Å for the Pt—Cl bond, 0 025 Å for the

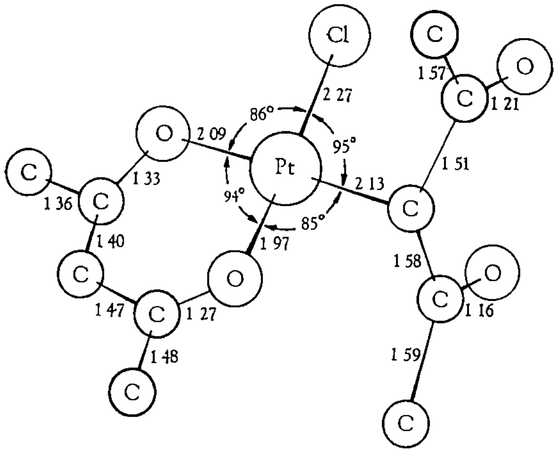


Fig 1 A view of the complex ion *bis*acetylacetonato-chloroplatinum (II) showing interatomic distances and certain angles

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* Now in the Department of Chemistry, University of Manchester

Pt—O and Pt—C bonds and 0 10 Å for all carbon–carbon and carbon–oxygen bonds Least squares refinement of the X-ray data is continuing and we shall report more accurate values for the bond-lengths and bond-angles elsewhere

The co-ordination of the platinum is square planar with only one acetylacetone co-ordinating through its oxygens, the other bonding through the carbon atom The lengths of the platinum–oxygen bonds *cis*- and *trans*- to the chlorine may be significantly different. The chlorine is six-co-ordinated by potassium ions at distances of 3 22 Å (twice), 3 25 Å (twice) and 3 61 Å (twice) There are two crystallographically and stereochemically non-equivalent potassium ions in the molecule K_1 is six-co-ordinated by pairs of oxygen atoms at 2 73 Å, 2 76 Å and 2 89 Å while K_2 is surrounded by a pair of oxygens at 2 84 Å and two pairs of chlorines at 3 22 Å and 3 25 Å respectively All other intra- and inter-molecular distances are of the usual order

Infra-red and nuclear magnetic resonance analyses offer some evidence that the platinum ion has essentially the same structure in solution as in the crystal

(1) The infra-red spectrum of KPt(Acac)$_2$Cl in deuterium oxide shows only minor changes, due to solvent perturbation, in the carbonyl-stretching region from those in nujol In contrast to the infra-red spectra of a number of transition metal acetylacetonates[5] KPt(Acac)$_2$Cl shows the presence of some strong bands above 1,650 cm^{-1} In nujol these actually occur at 1,653 cm^{-1} and 1,695 cm^{-1} and can be assigned to the free ketonic C=O stretching modes Two distinct types of C—H's in the complex are also indicated by the infra-red data of the γ-carbon–deuterium substituted molecule

(2) The complex behaves as a uni-univalent electrolyte and the molecular weight determined cryoscopically corresponds to the monomeric formulation

(3) The H^1 nuclear magnetic resonance spectrum of the complex at 40 Mc/s shows the presence of two separate methyl absorptions of equal intensity, with chemical shifts of 1 94 and 1 46 p p m relative to dioxane The splitting of the methyl protons by coupling to the platinum (platinum-195, abundance 33 7 per cent, spin ½) was readily observable for one of the groups ($J_{Pt\text{-}H} = 5\ 6$ c p s) but not for the other ($J_{Pt\text{-}H} \sim 2\ 5$ c p s) On the basis of the structure shown in Fig 1, three groups of methyl resonance frequencies might have been anticipated since there seems no *a priori* reason for equivalence of the methyl groups of the oxygen-co-ordinated acetylacetone The chemical shift between them is not expected to be large, however, and the fact that they give rise to but one group in the nuclear magnetic resonance spectrum must be due to a chemical shift below the limit of resolution The signals from the γ-carbon–protons were not of sufficient intensity to be interpreted

Werner[1] noted that solutions of the potassium salt yielded coloured precipitates with cupric, ferric and cobaltous chlorides We have examined the copper complex, which may be formulated as

$$\mathrm{Cu[PtCl(Acac)_2]_2}$$

The infra-red spectrum of the solid is considerably changed from that of the potassium complex, the highest frequency in the carbonyl region being 1,620 cm^{-1} Unfortunately, the compound is insoluble in water and organic solvents so that no infra-red or nuclear magnetic resonance measurements have been carried out on solutions. In spite of this it seems reasonable to believe that the copper is co-ordinated to the free oxygens of the carbon-bonded acetylacetone

The unequivocal determinations of the stereochemistry of KPt(Acac)$_2$Cl and the unusual bonding raises a number of interesting questions in relation to the structure and stability of d^8 complexes in general In addition to the above complexes, the compound KPt(Acac)$_3$ has been isolated The infra-red spectrum is complex and again has unusually high C=O stretching frequencies, the nuclear magnetic resonance spectrum shows the presence of two groups of methyl protons of intensity ratio 2 1 and with Pt—H coupling constants of 6 7 and ~2 c p s respectively. This may be taken to indicate that the stereochemistry of the complex is square planar with one 'normal' oxygen co-ordinated—and two γ-carbon co-ordinated—acetylacetone residues or, possibly, is five co-ordinate with two oxygen co-ordinated and one carbon co-ordinated ligand The corresponding Ni^{2+} anion is paramagnetic with a moment ($\mu_{\text{eff}}^{295°K} = 3\ 1$ B M) typical of that usually found for octahedral nickel complexes, its infra-red spectrum shows no anomalously high C=O absorption frequencies This probable change of co-ordination number in going from nickel (II) to platinum (II) complexes is similar to the structure differences between the monomeric Pt(Acac)$_2$ and the trimeric Ni(Acac)$_2^6$ Factors which may be involved in determining the co-ordination number of complexes of the first, second and third row of the transition elements have been summarized earlier[7]

Unusual co-ordination of acetylacetone through the γ-carbon atom has already, as was mentioned earlier, been observed in the case of Pt (IV) complexes by Swallow and Truter[4] It is interesting to note that the orientation of the acetylacetone group is quite different from that which we report here In considering the structure of the Pt (IV) complexes, it was suggested that the acetylacetone anion may be thought of as a substituted methyl group In the case of Pt (II), alkyl derivatives of the type L_2PtRX (R is the alkyl group, X a halogen and L a phosphine ligand) have been recently reported[8]. The stability of these complexes has been associated with the presence of the strong π-bonding phosphine ligands, which affect the stability of the complex to radical decomposition If one of the acetyl-acetonates in KPt(Acac)$_2$Cl is considered to be a substituted alkyl, a marked contrast of the present class of complex with those previously reported is obvious since acetylacetone is not generally considered to be capable of forming strong $p\pi$–$d\pi$ bonds with the metal This difference in stability may be correlated with the more probable decomposition of an anion in the case of acetylacetone

Part of this work was supported by the U S Army through its European Research Office

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COINCIDENCE OF SUDDEN IONOSPHERIC DISTURBANCES WITH THE EXPLOSIVE PHASE OF SOLAR FLARES

By RICHARD HANSEN and JOSIP KLECZEK*

High Altitude Observatory, Boulder, Colorado

In a recent communication, Covington and Harvey[1] demonstrated the coincidence of the 'explosive' phase of solar flares with 10.7 cm solar noise bursts. Their investigation was based on a list of flares, optically observed at the Lockheed Observatory, which Athay and Moreton[2] reported as having had apparent ejections at velocities of 500–1,500 km/sec. The time of ejection has been called the 'explosive' phase as distinguished from the times of beginning and maximum brightness in the complete evolution of certain complex flares. We wish to show that the explosive phase is also accompanied by the beginning of sudden ionospheric disturbances. Further, we present two examples of solar limb flares with the explosive phase. Finally, a possible explanation of ionospheric 'sluggishness' is given.

Since the International Geophysical Year, the McMath–Hulbert, University of Hawaii, Sacramento Peak, and High Altitude Observatories have operated a network of identical instruments recording ionospheric disturbances, as evidenced by sudden cosmic noise absorption (SCNA) and sudden enhancement of atmospherics (SEA). The equipment and principles of operation have been described by Lee[3].

All eleven of the 'explosive' phase flares reported by Athay and Moreton occurred during periods of concomitant ionospheric observation, nine were accompanied by distinct effects. In two of the nine cases (*C* – August 24, 1959, and *K* – October 12, 1960) direct measurements of X-rays in the range 20–80 keV were made above the Earth's atmosphere[4]. The relationship between the starting time of the flare, its explosive phase, the starting time of the 10.7-cm burst, and the starting time of the SCNA or SEA is represented in Fig. 1. To provide identification, each event retains the same letter notation as the flares in refs. 1 and 2. Coincidence of the sudden ionospheric disturbances with explosive phases of the flares and with 10.7-cm bursts is evident. Our result corroborates the statistical studies by Dodson, Hedeman and Covington[5], Kawabata[6], Hachenburg and Kruger[7] of the association between ionospheric disturbances and centimetre-wave bursts. The association suggests a common physical cause, namely, high-velocity electrons[8] accelerated during the explosive-phase with the "flare X-rays generated in the same region of origin of emission as centimetre-wave bursts"[4].

Further evidence for ejection of particles is given by limb flares with explosive phase. Two examples will be described separately.

The cosmic-ray flare of November 20, 1960, has already been reported in the literature[9–11]. Its most noteworthy optical feature was a high-speed ejection, having an apparent transverse velocity of about 700 km/sec (Fig. 2*a*). The ejection began at 2022

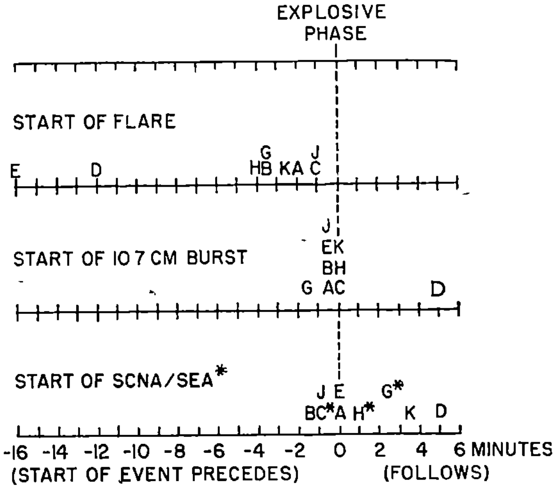
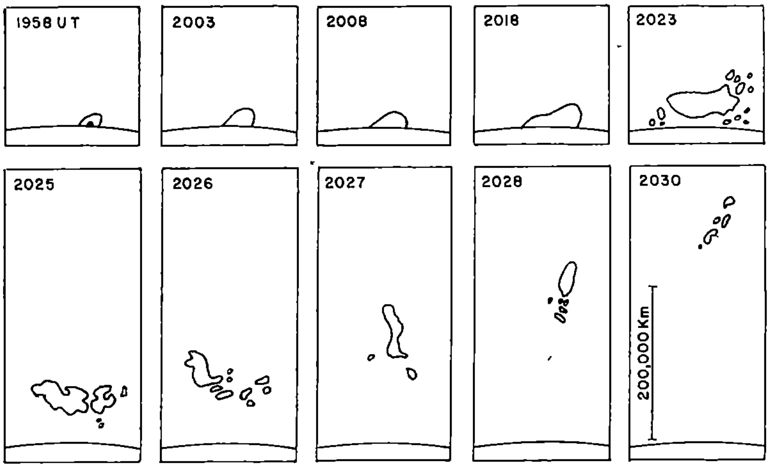


Fig. 1. Upper, distribution of the difference in time of start of the flare and of the explosive phase; middle, distribution of the difference in time of the start of the 10.7-cm burst and the explosive phase of the flare; lower, distribution of the difference in time of the start of the sudden cosmic noise absorption (SCNA) or sudden enhancement of atmospherics (SEA*) and the explosive phase of the flare. This is largely a reproduction of Fig. 1 from Ref. 1



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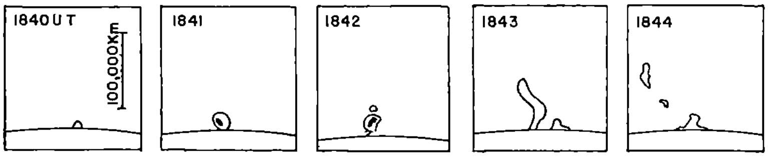

Aug 2, 1958

Fig. 2. Tracings of two solar limb flares with explosive ejections. The event of November 20, 1960 (25° N., 90° W.), is shown above and August 2, 1958 (13° S., 90° W.), below. All observations were made at the High Altitude Observatory, Climax

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* American Astronomical Society visiting professor from the Astronomical Institute, Ondrejov, Czechoslovakia.

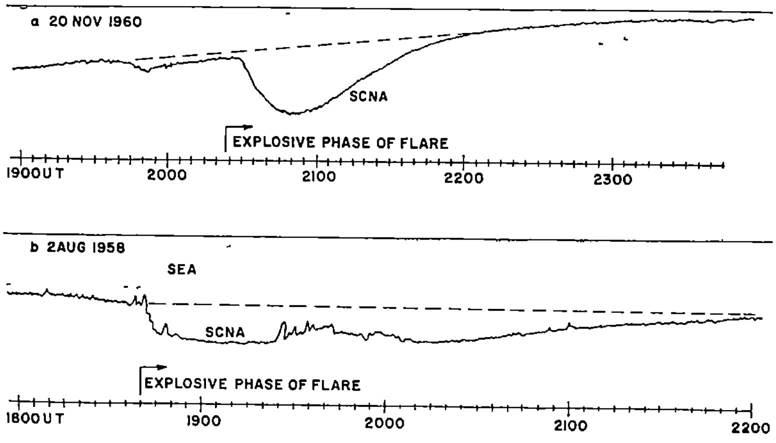

Fig. 3. Development of the ionospheric effects (SCNA and SEA) associated with the explosive phase of the solar flares shown in Fig. 2. The undisturbed trend of the 18 Mc/s cosmic noise is indicated by dashed lines

U.T. with the 10.7-cm burst starting at 2023 U.T. and the ionospheric disturbance starting from 2023 U.T. (SID and SEA) to 2028 U.T. (SCNA) (Fig. 3*a*). We believe this ejection to be the profile counterpart of the dark clouds reported by Athay and Moreton as being expelled from disk flares having explosive phase.

A very similar limb flare was observed at the Climax station of the High Altitude Observatory on August 2, 1958. A small, bright feature first appeared on the west limb at 1840 U.T., by 1842 U.T. it had clearly separated as an ejectual feature, continuing nearly radially outward from the Sun until 1847 when it was no longer visible (Fig. 2*b*). The transverse velocity was approximately 500 km/sec. Again, the microwave burst (1840 U.T.) and ionospheric effects (1841 U.T.) began essentially simultaneous with the ejection (Fig. 3*b*).

Some years ago, Ellison[12] noted the tendency for the maximum of the ionospheric effects to lag (by approximately 6 min) the maximum brightness of the optical flare. He attributed their delay to ionospheric 'sluggishness' due to the relaxation time associated with the production and decay of ionization in the ionosphere. The same author, however, has also directed attention to the 'flash of radiation' which occurs in the early stages of some great flares, intermediate between their first appearance and time of maximum brightness. This 'flash-phase' is certainly identical to what Athay and Moreton have called the 'explosive-phase', and the correct explanation of ionospheric 'sluggishness' is probably that the generation of solar X-rays is physically associated with the ejection of high-energy electrons at the explosive phase rather than with any other phase in the complex evolution of flares.

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SPECTRA OF TURBULENCE AT HIGH REYNOLDS NUMBER

By Dr. M. M. GIBSON
Department of Mechanics, Johns Hopkins University, Baltimore

ENERGY spectra of streamwise and radial turbulent velocity components have been measured on the axis of a round air jet at moderately high Reynolds number, with the object of providing evidence for the Kolmogoroff universal equilibrium hypothesis[1].

Turbulent velocity fluctuations were measured initially with a conventional constant current hot-wire anemometer but afterwards, as the equipment became available, a constant-temperature hot-wire anemometer with a linearizing circuit was used. The turbulence signal was analysed with a 'Kronhite 330' variable band pass filter and a Hewlett–Packard 302*A* superheterodyne wave analyser, results from the two instruments overlapping in the wave number-range $0.2 < k < 1 \text{ cm}^{-1}$. In the use of the constant current hot-wire anemometer systematic errors arise from the inherent non-linear static (King's law) and dynamic response of the instrument to large amplitude velocity fluctuations[2] and from trigonometric non-linear response to large fluctuations in the direction of the local cooling velocity[3]. The second of these effects was reduced by using fine platinum–rhodium wires (0.000025–0.00005 in. diam.). Rough estimates of the error introduced by the non-linear static response may be made and it appears probable that the errors due to the first two effects tend to be self-cancelling in terms of the total turbulence signal, although the shape of the energy spectrum may be somewhat distorted.

Errors associated with large fluctuations in magnitude may be reduced by using a constant-temperature hot-wire anemometer with a linearizing circuit at the cost of random errors due to drift, with temperature, of the associated d.-c. coupled feedback amplifier circuits and a considerably reduced band-width due to the increased, and only approximately known, thermal noise. The errors associated with the response to the local flow direction have been examined by Rose[3] and may be corrected for the total signal.

Further systematic errors common to both systems were wire-length effects[4] (reduced in this constant current case by use of very short wires), and the assumption of the Taylor hypothesis to relate wave number to frequency $\left(k = \dfrac{2\pi n}{\bar{U}}\right)$

The results presented here have not been corrected for any of these effects, and in this connexion it is of considerable interest to compare the data obtained by the two methods. This has been done for the streamwise energy spectrum function $F_1(k)$ in Fig. 5. It is seen that agreement is surprisingly good, systematic divergence of the two curves being just apparent at very low wave-numbers. Numerical values presented here have been derived from the measurements with the constant-temperature hot-wire equipment considered to be, on the whole, the more accurate of the two.

The jet Reynolds number based on the 15·25 cm orifice diameter and the initial mean velocity was 5×10^5. On the axis, 50 diameters from the nozzle, the local mean velocity $\overline{U} = 600$ cm/sec, root mean square turbulence-levels in the streamwise and radial directions $\frac{u'}{\overline{U}} = 25$ per cent, $\frac{v'}{\overline{U}} = 26$ per cent, and the turbulence Reynolds number $R_\lambda = 780$, the value of λ being obtained from the relations valid strictly for isotropic turbulence only.

Figs. 1 and 2 are logarithmic plots of the energy spectrum functions $F_1(k)$ and $F_2(k)$ obtained directly by the two hot-wire methods with the definitions

$$\int_0^\infty F_1(k)\mathrm{d}k = \overline{u^2} \text{ and } \int_0^\infty F_2(k)\,\mathrm{d}k = \overline{v^2}$$

Fig. 3 is a semi-logarithmic plot of the energy spectrum function $F_1(k)$ of Fig. 2 and the corresponding dissipation spectrum $k^2F_1(k)$. The two spectra are reasonably well separated in wave-number space and the existence of an inertial sub-range therefore seems plausible. The curves of Figs. 1 and 2 are well fitted by a $-5/3$ power law over approximately two decades of wave-number for $F_1(k)$ and rather less for $F_2(k)$.

A method of checking local isotropy is to derive the spectrum function $F_1(k)$ from the measured $F_2(k)$ using the relation for isotropic turbulence[5]

$$\frac{\mathrm{d}F_2(k)}{\mathrm{d}k} = -\frac{k}{2}\,\frac{\mathrm{d}^2F_1(k)}{\mathrm{d}k^2}$$

in integral form. This has been done for the constant temperature hot-wire data of Fig. 2, the derived and measured values of $F_1(k)$ being plotted, for comparison, in Fig. 4. The moderately good agreement for $k > 0·2$ is, considering the limited accuracy of the method, at least an indication of local isotropy. The anisotropy of the large-scale turbulence is shown quite clearly by the systematic divergence of the experimental points from the derived curve at low wave-numbers. The same method applied to the constant current hot-wire data of Fig. 1 yielded less satisfactory results, although the error was in the opposite direction, due to the unexpected and unexplained low measured value of v'.

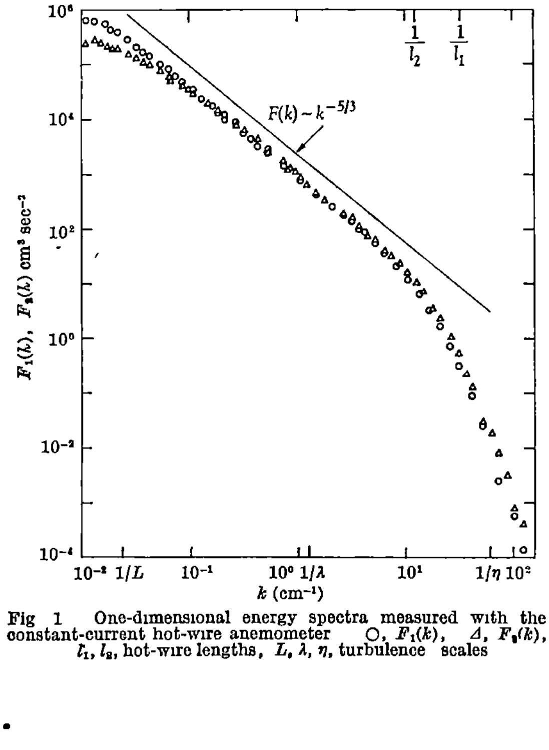

Fig. 1. One-dimensional energy spectra measured with the constant-current hot-wire anemometer. ○, $F_1(k)$, △, $F_2(k)$, l_1, l_2, hot-wire lengths, L, λ, η, turbulence scales

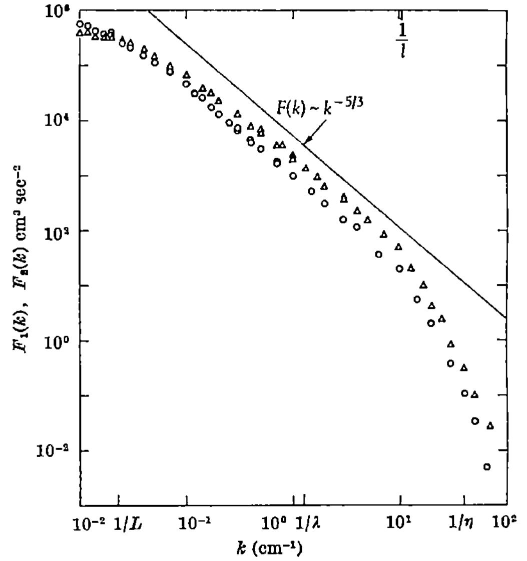

Fig. 2. One-dimensional energy spectra measured with the constant-temperature hot-wire anemometer. ○, $F_1(k)$, △, $F_2(k)$

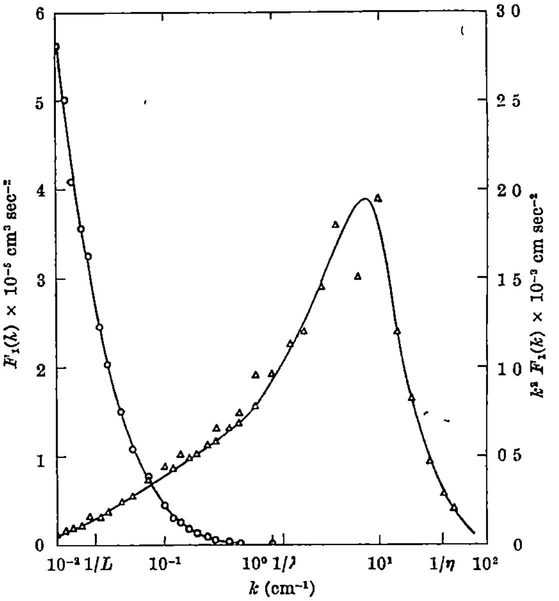

Fig. 3. ○, Energy spectrum $F_1(k)$, △, dissipation spectrum $k^2F_1(k)$

Assuming, then, local isotropy and the form of the three-dimensional energy spectrum predicted by the Kolmogoroff hypothesis for the inertial sub-range

$$E(k) = A\,\varepsilon^{2/3}k^{-5/3}$$

where

$$\int_0^\infty E(k)\,\mathrm{d}k = \frac{\overline{3u^2}}{2}$$

for isotropic turbulence, we obtain, with the relation $E(k) = -k\frac{\mathrm{d}}{\mathrm{d}k}[F_2(k) + \frac{1}{2}F_1(k)]$ (ref 5), a value for the postulated universal constant A compared in Table 1 with those found by other authors

Table 1

Ref	Flow	R_λ	A
Present work	Jet	780	1 57
6, 7	Tidal channel	3,600	1 44
8	Grid turbulence	750	2 70
9	Pipe	290	1 96

Bearing in mind the different conditions under which the data of Table 1 were obtained and the associated experimental difficulties, the four values of A reported are remarkably consistent and appear to bear out the Kolmogoroff theory for an inertial sub-range Some reservations must, however, be expressed with regard to the last two results Those of ref 8 appear to contradict the local isotropy hypothesis although, unfortunately, the wind tunnel used in this investigation was demolished before these unexpected effects could be properly examined The turbulence Reynolds number of the pipe flow of ref 9 scarcely seems high enough for the existence of an inertial sub-range and the shapes of the cross-stream spectra seem to bear this assumption out.

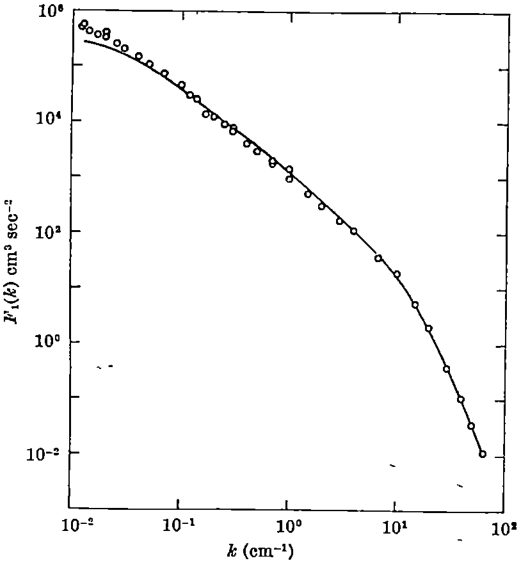

Fig 4 Solid line, $F_1(k)$ determined from $F_2(k)$, O, measured $F_1(k)$

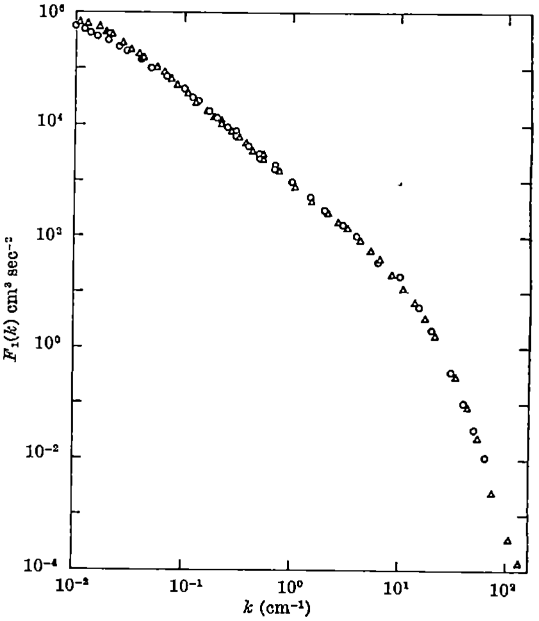

Fig 5 O, Results from constant-temperature hot-wire anemometer, Δ, results from constant-current hot-wire anemometer

Further measurements are clearly called for, and some are now being made in a region of the jet where the flow is highly non-isotropic and the turbulent shearing stresses are large

I thank Prof S. Corrsin for his advice and Dr W G Rose and Mr D A Kennedy for many helpful suggestions.

This work was supported by the U S National Science Foundation

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NATURE OF NUCLEAR DEBRIS IN SEA-WATER

By E C. FREILING and N. E. BALLOU

U S Naval Radiological Defense Laboratory, San Francisco, 24, California

IN a previous communication[1] 'fractionation' was defined as "any alteration of radionuclide composition occurring between the time of detonation and the time of radiochemical analysis which causes the debris sample to be non-representative of the detonation products taken as a whole" Fractionation resulting from the initial nucleation, condensation and separation processes was called 'primary fractionation' and discussed at length Subsequent fractionation due to contact of debris with radioactively inert surroundings was called 'secondary fractionation' This article is concerned with secondary fractionation in nuclear detonations occurring in the presence of sea-water Specifically, it summarizes studies carried out at the U S Naval Radiological Defense Laboratory up to the time of the moratorium on nuclear testing (October 1958) on the distribution of detonation product radionuclides between soluble, particulate and colloidal states It thus helps to establish the nature of radioactive contaminants of sea-water from nuclear detonations This information is pertinent to questions of the biological fate and the contamination and decontamination characteristics of such radioactive species. The work was carried out by a number of investigators, and involved investigations of both simulated and actual debris.

This report is essentially a progress report. Although the results presented are therefore not to be regarded as complete, they represent the best available information on this hitherto untreated subject. The extent of agreement among results from different detonations and from laboratory experiments simulating such events is greater than might at first be predicted.

Debris Formation

Little is known of the fundamental processes of nuclear debris formation in a sea-water environment. In the absence of large quantities of ballast or bottom material, the initial processes can probably be visualized, for orientation, as being similar to those of an air burst. The debris will consist initially of vaporized and dissociated water, sea-water salts and device materials. By applying an assumed efficiency to the thermal yield of a 10-*KT* device, detonated on the ocean surface, one calculates that the order of 1,000 tons of sea-water will be vaporized. If this device contains 1 ton of iron, the fireball will then contain 19 tons of chlorine, 11 tons of sodium and potassium, 1·6 tons of calcium and magnesium, 1 ton of iron, 0·9 ton of sulphur and less than 0·1 ton of any other sea-water constituent. Engulfed air will also be present. As this system cools, nucleation of the debris should be governed by iron, calcium and magnesium oxides. Thus, Stewart[2] estimates iron nucleation in an air burst would occur at 1,920° K, well above the boiling point of sodium chloride (1,686° K) and the sublimation point of sodium monoxide (1,550° K) but below the melting points of magnesium oxide ($\sim$2,880° K) and calcium oxide (2,840° K). Initial nucleation would take place in an atmosphere of undissociated water (which at this point can be neglected) and at concentrations not too different from those of an air burst. The initial nuclei would therefore be expected to consist of particles of calcium, magnesium and iron oxides of less than 20μ diameter, perhaps with a log normal, exponential or bimodal frequency distribution[2]. These would contain the refractorily behaving radionuclides. On further cooling, these would serve as nuclei for deposition of salt crystals, more volatile radionuclides, and eventually water or ice. Return to the sea-water would then be accompanied by dissolution of the condensed salt and late condensing soluble fission products, leaving colloidal particles bearing most of the activity. Due to the large surface to volume ratio, leaching of soluble radionuclides should occur with high efficiency, the more so since most of these would also be expected to have concentrated in the surface in particle formation, leaving the insoluble radionuclides in the colloidal state.

In the case of an underwater burst, a rapidly expending shell of vaporized device material first pushes the sea-water back, forming an expanding bubble. If the burst is sufficiently deep, this expansion reaches a maximum, and the bubble begins to contract. During these processes, the vaporized device material will be condensing and mixing with the vaporized sea-water. Unless bottom material is incorporated into the bubble, small particles are again formed. The greatest difference between the underwater burst and the surface burst, so far as the nature of the debris is concerned, is in the degree of primary fractionation. The degree of primary fractionation is dependent on the depth of burst. Thus, in the Shallower Underwater Burst examined here, slightly fractionated water, which was 25 per cent poor in strontium-89 in respect to the bulk of the fission products, appeared at surface zero. The missing strontium-89 appeared in the accompanying base surge, which was rich in strontium-89 by a factor of 2,000. In this case too, then, a large proportion of colloidally bound activity is to be expected in the water at surface zero.

If bottom material is incorporated, as was the case for the Lagoon Burst examined here, large particles and primary fractionation are naturally expected.

Except for Table 4, the results in this report are presented in forms which eliminate any effect of primary fractionation and illustrate only the effect of secondary fractionation.

Ultrafiltration

In view of the foregoing considerations a method of separating colloidal material from solutions is evidently required for the study of nuclear debris in sea-water bursts. High-pressure ultrafiltration through Visking dialysis tubing was used throughout these studies. The apparatus was based on a model described by Cummins[3], but metal surfaces were coated with inert material to minimize surface adsorption and voltaic cell formation. This has eventually led to the design of a plastic-lined ultrafilter by Callahan and Lai[4], although this came too late for the present work. Membrane testing has been carried out by Greendale and Ballou[5], and Chandler[6] and by Callahan and Lai[4]. Greendale and Ballou showed that the membranes would pass molecules of 12 Å diameter but retain particles of 30–40 Å diameter. The average pore diameter is rated by the manufacturer as 24 Å. In 1955, Chandler tested small fractions of the filtrate of radioactive solutions and found an ion retardation effect which was confirmed by Callahan and Lai. It is therefore important to bear in mind that ultrafiltration does not produce sharp separations but a gradation of retardations which increase with the size of the solute or colloid and may be influenced by the charge of the latter and the nature of the filtering medium as well.

Underwater Arcing Experiments

Greendale and Ballou[5] simulated the conditions of an underwater nuclear burst by vaporizing individual fission product radionuclides under sea-water in a carbon arc. The resulting mixtures were separated into particulate, colloidal and soluble fractions by centrifugation and by ultrafiltration through 'Cellophane' membranes. The results of duplicate runs (quadruplicate in the case of Sr) are shown in Table 1. The elements iodine, strontium, antimony and cæsium were found to be mainly soluble. Molybdenum was present chiefly as a colloid or particulate. Cerium, niobium, ruthenium, yttrium and zirconium were essentially particulate.

Underwater Burst

Physical-state investigations of debris from an underwater nuclear burst were carried out by Ballou, Richter, Wiltshire and Singer[7]. Surface-water samples and deep-water samples from this event were collected in polyethylene bottles, and aliquots of these were withdrawn for physical-state separations up to one month after zero time. Centrifugation was used to separate out a 'solid' fraction,

Table 1. PHYSICAL STATES OF SELECTED FISSION PRODUCT ELEMENTS FOLLOWING VAPORIZATION UNDER SEA-WATER

Element	Physical state (per cent) Soluble	Colloidal	Particulate
Ru	0.7 ± 0.5	3.5 ± 0.5	96 ± 1
	0 ± 1	6 ± 1	94 ± 1
Ce	1 ± 2	4 ± 6	95 ± 3
	2.7 ± 1.4	4 ± 1.7	94 ± 1
Cs	80 ± 9	0 ± 9	20 ± 2
	61 ± 7	14 ± 8	25 ± 1
Zr	0 ± 2.4	0 ± 3.4	100 ± 5
	2.5 ± 2.5	5 ± 2.5	92 ± 3
Sr	85 ± 2	3.6 ± 2	11.5 ± 0.2
	95 ± 9	0 ± 10	5 ± 0.4
	85 ± 11	8 ± 12	7 ± 1
	83 ± 2	0 ± 3	17 ± 0.5
I	86 ± 6	11 ± 6	3 ± 0.2
	93 ± 10	6 ± 10	1.6 ± 0.2
Y	0.4 ± 0.2	2.2 ± 0.4	98 ± 1
	0.2 ± 0.1	6.6 ± 0.2	93 ± 1
Nb	0.1 ± 0.5	0.5 ± 1.2	99 ± 3.5
	0.2 ± 0.6	0.1 ± 0.9	100 ± 1.7
Mo	29 ± 1	19 ± 1	52 ± 3
	31 ± 1	0.4 ± 1.6	68 ± 1
Te	37 ± 0.6	50 ± 2	13 ± 0.2
	53 ± 3	36 ± 6	11 ± 0.7
Sb	81 ± 1	6 ± 1	13 ± 1
	65 ± 0.3	23 ± 0.4	12 ± 0.1

and ultrafiltration of the resulting supernatant solution served to separate out a colloidal fraction. No visible solids were observable in the samples, and the significance of the differentiation between solid and colloidal fractions was afterwards discounted.

The percentage of total activity in the soluble phase as a function of time was determined by comparing β-disintegration-rates of gross samples with those of ultrafiltrates from successive separations. The decay curves of gross unseparated samples and of ultrafiltrates were quite similar, and consequently the percentage of total activity in the ultrafiltrate was rather insensitive to the length of time between the separation into fractions and the assay of activity. The results, plotted in Fig. 1, show that during the first day or so following detonation, approximately 35 per cent of the total radioactivity was in the soluble fraction. Separations at later times gave increasing relative amounts of radioactivity in the soluble fraction, and by two weeks after detonation this amount was up to approximately 60 per cent. This rise is attributed to the slow conversion of some of the constituent elements into a greater proportion of soluble material, rather than to the element composition changing through radioactive decay. Within the precision of the experiment, there is no apparent difference between samples, whether collected at the surface or at various depths.

The distribution of a number of radionuclides between soluble and insoluble fractions was determined on three surface samples. The percentages of the activity of each radionuclide in the ultrafiltrates at common reference times is given in Table 2. Sufficient data could not be accumulated to permit correlation of the above solubility behaviour of total radioactivity with that of individual constituents.

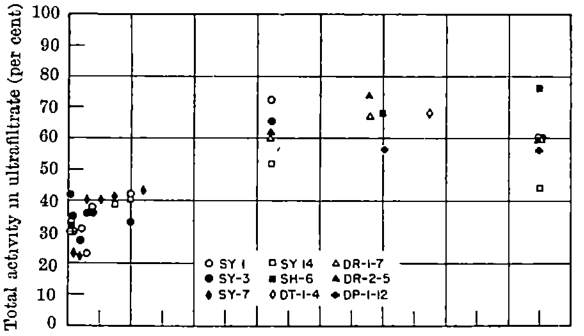

Fig. 1. Percentage of total activity in ultrafiltrate for ultrafiltrations at various times after detonation, Deep Underwater Burst (*SY*, surface-water sample; *DT*, *DR*, *DP*, deep water sample)

Table 2. PERCENTAGES OF ACTIVITY OF RADIONUCLIDES IN ULTRAFILTRATES FROM SURFACE-WATER SAMPLES, UNDERWATER BURST

Sample	Centrifugation time (h from zero time)	Relative activity in ultrafiltrate at centrifugation time (per cent) ^{95}Zr	^{99}Mo	^{239}Np	^{237}U	RE*	^{132}Te	^{140}Ba
SY-1	1	35	64	—	54	33	0	35
	7	—	—	42	—	—	—	—
	19	43	66	44	80	19	0	—
SY-3	7	—	—	41	—	—	—	—
	19	—	—	53	—	—	—	—
SY-7	10	48	67	67	77	42	0	—
	19	—	—	52	—	—	—	—
	116	43	63	—	71	39	0	—
Average		42	65	50	71	34	0	35

* RE, gross rare earths and yttrium

With the exception of tellurium, in all cases where comparisons can be made, the fraction of activity in the ultrafiltrate in the arcing experiments described above is lower than that observed here. This may be due to the presence of milligram quantities of precipitate (hydrous iron oxides and carbon) in the arcing experiments.

Shallower Underwater Burst

Investigations similar to those performed by Ballou *et al.*[7] were made on a surface-water sample from a somewhat shallower underwater burst[8]. This sample was collected in a polyethylene bottle 2 h after shot time in the vicinity of surface zero. The sample contained no visible solids.

A series of sedimentation, centrifugation and filtration experiments were performed on this sample in an effort to determine the distribution of gross γ-ray activity with particle size. From the results of these experiments, the depletion of gross γ-ray activity produced by removing particles up to a given size was plotted against the calculated size of the particles. The results are shown in Fig. 2. An integral curve is drawn through the experimental points and a differential histogram has been constructed. The histogram is seen to decrease gradually with increasing particle size from 0.002 to 20μ.

Duplicate aliquots of this sample were ultrafiltered at various times over a period of 2–13 days after zero time. The fraction of gross γ-ray activity on the membrane was determined by comparing the activities of the membrane and of a standard aliquot of the gross sample immediately after ultrafiltration. The measurements were made in a γ-ray ionization

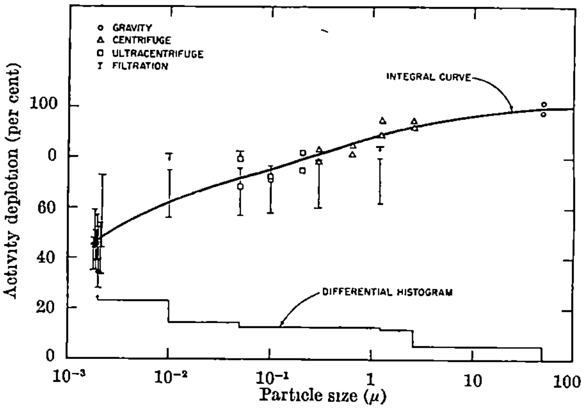

Fig. 2. Activity-particle size plot for $H+2$ surface-water sample, Shallower Underwater Burst

chamber Roughly equal divisions of gross activity were found after separation This is in agreement with the results from the Underwater Burst shown in Fig. 1 Decay measurements indicated that membrane activity decayed more rapidly than the activity of the ultrafiltrate, showing that fractionation was induced by the ultrafiltration process When the γ-ray activities of all membranes were measured at the same time, it was found that the membranes obtained later were more radioactive This observation could be explained by the gradual chemical conversion of radioactive species from the soluble to the insoluble state or by a change of gross radionuclide composition due to radioactive growth of a daughter radionuclide which associates itself with the insoluble (membrane) fraction In view of the results for the Underwater Burst and the known presence of the ^{140}Ba-^{140}La pair, the latter explanation is preferred

Radiochemical analysis for selected radionuclides was performed on duplicate aliquots of the gross samples, ultrafiltrates, and dissolved membranes. Results from successively separated fractions did not reveal any change in the physical state distribution of any radionuclide with time, but mass balance deficiencies prohibit firm conclusions in some cases The results from successive ultrafiltrations have therefore been averaged The results for each radionuclide are individually presented and discussed here and compared with the results of the two previous investigations Included in each discussion is the mass balance achieved and the effect of an additional membrane on the physical state distribution and mass balance observed Mass balance deficiency may be taken as a measure of contamination potential, since it presumably arises from adherence of the radioactive species to the walls of the polyethylene container or to the surfaces of the ultrafilter apparatus Results of experiments to determine the degree of deposit of several radionuclides on polyethylene surfaces from sea-water solutions are given in ref 8

^{89}*Sr* An average value of 4 ± 1 per cent of the strontium-89 was found to be in the insoluble phase, and an average mass balance of 116 ± 5 per cent was obtained The results show a somewhat greater solubility than that found in the underwater arcing experiments, but not outside the precision of the methods The results were unaffected by the presence of a second membrane

^{95}*Zr*–^{95}*Nb* Analyses were performed for both of these radionuclides, and no distinction between their behaviour was evident The presence of a second membrane increased both the mass balance and the percentage of activity appearing in the colloidal fraction With either a single or double membrane, 0–8 per cent of the activity was found in the soluble state, in fair agreement with the underwater arcing results With only a single membrane, an average of 48 per cent was found to be insoluble, but the results spread from 34 to 66 per cent The results from the deeper underwater burst are within this range

^{99}*Mo* Mass balance was consistently good, averaging 102 ± 5 per cent Using a single membrane, an average value of 68 ± 5 per cent was found to be soluble, in good agreement with the deeper underwater burst results With a double membrane, 26 ± 5 per cent was found to be soluble, in agreement with underwater arcing results

^{103}*Ru* It was found that 82 ± 5 per cent of the ruthenium was insoluble, the mass balance being 105 ± 6 per cent No double membrane effect was noted The solubility is therefore greater than indicated by the value of 1 per cent from the underwater arcing experiments

^{132}*Te* The insoluble fraction was found to contain 64 ± 9 per cent of the activity and the mass balance was 81 ± 12 per cent No double-membrane effect was noted These results are in better agreement with those from underwater arcing than with those from the deeper underwater burst

^{140}*Ba* This radionuclide showed a much higher solubility than the single determination made for the deeper underwater burst would indicate Only 5 ± 1 per cent was found in the insoluble fraction, the mass balance being 88 ± 4 per cent No double-membrane effect was noted The results are similar to those obtained for strontium-89

Total rare earths The insoluble fraction contained 67 ± 3 per cent of the activity, in accord with the deeper underwater burst results, but the mass balance was only 71 ± 3 per cent No double membrane effect was noted ^{141,144}Ce was found to behave similarly This behaviour is not inconsistent with the results from the underwater arcing experiment, where it was found to be soluble to the extent of 3 per cent or less

^{237}*U*. The insoluble fraction contained 46 ± 5 per cent of the activity, but the percentage of activity found in the soluble fraction was quite variable The deeper underwater burst results also indicate considerable variability No double membrane effect was noted

^{239}*Np* With single membranes, 50 ± 2 per cent of the activity was found to be insoluble, in agreement with the deeper underwater burst results. With a double membrane, however, 93 per cent was found to be insoluble As in the case of uranium-237, the percentage of activity found in the soluble fraction was quite variable

Lagoon Burst[8]

Another set of similar investigations has been carried out on an underwater burst which contacted the bottom of a coral lagoon Sample collection and treatment were similar to that performed on the shallower underwater burst, with two exceptions First, in addition to a surface water sample collected at 2 h after detonation time ($H + 2$), physical-state studies were also performed on a sample collected from the vicinity of surface zero at 20 h after detonation time ($H + 20$) Secondly, the presence of visible solids in the $H + 2$ sample allowed the initial separation of a particulate phase prior to ultrafiltration This was accomplished by filtration through a siliconed glass-wool plug in a siliconed funnel In order to achieve better definition of the particle size distribution, additional filtrations were made with 'Millipore' filters of various rated pore sizes

The distribution of gross γ-ray activity with particle size is shown in Figs 3 and 4 In the $H + 2$ sample, activity peaks corresponding to two different particle sizes are seen, one at about 10μ and one in the neighbourhood of 0 002μ The larger particle peak is missing from the $H + 20$ sample Comparison of gross activities of the filtrate and particle size fractions showed that the 10μ peak was effectively removed by the filtration with a siliconed glass wool plug

The radionuclide distributions found at 31 h after detonation in the surface water sample collected at $H + 2$ are given in Table 3 Solid phase refers to

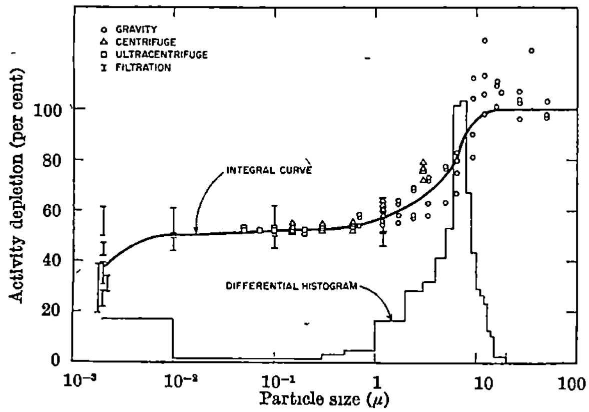

Fig 3 Activity-particle size plot for $H+2$ surface-water sample, Lagoon Burst

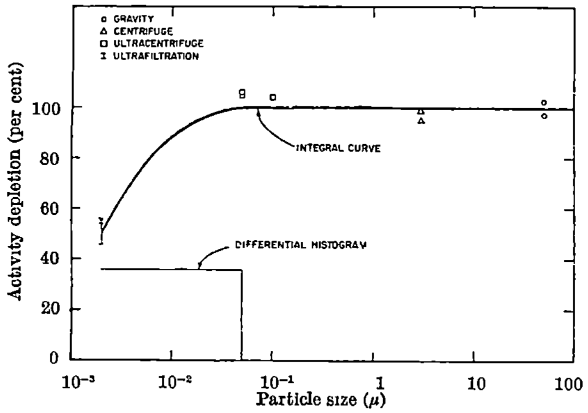

Fig 4 Activity-particle size plot for $H+20$ surface-water sample, Lagoon Burst

material removed by filtration through siliconed glass-wool, colloidal phase to material removed by ultrafiltration, and soluble phase to material remaining in the ultrafiltrate Standard deviations were calculated from duplicate analyses Deviation of sums from unity indicates lack of mass balance or tendency to contaminate

Except for molybdenum-99, the results obtained by analysis of the soluble phase are in fair agreement with the underwater arcing results As pointed out earlier, milligram quantities of hydrous iron oxide were present in the arcing experiments Although different precipitates were present in samples from the arcing experiments and from the coral lagoon burst, the presence of any precipitate would tend to make solubility results from these two situations more nearly alike Comparison of results on the other two phases, however, shows no consistency of agreement The soluble phase values for ^{89}Sr, ^{95}Zr, ^{95}Nb, ^{99}Mo and ^{140}Ba are in fair agreement with the shallower underwater burst results using a single membrane

Table 3 RADIONUCLIDE DISTRIBUTIONS 31 H AFTER DETONATION IN LAGOON BURST $H+2$ SURFACE-WATER SAMPLE

Radionuclide	Fraction distribution Solid phase	Colloidal phase	Soluble phase
^{89}Sr	0 00 ± 0 00	0 11 ± 0 01	0 99 ± 0 00
^{95}Zr	0 38 ± 0 01	0 16 -	0 03 ± 0 00
^{95}Nb	0 45 ± 0 00	0 39 ± 0 01	0 00 ± 0 00
^{99}Mo	0 28 ± 0 00	0 06 ± 0 01	0 60 ± 0 00
^{103}Ru	0 60	0 25 ± 0 00	—
^{132}Te	0 60 ± 0 00	0 18 ± 0 00	—
^{140}Ba	0 00 ± 0 00	0 01 ± 0 01	0 99 ± 0 01
TRE*	0 83 ± 0 02	0 14 ± 0 06	0 03 ± 0 00
^{237}U	0 02 ± 0 00	0 11 ± 0 01	0 04 ± 0 06
^{239}Np	0 47 ± 0 00	0 46 ± 0 01	0 02 ± 0 00

* TRE, Total rare earths

Table 4 COMPARISON OF GROSS $H+20$ SAMPLE COMPOSITION WITH COMPOSITION OF $H+2$ SAMPLE FILTRATES

	Gross $H+20$ Sample Composition	$\frac{\text{Activity per ml of } H+2 \text{ sample filtrate}}{\text{Activity per ml of gross } H+20 \text{ sample}}$ $H+31$ Filtration	 $H+41$ Filtration
^{89}Sr	1 00	0 547	0 547
^{95}Zr	1 00	1 68	1 40
^{95}Nb	1 00	1 52	1 30
^{103}Ru	1 00	1 32	1 13
^{140}Ba	1 00	1 23	1 48
TRE*	1 00	1 06	1 01

* TRE, total rare earths

In order to compare laboratory filtration results with the results of the natural separation brought about by sedimentation in the lagoon, the gross $H+20$ surface-water sample composition is compared in Table 4 with $H+31$ and $H+41$ filtrate compositions obtained from the $H+2$ surface water samples Except for strontium-89, the values in a column are fairly constant Radiochemical analysis showed that the $H+2$ surface water samples were fractionated with respect to strontium-89 Since the $H+20$ surface water sample was taken from a different location, the strontium-89 deviation may easily be attributed to fractionation The constancy of the other ratios indicates that the natural process was well approximated in the laboratory The lower values for the $H+20$ samples are probably due to natural dilution or to sampling an originally less-active zone

Table 5 VARIATION OF SOLID COMPOSITION OF $H+2$ SURFACE-WATER SAMPLES WITH TIME (RELATIVE ACTIVITY PER μg CALCIUM)

	$H+31$ Filtration	$H+41$* Filtration	Duplicate $H+120$ Filtration	Duplicate $H+120$ Filtration
^{95}Zr	1 00	(0 52)†	1 00	1 11
^{95}Nb	1 00	(5 0)	1 00	1 09
^{99}Mo	1 00	0 98	2 61	2 48
^{103}Ru	1 00	1 14	1 52	1 67
^{132}Te	1 00	1 01	1 73	1 63
TRE‡	1 00	1 01	0 97	(4 2)
^{237}U	1 00	5 03	8 22	10 7
^{239}Np	1 00	0 89	1 21	1 33

* No calcium analysis available Calcium assay estimated to be 10 per cent lower than the value for the $H+31$ filtration † Doubtful values are enclosed in parentheses ‡ TRE, total rare earths

Finally, the solid compositions per μg of calcium were calculated for radionuclides in solid fractions obtained from filtrations of the $H+2$ sample made at $H+31$, $H+41$ and in duplicate at $H+120$ These are related to $H+31$ solid composition in Table 5 for those radionuclides which were not predominantly soluble It is seen that the zirconium-95, niobium-95 and total rare earth compositions are constant The constancy of neptunium-239 composition is borderline All other radionuclide solid phase fractions increase with time, most remarkable being uranium-237

We thank the U S Atomic Energy Commission, Division of Research, for partial support of this work

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Solar Temperature at 2-mm Wave-length

ONE of the items developed for microwave diagnostic purposes on *Zeta* is a harmonic-mixing superheterodyne radiometer, working at a wave-length of about 2 mm. This radiometer has been used together with a small radiotelescope for measuring the apparent temperature of the Sun at a wave-length of 2·04 mm.

The radiotelescope was 43 in. in aperture, and was designed on the Coudé system to obviate the necessity of moving the receiver to follow the apparent motion of the Sun. It was also given a Cassegrain configuration to increase the effective focal-length to 163 in., as the focal-length of the primary mirror (18·7 in.) was so short that the Coudé system became geometrically impossible. The feed horn was thus behind the primary mirror, and connected by a run of oversize wave-guide (*WG* 16, 1 in. × ½ in., outside dimensions) to the receiver below the telescope. An equatorial mounting with synchronous motor drive was provided.

Recordings of received power when the aerial was aimed at the Sun were made on two cloudless days in August 1961 as the Sun declined from its noon altitude towards setting. The received powers were converted into aerial temperatures by comparison with a gas discharge noise tube, itself calibrated against a heated wave-guide load, and these temperatures plotted logarithmically against the secant of the Sun's zenith angle. A least-squares-fit straight line was drawn through the experimental points, and its intercept on the temperature axis taken to be the aerial temperature which would be obtained if the aerial were above the atmosphere. The slope of the line gave the vertical atmospheric attenuation, 3·10 db and 2·57 db on the two days, and this measured attenuation was compared with a calculated Van Vleck value, using upper atmosphere radiosonde data provided by the Meteorological Office. The calculated attenuation was found to be 1·5 times the measured value, in approximate agreement with results obtained elsewhere[1]. The temperature plots are shown in Fig. 1.

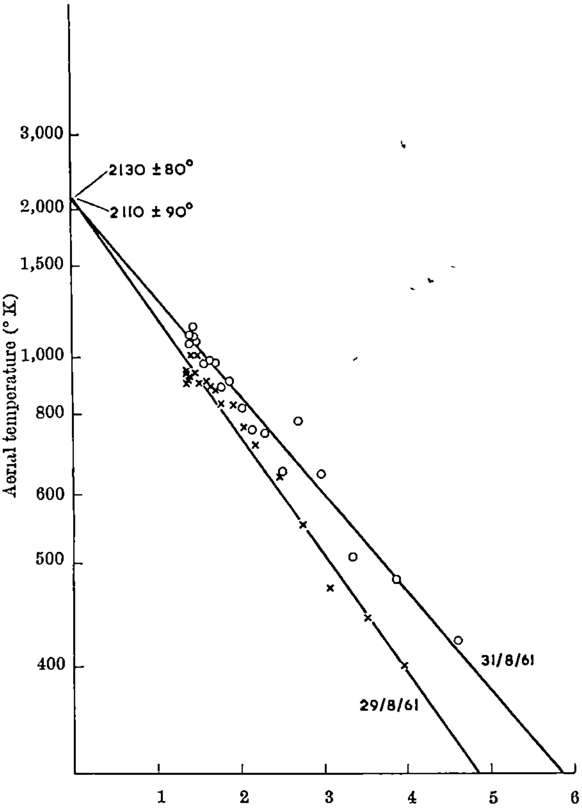

Fig. 1. Aerial temperature plots

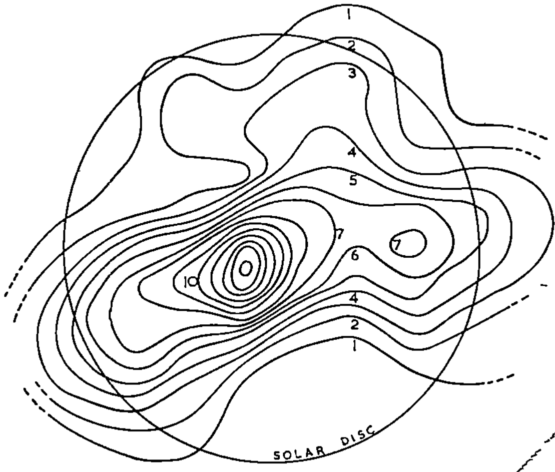

Fig. 2. Aerial gain pattern

The aerial was calibrated for the angular dependence of gain by transmitting a known power from an aerial of known gain at a distance of 930 m, which is more than twice the Rayleigh distance for the telescope, and measuring the power received as the telescope beam was scanned past the transmitter aerial. The gain pattern (Fig. 2) was integrated by planimeter over 14 contours, after superimposing the solar disk, and the result used for determining the true solar temperature from the observed aerial temperatures. Allowance was made for the atmospheric attenuation over the 930-m path.

The final result obtained was

$$T_{\mathrm{sun}} = 5{,}670 \pm 230^\circ \mathrm{K}$$

The probable error quoted includes individual errors due mainly to 'statistical' error from the least-squares fit, calibration of standard noise sources, standard aerial gain measurement. All other measurements are believed to be better than ± 1 per cent.

A drift curve, produced by locking the aerial and allowing the Sun to drift through the beam, is shown in Fig. 3 (receiver output time constant 16 sec). Theoretical points assuming the solar disk to be of uniform brightness from centre to limb are also shown.

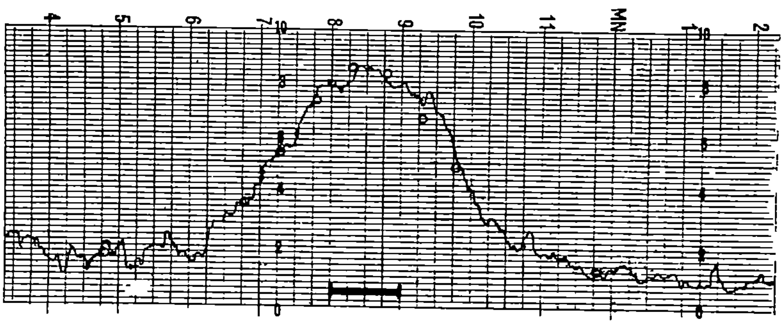

Fig. 3. Drift curve recording

This result may be compared with that reported by Tolbert *et al.*[1] of 5,433° ± 500° K at a wave-length of 2.15 mm.

These results appear to indicate that the Athay radio model of the chromosphere[2] requires some adjustment, in particular that the temperature of the 'cold' component of the chromosphere in the model is some 800° K too high.

I thank members of the Royal Radar Establishment, Malvern, who designed and constructed the radiometer, the N.I.R.N.S., Harwell, the Meteorological Office, and the Culham Laboratory.

D. J. H. Wort

U.K. Atomic Energy Authority,
Culham Laboratory,
Culham,
Nr. Abingdon.

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Polarization in the Central Component of Centaurus *A*

Using the recently completed 210-ft. radio telescope[1,2] of the Australian National Radio Astronomy Observatory at Parkes, New South Wales, we have detected linear polarization of the radio emission from the central component of Centaurus *A*. Many maps of this object have been made[3,4], and more recently Hindman and Wade[5] and Bolton and Clark[6] have shown that it consists of two major extended sources several degrees in extent, together with a central core. The central component, which is closely associated with the galaxy *NGC* 5128, has been further resolved into two parts by Twiss, Carter and Little[7] and Moffet[8] using interferometers and by Little[9] using a fan-beam.

Bracewell[10] found that the fan-beam and existing interferometric data could be accounted for if the two sources were arranged on a diagonal running from north-east to south-west. An alternative disposition of the sources, at opposite ends of the dark band crossing *NGC* 5128, agreed with the equivalent ellipse established by Mills[11] and suggested a three-dimensional distribution in the form of a toroid. However, from the depth of the minimum observed between the peaks, it can be shown that such a toroid would have to be extremely thin (less than about 10″), and Little, Cudaback and Bracewell[12] and Little and Bracewell[13] concluded that it would be a more acceptable configuration if the two peaks were separate, more or less globular volumes. Maltby[14] determined the position angle of the line joining the two sources as 46.5°.

The separation of the two central sources is 7 min. of arc and they are partially resolved by the 6.7 min. beam of the 210-ft. reflector at 10.0 cm. As part of the research programme of the Observatory, studies of the object are being made at all available wave-lengths. Immediately prior to the present observations, equipment for feed rotation had been installed and tested at 10 cm on a number of known polarized and unpolarized sources by Dr. J. A. Roberts.

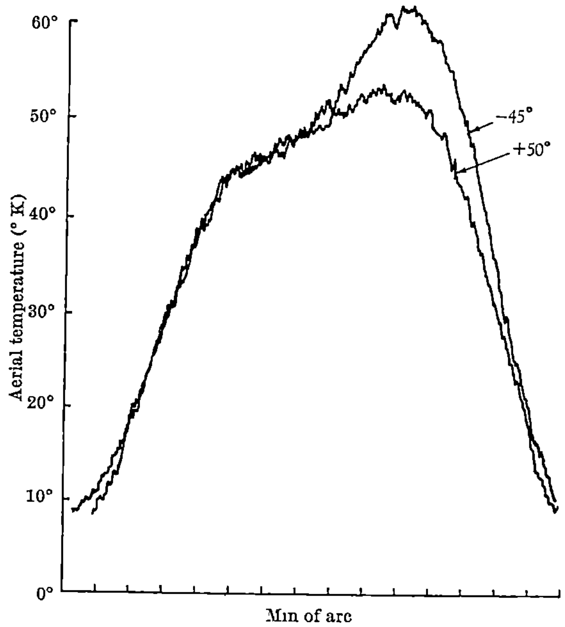

Fig. 1. Diagonal scans from north-east to south-west with polarization position angles of +50° and −45°

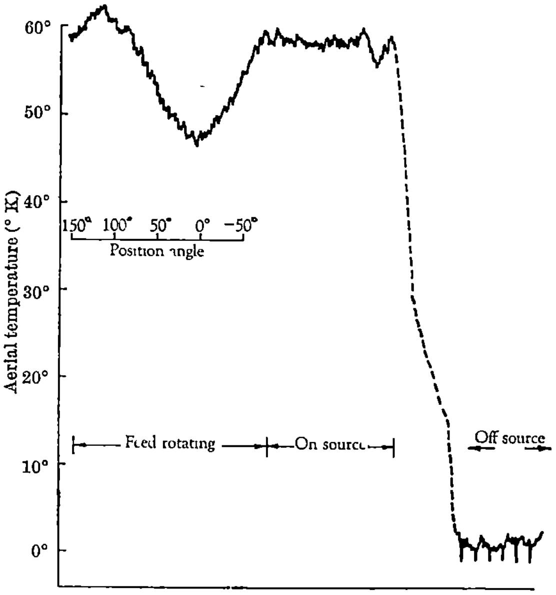

Fig. 2. Record taken near the maximum of the source with rotating feed horn

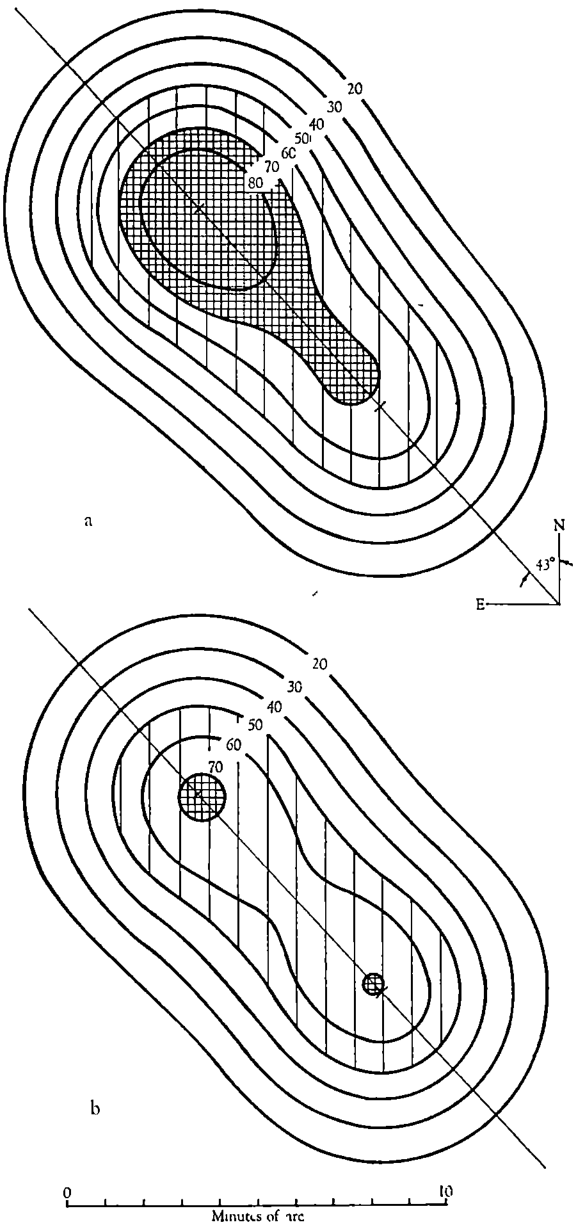

Fig 3 Contour diagrams of Centaurus *A* at 10 0 cm with polarization position angles chosen to exhibit maximum variation of the polarized component The position angle of the electric vector is (*a*) 115°, (*b*) 25° Contour unit, 0 7° K

Fig 1 shows typical scans made diagonally from north-east to south-west with the electric vector of the receiving horn in position angles 50 and − 45° It appears that the south-west component is unaffected by the change in polarization whereas the north-east component is

This conclusion was confirmed by rotating the feed horn through slightly less than 180° while tracking various points of the source An example is shown in Fig 2 This example shows a degree of polarization of 13 per cent, with the electric vector of the feed horn oriented in position angle 115° for maximum reception

Contour diagrams were constructed for position angles 50°, 0°, − 45° and − 90° These results are summarized in Fig 3, which shows the extreme contour diagrams for the position angles of 115° and 25° These diagrams represent our conclusion that the observations are consistent with the presence of two point sources lying on a line in position angle 43°, 7 min of arc apart, the north-east source being 15 per cent polarized in position angle 115 degrees The observations were matched by two gaussian humps of half-widths ~ 6′.7 and peak values of 78 and 66 contour units for the north-east (unpolarized part) and south-west components respectively To the accuracy with which the telescope is presently calibrated (~ 1′ arc) the two sources appear to be symmetrically placed with respect to *NGC* 5128

The degree of polarization is higher than has been observed for other cosmic sources[15], for example, the Crab nebula exhibits 3 per cent polarization at 10 cm and Cygnus *A* and Cassiopeia *A* have less than 1 per cent polarization At 3 cm the Crab nebula shows 7 per cent and Cygnus *A* 8 per cent polarization After allowance for the depolarizing effect of propagation through a medium containing a magnetic field the degree of polarization may be quite high, and it must be concluded that systematic orientation of magnetic field in the region of origin persists over vast volumes Assuming a distance of 4 megaparsecs to Centaurus *A*, and an angular diameter of 1 min of arc for a source, its diameter is about 1 kiloparsec It is apparent that such extensive magnetic fields play an important part in the behaviour of a galaxy and it should become possible to map the field pattern surrounding a galaxy by polarization observations

One of us (R N B) thanks Dr E G Bowen for permission to take part in the observations

Note added in proof Since this communication was written it has come to our notice that C H Mayer, of the Naval Research Laboratory, Washington, D C , has independently detected linear polarization in the central component of Centaurus *A* At 3 15 cm he finds a degree of polarization similar to that reported here

R N Bracewell*
Radio Astronomy Institute,
Stanford University,
Stanford, California

B F C Cooper
T E Cousins
Radiophysics Laboratory,
Commonwealth Scientific and Industrial
Research Organization,
Sydney

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* At present on sabbatical leave at the School of Physics, University of Sydney

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PHYSICS

Interferometric Measurement of Electron Density in *Sceptre IV*

THE density of free electrons in a plasma may be determined from its refractive index If electromagnetic waves are used, the frequency of which is greatly in excess of the plasma frequency and the electron cyclotron frequency, the refractive index is given by[1]:

$$(\mu_p - 1) = -4\ 46 \times 10^{-4}\ \lambda^2 N_e$$

μ_p is the refractive index, λ the wave-length (Å) and N_e the number of electrons per cm^3. Optical interferometer techniques have previously been used to measure electron densities of about 10^{17} cm^{-3} in shock waves[1-3] and of the order of 10^{14} cm^{-3} in radio-frequency discharges[4] This communication describes experiments to determine spatial and temporal variations in the electron density in a hydrogen discharge in *Sceptre IV*[5] over the density range 10^{14}–10^{16} cm^{-3} For the conditions used, the contributions of atoms and ions, both of hydrogen and impurities, to the refractive index can be neglected

The common-path interferometer[6] used for this experiment has several advantages It is easy to set up, requires only a moderately narrow wave-length spread and, as each beam passes twice through the plasma, the fringe shift for any electron density change is twice that in a single pass instrument such as a Mach–Zehnder interferometer No balancing of optical paths is required, and it is possible to compensate for any vibrations of the mechanical parts of the system. Fig 1 shows the optical arrangement, with the reference beam passing through the centre of the discharge tube and the test beam filling the aperture of the window, including two small evacuated tubes which provide information on the changes in density along the reference beam The diameter of the discharge tube bore is 30 cm The concave mirror, fixed on anti-vibration mountings on the iron core linking the torus, reflects the two beams back to the interferometer, which produces a pattern of horizontal fringes within a vertical image of the window aperture The dark zero-order fringe appears at the position at which the reference beam strikes the aperture This image is focused on to the plate of a rotating mirror camera after passing through a Wadsworth monochromator acting as a filter The interferometer was used with a cylindrical beam-splitting lens to make most effective use of the light from a 2 5 kW A E I xenon lamp Light from the discharge was effectively prevented from obscuring the interferogram by the polarization selection of the interferometer, by the monochromator and by means of suitable diaphragms

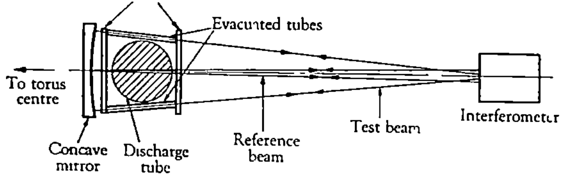

Fig 1 Optical arrangement

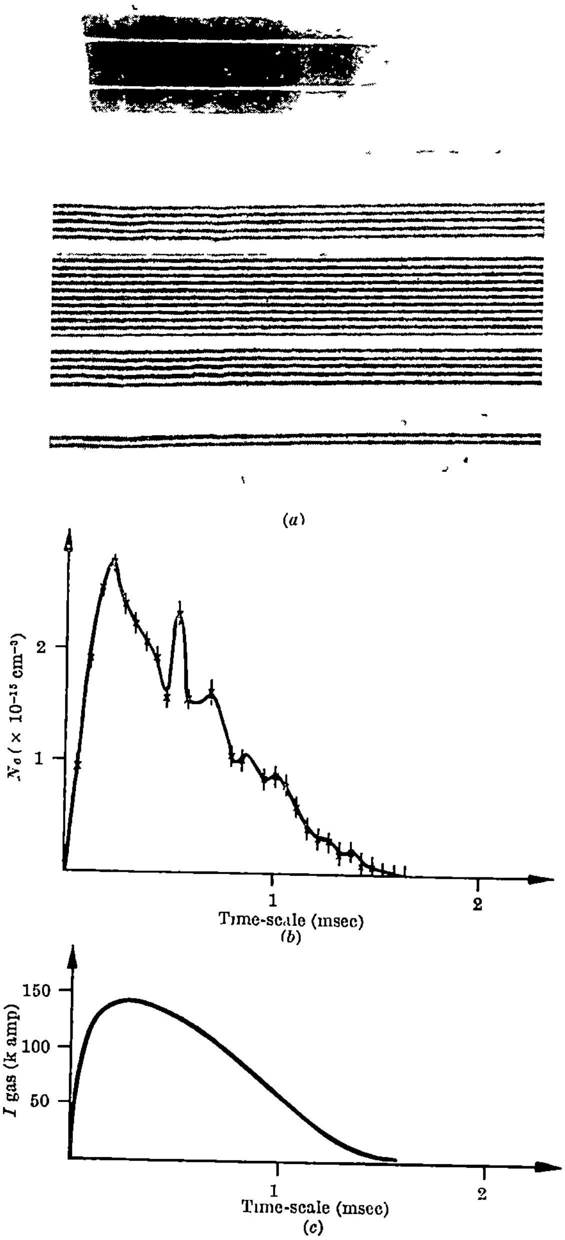

Fig 2 *a*, Rotating mirror photograph and interferogram, *b*, mean electron density along line across the centre of the tube *c*, gas current Discharge conditions $C = 912\ \mu$F, $V_c = 20$ kV, 8 1 turns ratio, 2 4 × 10^{-2} mm mercury of hydrogen, B_φ = 1,500*g*

The discharge light, observed through the same window by a different optical path, was recorded simultaneously on the plate and can be seen (in the form of a negative) above the example interferogram in Fig 2 The clear bands in both the discharge and fringe pictures are caused by copper bars across the window The fringes in the two small tubes can be seen on either side of the main fringe picture The orientation of the photograph is such that both discharge and fringe pictures are inverted A downward fringe shift in the evacuated tubes implies an increase in electron density at the centre of the discharge tube A downward shift at any other point in the interferogram implies an increase in the difference between the density at that point and that at the position of the zero order fringe, that is, a reduction in density The resolution time is about 30 μsec The mean optical wave-length chosen was 6200 Å

The upper curve in Fig 2 shows the mean electron density for the line across the centre of the tube obtained from the fringe shifts in the small tubes, and the lower curve is a current wave-form taken for the same conditions but for a different discharge The interferogram was taken for a discharge with a filling pressure of $2 \cdot 4 \times 10^{-2}$ mm mercury of hydrogen, an initial axial magnetic field of 1,500 g and a peak gas current of 140 k amp The maximum fringe shift is about 0 4 of an order The fluctuations in the density shown in Fig 2 are caused by movements of the region of peak density and by changes in the overall density The errors given in Fig 2 are absolute errors, the difference in density between two adjacent times is more precise and is indicated by the curve drawn through the points

The density peaks correspond closely to strong light emission from the centre of the tube An Abel integration across the tube to find the radial distribution of electron density gives a peak density at the centre of $3 \cdot 8 \times 10^{15}$ cm^{-3} At this time, the number of electrons in the tube was within 10 per cent of the initial number of atoms The maximum density was followed by 'pump-out'[7] and some form of rapid injection when the overall density rises before decaying to less than 10^{14} cm^{-3} in 1 6 msec from the start of the discharge These results, together with similar measurements at initial pressures of down to 2×10^{-3} mm mercury of hydrogen, will be described in a more detailed paper

We thank Dr A A Ware for advice, Messrs D Warren and A Ennos for their assistance in the experiments and Messrs L A Green and A C Richardson for operating *Sceptre IV* This work was supported in part by a U K Atomic Energy Authority contract We also thank Dr T E Allibone, director of the Laboratory, for permission to publish this communication

J DYSON
R V WILLIAMS
K M YOUNG

Associated Electrical Industries, Ltd ,
Aldermaston Court,
Aldermaston, Berks

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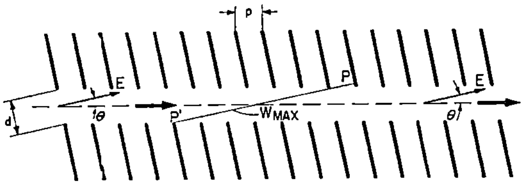
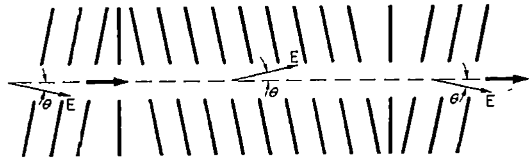
High-Voltage Acceleration Tubes utilizing Inclined-Field Principles

THE 'total voltage effect', which limits the voltage across an evacuated tube in a manner distinct from that of ordinary insulation, generally prevents a linear increase in voltage with an increase in tube-length for fixed voltage gradient along the tube Thus, although a tube designed for operation at 3 million volts may work very well, a similar tube twice as long does not usually satisfactorily insulate 6 million volts, even though the voltage gradient remains the same This breakdown, the precise nature of which is still obscure, is caused by the regenerative multiplication of secondary particles initiated within the tube by various causes such as cosmic rays, the primary beam, and field emission

This communication describes the performance of three large-aperture high-voltage tubes which have been developed to avoid the total voltage effect This was accomplished by inclining the central plane of the electrodes to the axis of the tube, thereby reducing the length of the electric lines of force to only a fraction of the insulating length of the tube (Fig 1*a*) It is desirable that secondary particles, particularly electrons, produced within the tube be limited to a maximum energy which is as low as feasible Since it may be considered that the electric field is uniform and that secondary particles are liberated with negligible energy, Fig 1*a* shows that a secondary particle attaining maximum energy is one liberated at point *P* on one electrode so that it barely misses interception on the adjacent electrode The angle of inclination, θ, and dimension, *d*, of the tube opening in the direction of inclination can be so chosen that this particle will barely be intercepted by another electrode



a NEUTRAL BEAM TUBE



b CHARGED PARTICLE ACCELERATION TUBE

Fig 1 Cross-sections of the central regions of two different inclined-field tubes showing the geometrical arrangements of the plane electrodes

The maximum kinetic energy acquired by a secondary particle will then be

$$W_{\max} = \left(p + \frac{d}{\sin\theta}\right) eE$$

where p is the axial inter-electrode spacing and E is the axial voltage gradient For a representative tube test $W_{\max}$ was calculated to be 360 keV when the total tube voltage was 5,760 kV As the expression is not affected by the aperture dimension perpendicular to the plane of the sections shown in Fig 1, slotted electrodes were used to increase the aperture area and the conductance of the tube

In this simple form a tube was successfully used to provide passage for an intense neutral beam However, this tube is evidently unsuitable for the acceleration of charged particles But, by alternating the direction of the inclined field in a calculated manner as illustrated in Fig 1*b*, an injected charged particle beam was accelerated through the modified tubes along a path which was very nearly axial Secondary particles still travel along the lines of force and are removed from the tube before they can enter a section of opposite inclination, except those formed near an intersection which may have a somewhat longer path It is worth noting that the operation of the inclined field tube depends upon the charge of the primary beam and not on the particle mass and therefore such tubes may also be used to accelerate an injected electron beam

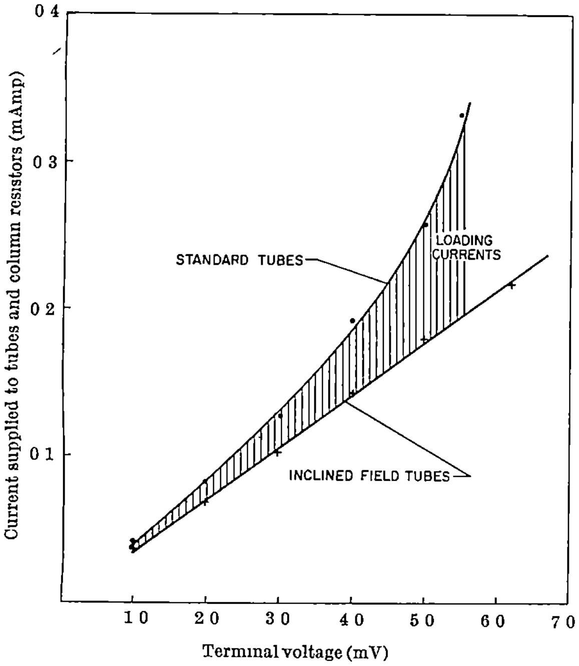


Fig 2 A comparison between the performance of standard and inclined-field tubes The shaded region shows the loading currents associated with standard tubes

The immediate removal of the secondary particles has various advantages Previously, even with the greatly improved acceleration tubes having electrodes tapered in diameter of aperture, which were developed by Prof J G Trump *et al* , many secondary electrons were accelerated along the total length of the tube Due to their high energy, these electrons produce undesirable and penetrating radiation On the other hand, the maximum energy of the X-radiation spectrum from the inclined field tubes was shown, by scintillation counter measurements, to be in close agreement with the calculated shortening of the secondary particle trajectories In addition, when standard acceleration tubes are used, the current drain due either directly or indirectly to the secondary particles must be compensated by an increase in the output current of the power supply maintaining the voltage across the tube This phenomenon, often known as loading, has generally limited the maximum current capability of electrostatic accelerators at the high end of the voltage-range The shaded region in Fig 2 shows, for standard tubes in a tandem accelerator, the rapid increase in regenerative loading with terminal voltage The inclined-field tubes show negligible loading as evidenced by the linearity of the lower curve, which is the effect of the column resistor current and would occur without the presence of the tubes In both cases the same beam current was accelerated

By using two of the inclined-field tubes each with an insulating length of 12 ft in a tandem accelerator, an analysed proton beam of 3 9 µamp was attained at 15 6 MeV. The background radiation from the machine was not only less penetrating than with conventional tubes, but also was reduced in intensity by a factor of about a thousand Maximum voltage could be applied immediately without the usual tube conditioning time No tube sparks were observed and the upper voltage limit was determined by factors other than the acceleration tubes

Since these tests show that the limitation in beam current due to regenerative side-effects was eliminated, it may be expected that far higher currents can now be accelerated In addition, the effective elimination of the total voltage effect makes it possible to increase maximum tube voltage linearly with tube-length The development of the inclined-field tube is continuing and more detailed results are in preparation for a subsequent publication

R J Van de Graaff
P H Rose
A B Wittkower
High Voltage Engineering Corporation,
Burlington, Massachusetts

Direct Observation of Detonation in Compressed Nitromethane

Johansson and Sternhoff[1] have shown that the detonation light from some TNT-based explosives is suppressed under pressure from an externally produced shock wave We have observed similar behaviour in nitromethane where, in this case, the homogeneous liquid can be considered to be free of voids which may influence the emitted light A somewhat more indirect experiment has been described[2], wherein a detonating nitromethane charge was observed normal to the open end With proper under-boostering, weak light was observed which, after a time, suddenly changed to the normal detonation intensity The weak light was attributed to detonation of material compressed by the initiating shock wave

In the experiments reported here, the nitromethane was contained in an aluminium box having front and back sides of 1 5-in thick 'Plexiglas' and was loaded just prior to firing in order to minimize the solvent effect of nitromethane on plastic Under these conditions the 'Plexiglas' remains quite transparent until free surface motion begins[3] The detonation event was observed through the 'Plexiglas' by a Cordin framing camera at exposure times of 0 25 µsec duration

The experimental assembly is illustrated in Fig 1 The initiating explosive was 'Composition *C*-3'

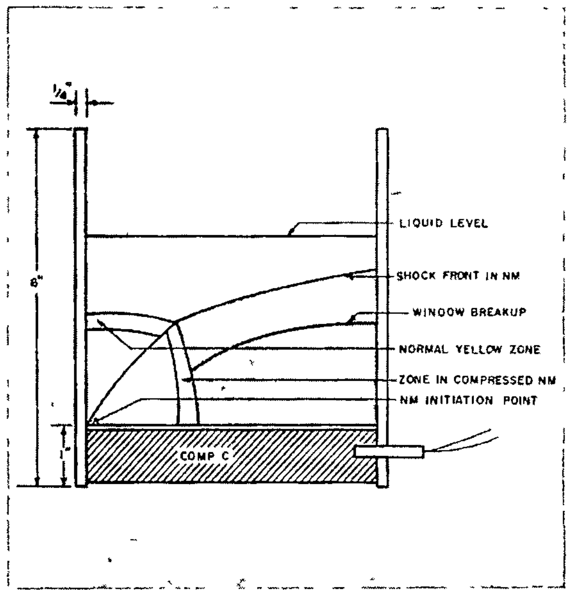


Fig 1 Diagram of experimental arrangement

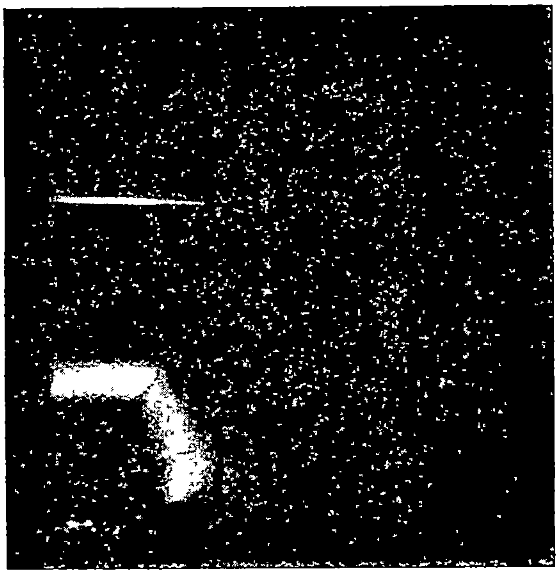


Fig 2 Detonation through normal (top) and shocked nitromethane

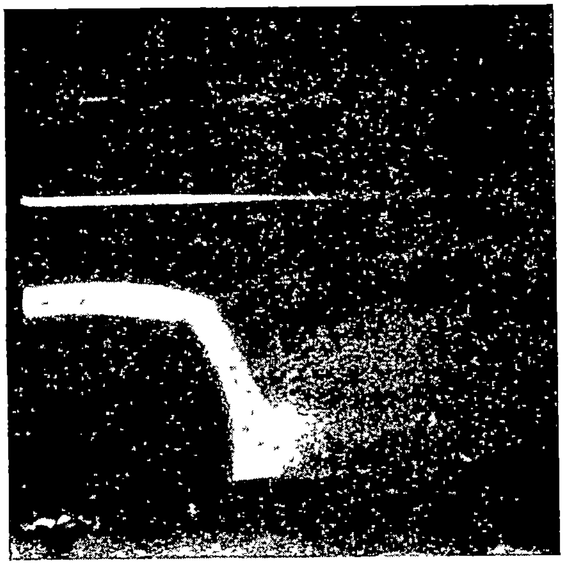


Fig 3 Interaction between detonation wave fronts in nitromethane

(detonation rate 7,600 m/sec), which drove a wiping shock wave into the nitromethane through the 1/8-in aluminium tank floor This shock was insufficient to initiate nitromethane directly but did so after reflexion from the opposite corner of the tank

The event was recorded on Eastman 'H S Ektachrome' film The film showed the normal bright yellow detonation proceeding upward into the unshocked liquid and a dim yellow, right-going, zone in the compressed explosive which maintained its identity throughout the sequence The experiment was reproducible Although the zones were easily distinguishable on colour film the brightness differences were not great and show to less advantage in black-and-white. An early frame is shown in Fig. 2 Fig 3, from the same sequence, was contrast-controlled during printing to show details of the window break-up and the interaction line behind the wave fronts The interaction line remained visible for some time and was, perhaps, etched into the 'Plexiglas' window Campbell *et al* [2] postulate a super-velocity detonation through the compressed region to explain the first dim light which they observed In view of these experiments, it seems reasonable to attribute weak detonation light to the reaction of compressed material Although there is some tendency for the wave in the compressed explosive to pull away from the normal front, it is not pronounced Compression to higher densities would be expected to increase that tendency

The photographs shown were taken without external illumination Similar experiments, which were illuminated with diffused backlight from an argon flash bomb, showed the compressed but undetonated nitromethane to be transparent while everywhere behind the two detonation fronts a black, opaque, reaction product cloud had formed We believe the opacity was due to free carbon from this oxygen-deficient explosive rather than opaquing of the 'Plexiglas' for two reasons (1) explosives having transparent reaction products have been used and in these cases the 'Plexiglas' windows transmitted backlight, (2) experiments with front-lighted nitromethane tanks, having white grid lines painted on the inside front surface of the 'Plexiglas' window, have shown that such grid lines remained clearly visible after passage of the detonation wave The lines remained visible until break-up of the window occurred.

H Dean Mallory
R A Plauson

Chemistry Division,
U S Naval Ordnance Test Station,
China Lake, California

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RADIATION CHEMISTRY

The Effect of *p*H on the Radical Yields in the γ-Radiolysis of Aqueous Systems

It is customary to describe the state of affairs about 10^{-8} sec after the passage of a fast electron traversing a liquid aqueous medium by equation (1) ·

$$G_{\text{-H}_2\text{O}}\text{H}_2\text{O} \rightsquigarrow G_{\text{'H'}}\,\text{'H'} + G_{\text{OH}}\,\text{OH} + G_{\text{H}_2}\,\text{H}_2 + G_{\text{H}_2\text{O}_2}\,\text{H}_2\text{O}_2 \quad (1)$$

in which $G_{\text{'H'}}$ and G_{OH} are the numbers of H atoms and OH radicals respectively which are available to react with solutes for each 100 eV energy deposited in the water Recent work[1,2] has shown that the precursor of the hydrogen atom is a solvated electron, e^-_{aq}, capable of reaction in this form with solutes such as N_2O[3], $ClCH_2COOH$[4], O_2[2], H_2O_2[2], etc The solvated electron is also able to react with hydrogen ions according to equation (2), when it is converted into a hydrogen atom

$$\text{H}^+_{\text{aq}} + e^-_{\text{aq}} \longrightarrow \text{H}_{\text{aq}} \quad (2)$$

Consequently, as the *p*H of a solution is reduced a solute has less chance of reaction with e^-_{aq} and more of reacting with H It is also known[3] that reduction of the *p*H below ~ 3 causes the production in equal yield of two additional entities, stoichiometrically equivalent to H and OH and tentatively identified as H_2^+ and OH The fact that the molecular yields,

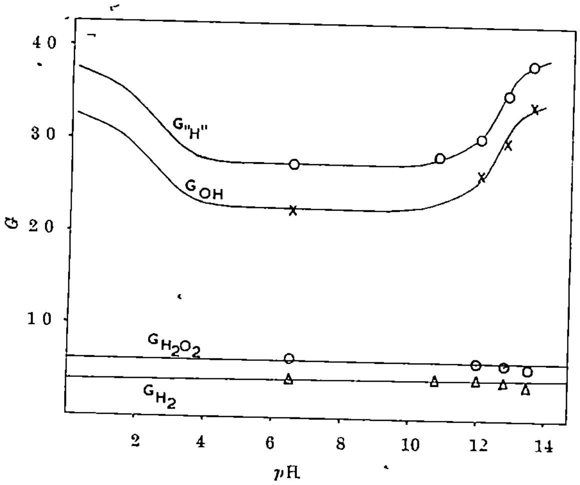

Fig 1 Variations with pH of $G_{'H'}$, G_{OH}, G_{H_2} and $G_{H_2O_2}$ for γ-irradiated liquid aqueous systems Lines at $p\text{H} < 6$ based on many previous investigations (see Fig 9, ref 3) Lines at $p\text{H} > 6$ based on the $Fe(CN)_6{}^{3-}$ — $Fe(CN)_6{}^{4-}$ — N_2O system described here and to which the experimental points refer

G_{H_2} and $G_{H_2O_2}$ do not diminish as the pH is reduced below 3, indicates that these entities are formed by the attack of the acid on some intermediate which otherwise (that is, at $p\text{H} > 3$) reverts to water, and the kinetics[3] indicate that the reversion is first order in intermediate concentration This intermediate may be either an excited water molecule, H_2O^*, or an isolated radical pair in a solvent cage, denoted by (H + OH) Its reaction with the acid is then represented by either equation (3) or (4)

$$H_2O^* + H^+_{aq} \longrightarrow H^+_{2\,aq} + OH_{aq} \qquad (3)$$

$$(H + OH) + H^+_{aq} \longrightarrow H^+_{2aq} + OH_{aq} \qquad (4)$$

Consequently, the conventional hydrogen atom yield $G_{'H'}$ is the composite quantity, $G_{e^-} + G_H + G_{H_2^+}$ The dependence of $G_{'H'}$ G_{OH}, G_{H_2} and $G_{H_2O_2}$ on pH below pH 7 is shown in the left hand part of Fig 1

$$H_2O^* + OH^-_{aq} \longrightarrow e^-_{aq} + OH_{aq} \qquad (5)$$

$$(H + OH) + OH^-_{aq} \longrightarrow e^-_{aq} + OH_{aq} \qquad (6)$$

We may envisage the reactions (5) and (6), which are the basic analogues of reactions (3) and (4), and we are therefore led to expect a perceptible increase of the conventional yields as the pH is increased above about 11 Satisfactory solutes for use in alkaline solutions are rare and we have therefore tackled this problem in the following manner First, we have shown that when acrylamide is used as a solute $G(H_2) = G_{H_2}$ and $G(H_2O_2) = G_{H_2O_2}$ undergo no sudden changes between pH 0 and 13 Secondly, we have shown that the only reactions occurring at $p\text{H} > 7$ in deaerated aqueous solutions containing potassium ferrocyanide, potassium ferricyanide and varying amounts of nitrous oxide are

$$Fe(CN)_6{}^{3-} + e^-_{aq} \longrightarrow Fe(CN)_6{}^{4-} \qquad (7)$$

$$N_2O + e^-_{aq} \longrightarrow N_2 + O^-(\rightarrow OH) \qquad (8)$$

$$OH + Fe(CN)_6{}^{4-} \longrightarrow OH^- + Fe(CN)_6{}^{3-} \qquad (9)$$

$$H_2O_2 + 2Fe(CN)_6{}^{3-} \longrightarrow 2H^+ + O_2 + 2Fe(CN)_6{}^{4-} \qquad (10)$$

Hence, for this system, we obtain the radical and molecular yields from equations (11)–(14) inclusive, in which the superscript, $- N_2O$, means no N_2O present and the superscript, $+ N_2O$, means that the N_2O concentration is sufficiently large for $k_8[N_2O] \gg k_7[Fe(CN)_6{}^{3-}]$.

$$G_{H_2O_2} = G(O_2) \qquad (11)$$

$$G_{H_2} = G(H_2) \qquad (12)$$

$$G_{'H'} = G(N_2)^{+N_2O} \text{ or } \{G(\text{ferri})^{+N_2O} - G(\text{ferri})^{-N_2O}\}/2 \qquad (13)$$

$$G_{OH} = G_{'H'} + 2\,G_{H_2} - 2G_{H_2O_2} = \{G(\text{ferri})^{+N_2O} + G(\text{ferric})^{-N_2O} + 4\,G(O_2)\}/2 \qquad (14)$$

The values of these yields are shown in the right-hand half of Fig 1, and their variation with pH is in accord with the proposed existence of reaction (5) or (6) At $p\text{H} \leqslant 6$ reaction (10) is replaced by the relatively slow reaction (15)

$$2\,Fe(CN)_6{}^{4-} + H_2O_2 \rightarrow 2\,Fe(CN)_6{}^{3-} + 2\,OH^- \qquad (15)$$

so that the measurement of the post-irradiation reaction and of the effect of N_2O on G(ferri) can be used to determine the radical and molecular yields The values at $p\text{H} = 6$ are shown in Fig 1

Two other pieces of evidence support this hypothesis Thus, Dainton and Peterson[3] showed that $G(N_2)$ from γ-irradiated aqueous solutions of N_2O undergoes an increase of about one unit as the pH is increased above 11 This was attributed to ionic dissociation of the OH radical, but this explanation is less free from objection than the simple assumption of the occurrence of either reaction (5) or reaction (6) Again, the existence of the acid-base reaction (16), which is closely related to reactions (5) and (6) and was first proposed by Baxendale and Hughes[5], has recently been unequivocally demonstrated[6]

$$H_{aq} + OH^-_{aq} \longrightarrow e^-_{aq} \qquad (16)$$

If H^+ and OH^- ions can each react with H_2O^* or (H + OH) it is to be expected that other solutes will exist which also have this property In fact aqueous solutions of some solutes at a pH within the range $3 < p\text{H} < 11$ on irradiation give a single product which seems to be derived from reaction of the solute with e^-_{aq} alone, while solutions of other solutes give products seemingly derived from reactions with both e^-_{aq} and H This situation is readily explicable A solute, such as nitrous oxide, may react rapidly with e^-_{aq} but only slowly with (H + OH) or H_2O^* and will therefore 'see' only e^-_{aq} Another solute may react rapidly with (H + OH) or H_2O^* as well as with e^-_{aq} but in reacting with the former generates a product which is converted back to the original reagent by the remaining radical of the radical pair Examples of this may be oxygen and $Fe(CN)_6{}^{3-\text{and}4-}$ when the relevant fast reactions are

$$H_2O^* \text{ or } (H + OH) + O_2 \rightarrow HO_2 + HO \rightarrow H_2O + O_2$$

$$H_2O^* \text{ or } (H + OH) + Fe(CN)_6^{3-} \rightarrow H_2O^+ \text{ or } H^+ + OH + Fe(CN)_6^{4-} \rightarrow H_2O + Fe(CN)_6{}^-$$

$$H_2O^* \text{ or } (H + OH) + Fe(CN)_6{}^{-4} \rightarrow H + OH^- + Fe(CN)_6{}^{-3} \rightarrow H_2O + Fe(CN)_6{}^{-4}$$

The solute in this case may be regarded as a quencher of H_2O^* or as a catalyst of H + OH recombination but in any event will only 'see' e^-_{aq} or OH There are yet other solutes which can react quickly with e^-_{aq} to form one product and with H_2O^* or (H + OH) to form another which is resistant to attack by the conjugate radical These will 'see' both e^-_{aq} and H_2O^* or (H + OH) An example is Cl CH_2COOH, which reacts with e^-_{aq} to form Cl^- ($G(Cl^-)$ at $4 < p\text{H} < 7 = \sim 3$) and with H_2O^* or (H + OH) to form H_2($G(H_2)$ at $4 < p\text{H} < 7 = \sim 1$)[4,7], which reacts only slowly with OH Such solutes should compete with OH^- ions for H_2O^* or (H + OH) and accordingly we expect that for several organic solutes $G(H_2)$ should decrease markedly around pH 11 and G (product derived from e_{aq}^-) show a complementary increase

F S Dainton
W S Watt

School of Chemistry,
University of Leeds

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CHEMISTRY

Complex Formation of Monomeric Amides with Lithium Perchlorate

It has been demonstrated that aqueous solutions of lithium bromide can induce the disruption of the ordered portions of fibrous and globular proteins[1-4] Since this phenomenon is observed for proteins of widely differing secondary and crystallographic structure and also amino-acid composition, the conclusion was reached that an interaction with the peptide linkage was involved The nature of the melting temperature–LiBr concentration curve for both α and β keratin[1,2] suggested that, in addition, an alteration in the nature of the peptide bond occurs

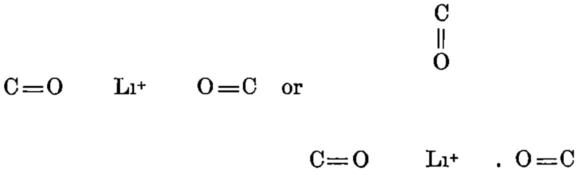
Lithium bromide and similar salts are known to form complexes with simpler compounds containing some of the characteristic features of the peptide groups[5-9] In particular, complexes of the general formula $LiX\ nCO(NH_2)_2$ with $n = 1$, 2, and 3 have been established for aqueous solutions of lithium bromide or lithium chloride with urea[7,8] A similar situation has also been demonstrated for aqueous solutions of lithium bromide with either *N*-methylacetamide or dimethylacetamide[9] Contrary explanations have been offered, and the interpretation of the results is complicated by the necessity of having a third component, water, present in the systems previously selected for study Since the melting of the fibrous proteins can also be demonstrated in non-aqueous solutions of lithium bromide[10], the examination of model compounds to elucidate the nature of the interaction is best accomplished by the elimination of water from the system

To this end we have investigated, by means of an infra-red spectroscopic technique, the interaction of anhydrous lithium perchlorate with *N* - methylpropionamide (NMP) Solutions of the anhydrous salt and NMP were prepared in a dry nitrogen atmosphere, the molar ratio of amide to lithium employed was approximately 3 1 This system, thus, possesses the advantage that adequate solubility of the salt in the amide can be obtained without the intervention of water

The spectral data for the pure amide and the solution are illustrated in Fig 1 A strong interaction between the lithium salt and the amide group is indicated Lithium bromide was found to give essentially the same results, so the effect can be attributed largely to the lithium ion The amide absorption band at 1 645 cm^{-1} attributed to C=O stretch (amide I) shows a marked change on addition of lithium perchlorate to the pure amide This carbonyl frequency is lowered in wave number, the single band observed with the pure amide (1,645 cm^{-1}) becomes a doublet (1,645 cm^{-1} and 1,620 cm^{-1}) The amide absorption band at 1,550 cm^{-1} attributed to N—H deformation coupled with C=N stretch (amide II) (ref 11), and its accompanying weaker absorption band (amide III), are slightly changed on addition of lithium perchlorate to the pure amide

The N—H stretching mode at 3,300 cm^{-1} of pure NMP is also significantly altered on addition of lithium perchlorate The strong band at 3,300 cm^{-1} is completely replaced by a strong band at 3,400 cm^{-1}, representing a change toward the higher frequency by 100 cm^{-1} In addition, the weaker NH vibrational band observed in the pure amide at 3,100 cm^{-1} becomes of even weaker intensity, almost to disappearance, in the lithium perchlorate–amide mixture

These results are strikingly similar to those obtained by Gerrard, Lappert, Pyzora and Wallis[12] in their investigation of the spectra of simple amides and the complexes that are formed with boron trihalides The results reported here can be interpreted in a similar manner The results suggest that the lithium ion interacts with the carbonyl group directly, rather than with the nitrogen atoms previously suggested for the urea complexes of lithium chloride[7] The splitting of the carbonyl frequency may be attributed to lithium to carbonyl bonding, the lithium ion being bonded perhaps to more than one carbonyl group



$$C{=}O \quad Li^+ \quad \overset{\overset{\displaystyle C}{\|}}{O}{=}C \quad \text{or} \qquad C{=}O \quad Li^+ \quad . \ O{=}C$$

A somewhat similar conclusion has recently been reached by Bello and Bello[13] from a preliminary

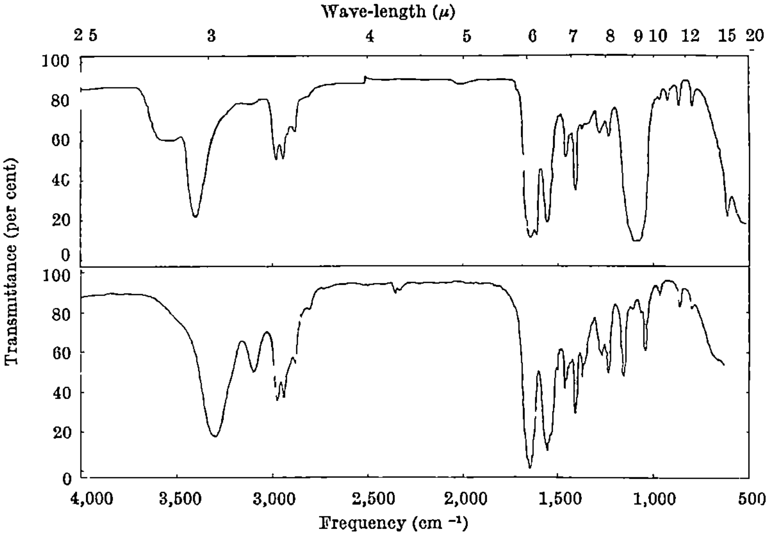


Fig 1 Upper curve, infra-red spectrum of pure *N*-methylpropionamide, lower curve, infra-red spectrum of anhydrous lithium perchlorate-*N*-methylpropionamide solution

structural analysis of the crystals precipitated from an aqueous solution of *N*-methyl acetamide and lithium bromide The formation of the polymeric forms of the amide would be consistent with the extremely high viscosity observed in the solutions[14] The disruption of the hydrogen-bonded network of the pure liquid amide, upon the introduction of the lithium ion, is consistent with the characteristic shift observed in the N—H stretching mode At the concentration of lithium perchlorate used, there is no spectral evidence of any residual interamide hydrogen bonds, indicating that essentially all the amide groups are bonded by means of the lithium ion

Studies in which small amounts of water were deliberately added to the system show that the splitting of the carbonyl band is observed only when the NMP–lithium perchlorate mixture is essentially free of water, as indicated by a very weak band at 3,600 cm^{-1} (O—H stretch) The presence of water will allow the lithium ion to form its hydration shell and thus reduce the effective concentration of lithium ion available for binding with the amide These results indicate that the disruption of the ordered structure observed in protein systems could very well be a result of a similar binding phenomenon not necessarily involving the water present

We thank Dr W G Bordum for supplying *N*-methylpropionamide, and Dr G Atkinson for helpful discussions

A F DIORIO

Macromolecules Section,
National Bureau of Standards,
Washington, D C

E LIPPINCOTT

Department of Chemistry,
University of Maryland,
College Park, Md

L MANDELKERN

Department of Chemistry,
The Florida State University,
Tallahassee, Florida

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Differential Thermal Analysis of Potassium Perchlorate

Gordon and Campbell published a paper[1] in which the differential thermal analysis of potassium perchlorate is illustrated from the point of view of the characterization of this compound rather than the details of its decomposition In this work, where the differential thermal analysis was carried out in a test-tube in an open furnace, a large endotherm following the initial exotherm after fusion was observed Later, Markowitz and Boryta[2] became interested in the details of decomposition as revealed by differential thermal analysis and pointed out that this endotherm is due to the molten sample rising up the sides of the tube resulting in separation of the sample from the thermocouple This gives rise to an apparent endotherm at that point These authors attempted to overcome the thermal effects which accompany the separation of the sample from the thermocouple by carrying out the thermal analysis in a closed furnace system Although the endotherm in question is not observed under these conditions, the details of the decomposition are missed, undoubtedly due to the large mass of sample used in these experiments and because of poor heat transfer conditions in the closed furnace system The approach in conducting the differential thermal analysis experiments in the present work is to adjust the sample mass and dimensions of the sample container so that the decomposing gases will not force the reacting mass up the tube This results in intimate contact of the sample with the thermocouple throughout the entire reaction, which is after all the best condition for the differential thermal analysis experiment It was found that when 500 mg and less of potassium perchlorate are contained in test-tubes with a ninside diameter of 16 mm separation of the sample from the thermocouple does not occur These experiments show that it is possible to obtain detailed differential thermal analysis curves for decomposition reactions which are accompanied by violent foaming and bubbling by adjusting sample weight and geometric experimental parameters In this work the detailed differential thermal analysis curve for the decomposition of potassium perchlorate is presented for the first time, which reveals two exothermal processes one during decomposition prior to the precipitation of potassium chloride and the other which occurs simultaneously with the precipitation of potassium chloride This was determined in differential thermal analysis experiments carried out in a metal block previously described[1] where provisions were made for observing the sample as it was heated

Fig 1 shows the results of a combination of differential thermal analysis and thermogravimetric analysis carried out simultaneously on an 86 mg sample of potassium perchlorate The sample mass used in these experiments is over 50 times less than that used by the referenced authors This curve is essentially identical with the differential thermal analysis curves obtained with 500-mg samples using the furnace block The differential thermal analysis curve shows an endotherm due to melting with a peak at approximately 606° After melting the reaction becomes exothermal The exothermicity decreases as the temperature approaches approximately 638° At 638° a sharp rise in exothermicity occurs with a peak at 641° followed by the rapid decay of the exotherm The thermogravimetric analysis curve shows that weight loss due to decomposition occurs simultaneously with the endotherm for melting and is complete at the peak of the final exotherm The thermogravimetric analysis curve shows a single, smooth, sigmoidal-shaped weight-loss curve with no inflexions corresponding to the thermal changes which occur during the reaction Decomposition occurs throughout melting as well as the exothermal regions of reaction Prior to the final exothermal band at 638° the samples underwent 67 per cent decomposition Observations made in separate experiments show that after melting and during the first exothermal step of decomposition following melting the decomposing mass is clear

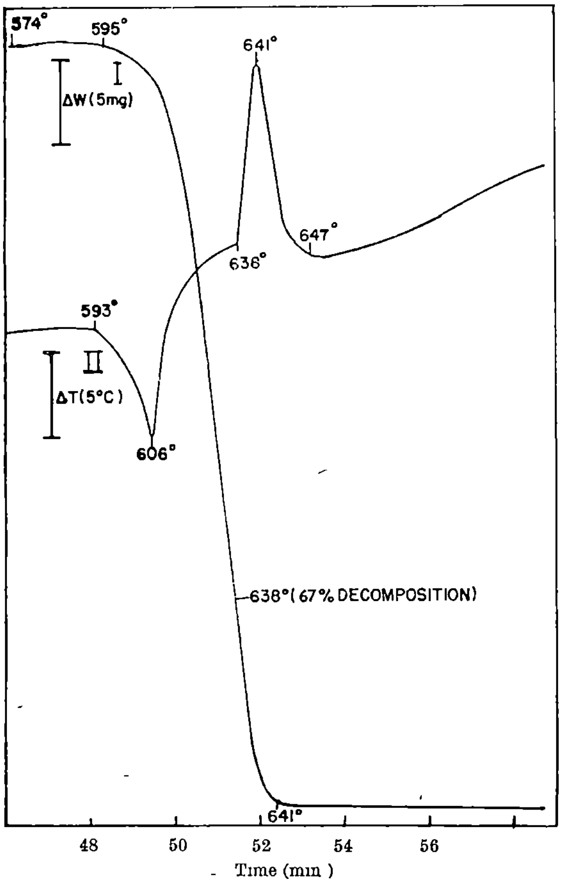

Fig 1 Simultaneous thermogravimetric analysis (I) and differential thermal analysis (II) curves for an 86-mg sample of potassium perchlorate heated in air at a rate of 10° C/min

The final exothermal band is observed to occur during the precipitation of potassium chloride Since the net decomposition of potassium perchlorate to form chloride liquid and oxygen is reported[2] to be endothermal the fact that an exothermal reaction occurs prior to 638° in the clear molten mass remains to be explained This is not easy since in spite of the fact that the decomposition of potassium perchlorate has been studied the mechanism of reaction is not well understood[3–5] The initial exotherm to 638°, however, is evidence that decomposition to KCl (liquid) is actually exothermic contrary to the previous belief that this reaction should be endothermal[2]

There were two series of differential thermal analysis experiments In the first series the apparatus which was previously described[1] consisted of a metal block in which test-tubes containing the samples and reference materials are located The metal block was heated at a rate of 10° C/min and the differential temperature vs sample temperature was recorded on a Moseley autograph, model 3 *X-Y* recorder A West Gardsman proportional controller was used to monitor the rate of heating In these experiments bare 28-gauge chromel–alumel thermocouples were located in the sample and reference materials The sample weight was approximately 500 mg and in both series of experiments doubly recrystallized Fisher scientific reagent grade potassium perchlorate was used The second series of experiments involved obtaining both the differential thermal analysis and thermogravimetric analysis curves simultaneously on about 86 mg of potassium perchlorate The apparatus used consisted of an analytical balance which was adapted for automatic recording by means of a linear differential transformer A rod which is supported on one end of the balance beam and which is in the vertical position above the balance is used to support sample and reference materials, which were contained in 16-mm test-tubes, approximately 1½ in long The sample and reference materials were mounted, one above the other Chromel–alumel, 38-gauge thermocouples were placed in both the sample and reference compounds The furnace was lowered over the test-tubes Changes in weight and differential temperature were recorded on a Bristol 2-pen time base recorder while sample temperature was simultaneously recorded on a Brown strip chart recorder The heating rate was programmed at 10° C/min In all cases replicate experiments were conducted

David A Anderson
Eli S Freeman
Pyrotechnics Chemical Research Laboratory,
Picatinny Arsenal,
Dover, New Jersey

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Gelatin as a Protective Colloid

It is well known that a solution of gelatin restrains the precipitation of most insoluble substances It has also been shown, in a study of the Liesegang phenomenon[1], that the gelatin composing a set gel exerts little or no restraint beyond eliminating mechanical mixing The experiments reported here suggest that gelatin solutions lose much of their restraining power before they set to gels

Two pure, inactive photographic gelatins were used, of which I had a shorter setting time and higher gel strength than II 200 ml of a 2½ per cent solution of gelatin containing 0 087 M potassium bromide was taken, and 2 ml of 0 300 M silver nitrate added to it (leaving an excess of 10 0 g/l potassium bromide) The solutions were stirred throughout and the growth of turbidity was followed by withdrawing samples at intervals and measuring the optical density to red light with a 'Spekker' photometer The lower the temperature the more slowly did the turbidity increase Below about 28°, however, a proportion of the silver bromide was precipitated immediately, as fairly large crystals, although the subsequent growth of density appeared normal Moreover, if the solutions were mixed at 43° C and then cooled to 26° C, the rate of growth of density was smaller than at high temperatures, as would be expected The lower the temperature of mixing, the more silver bromide was precipitated immediately so that the time needed to reach a fixed density fell rapidly This is shown in Fig 1, where the logarithm of the time needed to reach a density of 0 5 is plotted against the temperature of precipitation Gelatin II behaved similarly except that the break occurred at a slightly lower temperature A similar loss of restraining power was observed when potassium bromide was added to a large excess of silver nitrate in gelatin The phenomenon was not related to the onset of gel formation since a solution of 0 2 per cent of gelatin I also behaved in the same way (Fig 1), although it was too dilute to form a gel, even at 1° C

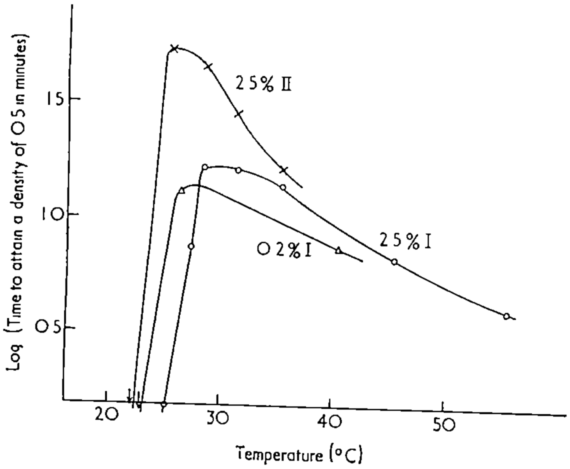

Fig. 1. Logarithm of the time needed to attain a density of 0·5 as a function of temperature

The break-down in protection is probably associated with the formation of aggregates of gelatin molecules, which Boedtker and Doty[2] showed to form in this temperature-range, even when the solution was too dilute to gel. Presumably the aggregates are unable to adsorb on the freshly forming precipitate quickly enough to afford protection in the initial stages of precipitation. Once adsorbed, however, they afford colloid protection and the later stages of ripening appear to be normal.

P. J. Hillson

Research Laboratories,
Kodak Limited,
Wealdstone, Harrow, Middlesex

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Optical Properties and Structure of Crazes in Transparent Glassy Polymers

It has been known for about a year that a large fraction of what appear to be planar reflective cracks in transparent polymers such as polystyrene, polymethyl methacrylate, and polycarbonate are not true cracks at all but rather are thin, abruptly bounded regions of material having different optical properties from the bulk polymer[1]. This material connects to the surrounding bulk polymer so that the pseudo-crack (henceforth termed a craze) will bear a tensile load in contrast to the true crack. Furthermore, such things as growth-rates under stress and ability to 'heal' under heat are quite different for the craze and the crack. Nevertheless, crazing produces stress heterogeneities, and for this reason at least is a precursor of cracking and fracture.

For reasons such as these it is obviously of importance to be able to differentiate between crazes and cracks and to know the details of craze structure and properties. Recently a way has been found here to measure quickly, conveniently and non-destructively the refractive index of the crack or craze *in situ*. From the index it is possible to differentiate between cracks and crazes and to obtain a great deal of information about the latter.

The method of index determination (Fig. 1) involves measuring the critical incident angle of a parallel light beam on the polymer specimen surface for which total internal reflexion occurs at the interface between the craze or crack and the bulk polymer. The beam, upon entering the specimen, is refracted to a new angle which is calculable knowing the refractive index of the bulk material. That portion of the beam which strikes the craze interface either is transmitted and passes on out the back side of the specimen or suffers total reflexion, whereupon a shadow of the craze appears on the back side. With the various angles involved and the bulk polymer index known, the craze or crack index is calculable from Snell's law of refraction. It is easily shown from Snell's law also that the calculations are not affected by the possible presence of thin layers of material of different index from the bulk either at the specimen surface or at the craze-bulk interface. (Such layers, for example, could arise from orientation or swelling effects.) It should also be stated here that for cracks or crazes of low index the incident angle on the craze-bulk interface cannot be made large enough with the set-up in Fig. 1 for light to be passed by the craze, due to the geometry of the system. This limitation was overcome by affixing 30° polymer wedges with flat polished faces on the front and back specimen surfaces with a suitable transparent adhesive ('Apiezon' grease works well.) The wedges were placed in such a way that their outward faces were at 60° to the craze plane whereas the former polymer–air surfaces were at 90° to the craze. This addition allowed high-incident angles at the craze plane and observance of shadow disappearance through the back wedge.

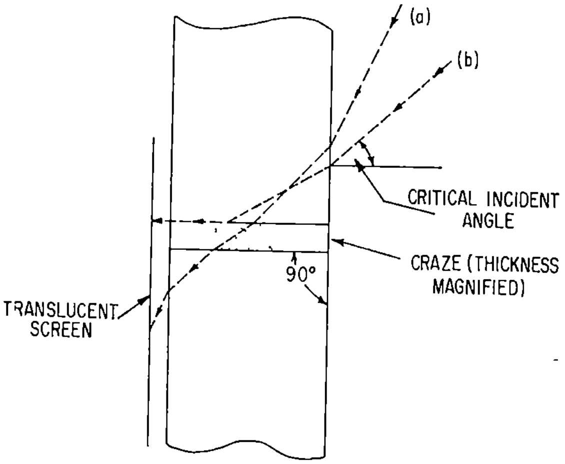

Fig. 1. Cross-sectional diagram of set-up for measuring critical angle for total reflexion at craze interface. Incident angle of parallel beam (*a*) is large enough for beam to pass through craze and register on screen. Beam (*b*) is at the critical angle and a shadow of the craze appears on the screen

Under favourable conditions critical incident angles can be determined by the naked eye to ± ½° with practice. It is also noteworthy that within these limits of accuracy at least the critical angle has not appeared to change with polarization of the incident beam. Finally, although the craze is generally thin enough to give rise conceivably to interference effects, none has been noticed during the measurements of critical angles.

Results with polycarbonate. To date investigations have dealt with 'Lexan$^{(R)}$' (General Electric Co. trademark) polycarbonate resin. The material commends itself for craze structure study because single planar crazes of large size (both in thickness and planar dimensions) can be produced by subjecting specimens to moderate bending strains under poorly plasticizing liquids such as n-C_6H_{14} or C_2H_5OH. On the other hand, large true mirror-smooth cracks may be propagated into specimens by judicious bending under carbon tetrachloride

Initially cracks induced by carbon tetrachloride give $n = 1\ 44$, the index of CCl_4 liquid Within a few minutes exposure to air the liquid in the crack evaporates out and n drops to 1 00, the index of air These results are a check on the method Polycarbonate crazes, on the other hand, yield widely varying indices depending on post-crazing treatment of the specimen Some results for ethanol-induced crazes follow

For a freshly produced craze $n = 1\ 46$, which is between that of the bulk polymer (1 58) and liquid C_2H_5OH (1 36) If the applied stress and the alcohol environment are both removed a healing process appears to occur over several days, the index rising eventually to 1 57 The rise appears to be linear on a log time plot, suggesting the occurrence of a disorientation process On the other hand, if the specimen is left strained after its removal from the alcohol, the index begins to drop rapidly and finally reaches a value of 1 27 Investigations of the kinetics here suggest that a rather rapid diffusion of alcohol back out of the craze is occurring, leaving a porous structure behind

Assuming polarization additivity on a weight fraction basis, volume additivity for polymer–alcohol mixtures, and a negligible effect of orientation on the specific polarizability of the polymer, it is possible to calculate craze density and composition from the Clausius–Mosotti equation[2] The only quantities needed are the craze indices and the indices and densities of the pure components (Polycarbonate density is 1 20 g/cc) On this basis the fresh, alcohol-saturated craze appears to be 40 per cent alcohol–60 per cent polymer by volume The still-strained, dried-out craze is calculated to be 50 per cent air–50 per cent polymer assuming no alcohol remains The two calculations are thus in reasonable agreement

The value of 60 per cent polymer content in the craze is buttressed by photomicrographs of the craze at the specimen surface It is found that suitable scratches imposed on a specimen prior to crazing are distorted when a craze developed across them, from the craze width and the scratch distortion it is calculated that a unit of material in the craze at the specimen surface is about 40 per cent longer than it was before crazing Although lateral contraction (that is, reduction in the dimensions of the craze plane) takes place in the craze at the specimen surface, the resultant volume contraction is negligible compared to the volume increase due to elongation in the stress direction upon craze formation The result is a polymer density decrease by 40 per cent The normal solubility of ethanol in polycarbonate is 8 per cent, so that the remainder of the rarefaction must be attributed to 3-dimensional tensile stresses existent in craze formation In this respect at least crazing differs from cold drawing, which appears to involve elongation by shearing at 45° to the tensile stress[3] with no resultant change in material density (unpublished observations on cold drawing of polycarbonate)

ROGER P KAMBOUR

General Electric Research Laboratory,
Schenectady, New York

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BIOCHEMISTRY

Isoxazolyl Penicillins and Penicillinase

THE isoxazolyl series of penicillinase-resistant penicillins has been described by Doyle *et al* [1] Many members of this series are acid-stable, and considerably more active against staphylococci than methicillin Three members of this series (*BRL* 1400 5-methyl-3-phenyl-4-isoxazolylpenicillin, *BRL* 1577 5-methyl-3-*p*-chlorphenyl-4-isoxazolylpenicillin and *BRL* 1621 5-methyl-3-*o*-chlorphenyl-4-isoxazolylpenicillin) have been investigated for antibacterial activity and for stability to, induction of, affinity for, and inhibitory action on, the penicillinases of *Staph aureus* and *B cereus*

Most of the cultures used were freshly isolated from clinical material, but in some cases stock strains were used 0 01 ml of a 1/10 dilution of a 6-h infusion broth culture was inoculated on to penicillin-containing infusion agar The plates were incubated overnight and the results recorded The results (Table 1) show that penicillin *G* was highly active against the Oxford staphylococcus and the other penicillinase-negative strain whereas the isoxazolyl penicillins were about 10 times less active Methicillin was even less active, having only about a fifth to a tenth of the activity of the isoxazolyl penicillins.

Table 1 MINIMUM INHIBITORY CONCENTRATIONS (μG/ML) OF PENICILLINS AGAINST *Staph aureus* STRAINS

Staph aureus	Penicillinase	Penicillin *G*	Methicillin	*BRL* 1400	*BRL*.1577	*BRL* 1621
Oxford	−	0 025	0 9	0 2	0 2	0 2
A 8	−	0 02	1 8	0 2	0 2	0 2
A 9	+	3 7	1 8	0 2	0 2	0 2
A 6	+	6 2	1 8	0 4	0 2	0 4
A 14	+	15	1 8	0 2	0 2	0 2
A 7	+	15	1 8	0 4	0 4	0 4
A 13	+	62 5	3 7	0 9	0 4	0 4
Cave	+	62 5	3 7	0 9	0 4	0 4
Crouch	+	62 5	3 7	0 9	0 4	0 4
E 3	+	250	3 7	0 9	0 9	0 9
Hunter	+	250	3 7	0 9	0 9	0 9

Penicillinase positive staphylococci, however, were all relatively resistant to penicillin *G* Methicillin was at least twice, and at the most 67 times, as active as penicillin *G* The isoxazolyl penicillins were about 5 times as active again as methicillin against these penicillinase-producing strains The question of cross-resistance between methicillin and the isoxazolyl penicillins will be discussed elsewhere

Table 2 RELATIVE INITIAL RATES OF HYDROLYSIS (PENICILLIN *G* TAKEN AS 100 PER CENT) AT A PENICILLIN CONCENTRATION OF 2 MG/ML

Penicillin	*S aureus E* 3	*B cereus* N R R L 569
G	100	100
Methicillin	0 7	1 4
BRL 1400	1 4	2 6
BRL 1577	1 6	1 1
BRL 1621	1 1	0 3

The breakdown of the various penicillins by penicillinases from *Staph aureus E*3 and *B cereus* N R R L 569 was measured by the hydroxylamine method of Boxer and Everett[2] as described by Knox and Smith[3] The results (Table 2) show that methicillin is the most resistant penicillin to staphylococcal penicillinase, being broken at 0 7 per cent of the rate of penicillin *G* The isoxazolyl penicillins, however, were less resistant to staphylococcal penicillinase than methicillin, being destroyed at about twice its rate, although the difference may not be significant With the *B cereus* penicillinase, however, both *BRL*. 1621 and *BRL* 1577 were more resistant to hydrolysis than methicillin although *BRL* 1400 was hydrolysed more readily than methicillin

The initial rate of destruction of each penicillin at various concentrations was measured by the hydroxylamine method[2] and the Michaelis constants (K_m values) were calculated by the method of Lineweaver and Burk[4]. Manson *et al.*[5] found that the K_m for penicillin *G* and the extracellular enzyme of *B. cereus* was approximately 20 μg/ml., and Novick[6] found that the K_m for penicillin *G* and the cell-bound enzyme of *Staph. aureus* was about 7 μg/ml. The K_m values determined here for the isoxazolyl penicillins and methicillin (Table 3) are about a thousand-fold larger than these values.

Table 3. MICHAELIS CONSTANTS OF VARIOUS PENICILLINS IN MG/ML.

Penicillinase	Penicillin			
	Methicillin	*BRL*.1400	*BRL*.1577	*BRL*.1621
Staph. aureus	7·7	5·9	6·5	*
B. cereus	5	16	13	10

* Concentrations of this penicillin greater than 3 mg/ml. showed no appreciable hydrolysis, hence the Michaelis constant could not be determined.

The concentration of penicillin necessary to cause half maximal and maximal induction of penicillinase above the basal value was determined as described by Knox and Smith[3] using *Staph. aureus E*3. The results (Table 4) show that although methicillin was much more active than benzyl penicillin, the isoxazolyl penicillins were even more active as regards the amount necessary to cause half-maximal and maximal induction. In addition, the increase in penicillinase content of fully induced cells was highest with the isoxazolyl penicillins. The figures given for penicillin *G* are probably of little significance since this inducing agent is destroyed during induction.

Table 4. INDUCING ABILITY OF THE VARIOUS PENICILLINS UPON THE PENICILLINASE OF *Staph. aureus E* 3

	Concentration μg/ml. to give maximal induction	Concentration μg/ml. to give half-maximal induction ('induction constant')	Ratio of induced to basal penicillinase activity at maximum
Penicillin *G**	300	6·6	12·4
Methicillin	3	0·27	36·0
BRL.1400	0·3	0·019	55·8
BRL.1577	0·3	0·017	65·8
BRL.1621	0·1	0·013	71·3

* See text.

The amount of inhibition caused by methicillin and the isoxazolyl penicillins was measured by using a modified form of the method of Saz, Lowery and Jackson[7]. The hydrolysis of 1 mg/ml. of penicillin *G* in the presence of 1 mg/ml. of the 'inhibitor' penicillin added simultaneously to the enzyme was followed at 37° C. As it was found that a given amount of inhibitor caused more inhibition as the enzyme concentration was decreased, the enzyme preparations used in these studies were adjusted so that they hydrolysed penicillin *G* at approximately similar rates (2 μg/min). No inhibition occurred using the staphylococcal enzyme, but it has been shown[8] that previous incubation of staphylococcal penicillinase with methicillin or *BRL*.1400 caused some inactivation. With the *B. cereus* enzyme methicillin inhibited the hydrolysis of penicillin *G* by 67 per cent and the isoxazolyl penicillins inhibited the hydrolysis of penicillin *G* by 29 (*BRL*.1400), 22 (*BRL*.1577) and 23 (*BRL*.1621) per cent.

The efficacy of methicillin, the first penicillin highly active against penicillinase-producing staphylococci, seemed, partly at least, to be due to its high degree of resistance to staphylococcal penicillinase. The isoxazolyl penicillins were found to be slightly less resistant to staphylococcal penicillinase than methicillin and both the isoxazolyl penicillins and methicillin had similar low affinities (high K_m values) for staphylococcal penicillinase as compared with the high affinity (low K_m) of penicillin *G*. Neither methicillin nor the isoxazolyl penicillins inhibited the hydrolysis of penicillin *G* by staphylococcal penicillinase, although they did inhibit *B. cereus* penicillinase. Thus the fact that the isoxazolyl penicillins were more active than methicillin against penicillinase-producing staphylococci could not be explained in terms either of stability to staphylococcal penicillinase or of affinity for the enzyme. However, the isoxazolyl penicillins were much more effective than methicillin in inducing staphylococcal penicillinase, giving low induction constants[9] suggesting high inducing affinity.

We acknowledge gifts of methicillin and the isoxazolyl penicillins from Beecham Research Laboratories and technical assistance from Miss B. Whittamore.

J. T. SMITH
J. M. T. HAMILTON-MILLER
R. KNOX

Department of Bacteriology,
Guy's Hospital Medical School,
London Bridge,
London, S.E.1.

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Hepatotoxic Action of Chromatographically Separated Fractions of *Aspergillus flavus* Extracts

THE presence in certain groundnut meals of a toxic substance which causes the death of young ducks and turkeys has recently been reported[1]. The toxin is produced by a strain of the mould *Aspergillus flavus* growing on the groundnuts[2,3]. There are two methods for detecting this substance: a biological test with ducklings and a chemical test in which a single, blue fluorescent spot is found on paper chromatograms of extracts of the mould or mould-infected meals. By chromatographing extracts of toxic strains of *A. flavus* on thin layers of silica-gel we have obtained at least twelve fluorescent components, five of which caused liver lesions when fed to ducklings.

Cultures of *Aspergillus flavus* were grown on a glucose-ammonium nitrate medium[4] in penicillin flasks. After 9 days at 27° C the mould was separated from the growth medium by filtration and shaken with chloroform to give a yellow-coloured extract. The growth medium was also extracted with chloroform in a liquid-liquid extractor and the combined extracts were evaporated to a small bulk under nitrogen gas to give a concentrate (about 2·0 mg/ml. total solids) suitable for preparative chromatography on thin layers of silica-gel.

A 275-μ thick layer of 'Kieselgel *G*' (Merck) was applied to glass plates with a Desaga spreader. These plates were dried at 105° C for 24 h and stored in an open rack; they were re-heated to 105° C for 1 h immediately before use. To separate material for duckling feeding experiments, replicate spots (5–10 μl.) of the chloroform extracts were suitably spaced

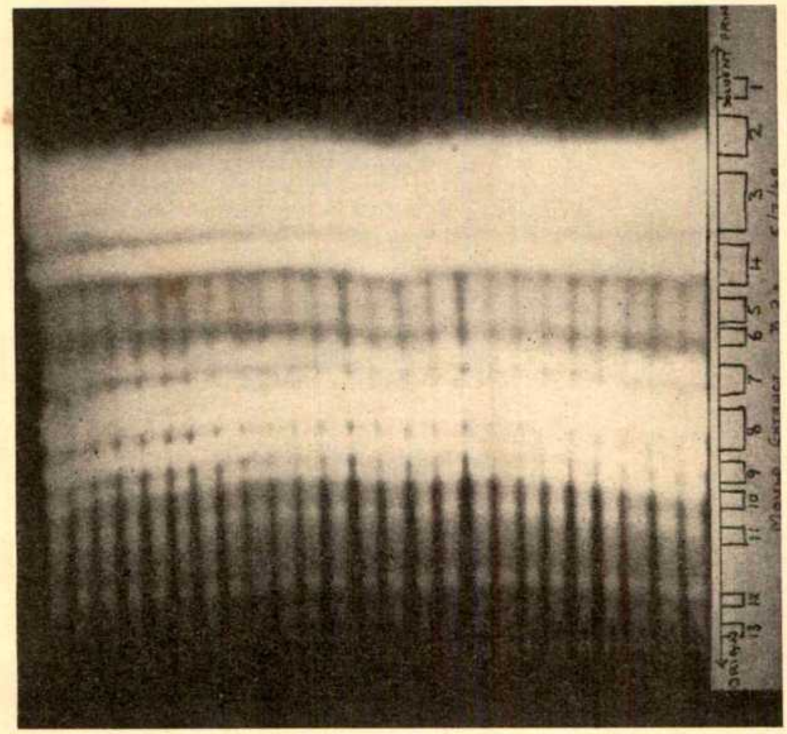

Fig. 1. Preparative thin-layer chromatogram of extract of toxic strain of *Aspergillus flavus*, photographed under ultra-violet light

along a starting line parallel to one edge of a 20 × 20 cm plate and chromatographed by the ascending method in small glass tanks equilibrated with chloroform/methanol/formic acid (95 : 5 : 1); separation took about 30 min.

Extracts of highly toxic strains of *A. flavus* were thus resolved into at least 12 clearly defined components which fluoresced under ultra-violet light (365 mμ). In Fig. 1, a typical preparative plate, the fluorescent components have been numbered in decreasing R_F order. Components 3 and 4 together account for about 60 per cent of the total fluorescence of applied extract. Components 4 and 9 had a blue-green fluorescence, in contrast to the dark blue fluorescence of the other spots. If the chromatogram was sprayed with 50 per cent sulphuric acid and gently warmed, the colour of the fluorescence changed from blue to yellowish-green. At the same time the more strongly fluorescent components became visible under ordinary light as yellow spots, which on stronger heating (200–250° C) became dark blue in colour. Under these conditions the rather weakly fluorescent components 1 and 2, running near the solvent front, first gave a characteristic rose-pink colour and on stronger heating a dark brown colour.

For biological testing the individual fractions were isolated by scraping the band of adsorbent associated with each row of fluorescent spots into a small beaker, extracting with chloroform and evaporating the extract to dryness under vacuum. When these fractions were rechromatographed in the same system, each gave essentially two components, one with the same R_F as the original spot and some fast-moving material, presumably fractions 1 or 2, which stained pink with sulphuric acid. Repeated thin-layer chromatography gave fractions which were substantially homogeneous. Fractions 10, 11 and 12, which were present only in small amounts, were not isolated.

Weighed amounts of the isolated components were suspended in water for administration to ducklings. Day-old Khaki-Campbell ducklings were fed 1 ml. of these suspensions per day for 5 consecutive days.

They were killed after 7 days and their livers examined histologically by Wannop's method[5]. Characteristic lesions, similar to those reported by Wannop[5] for turkeys with *X* disease, were observed in livers taken from birds fed fractions 3, 4, 5, 8 and 9. So far as could be judged by this histological test, all these fractions had about the same potency. Fractions 1, 2, 6 and 7 fed at the same level did not produce significant liver lesions.

Extracts of mildly toxic strains of *A. flavus* gave simpler chromatographic patterns in which component 3 or components 3 and 4 predominated (Fig. 2).

We conclude that the hepatotoxic activity of certain strains of *A. flavus* is associated with several discrete, fluorescent substances. With other methods of separation, this activity may prove to be even more complex in nature.

We thank Mr. P. Austwick, Central Veterinary Laboratory, for supplying the toxic strains of *A. flavus*; Mr. C. C. Wannop, Houghton

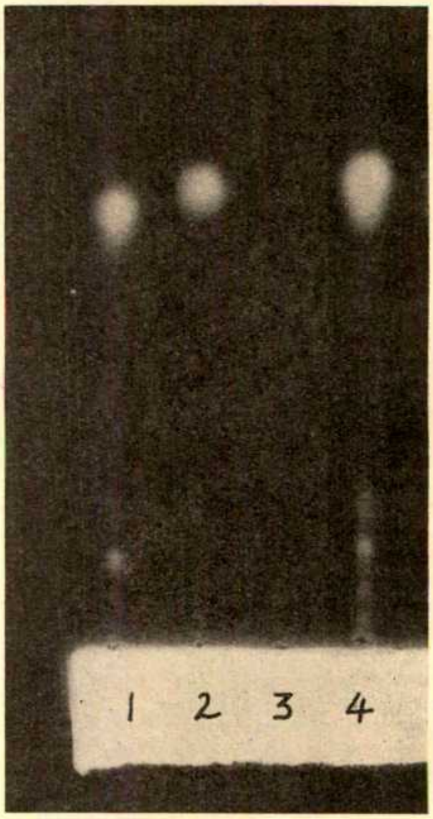

Fig. 2. Thin layer chromatograms of extracts of mildly toxic strains of *Aspergillus flavus* photographed under ultra-violet light

Poultry Research Station, for advice on duck liver histology, and Dr. L. Morris for advice on thin-layer chromatography.

[See also *Nature* of September 15, pp. 1060–1063.]

R. H. SMITH
W. MCKERNAN

Biochemistry Department,
British Glues and Chemicals, Ltd.,
Berkshire House,
168–173 High Holborn,
London, W.C.1.

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Radiometric Assay of Acetylcholinesterase

A METHOD for the microdetermination of acetylcholinesterase activity has been developed in which the formation of acetic acid labelled with carbon-14, liberated enzymically from the labelled substrate, is measured by a simple counting technique. The method has the advantage that it can be applied to very small samples of tissue at relatively constant *p*H. It has been applied successfully to samples of the order of 1 μl. of whole blood, blood plasma, or insect tissue deposited on cavity-type microscope slides. Since dilution of the sample is unnecessary or readily limited without affecting sensitivity, the technique provides a more reliable indicator of reversible cholinesterase inhibition by compounds such as carbamates than the conventional Michel method[1], for example.

Anhydrous sodium [1-^{14}C] acetate (6.8 mg containing 1 mc. carbon-14) was heated for 24 h at 60° C in a small sealed tube containing 0.47 ml. (5 mM) acetic anhydride. Complete isotope exchange between acetate and anhydride carboxyl occurred under these conditions (cf. Avivi *et al.*[2]). The [^{14}C]-acetic anhydride so produced was distilled *in vacuo* into a second small tube containing 139.7 mg [1 mM] of dried choline chloride, the tube sealed and complete acetylation achieved by heating the solution obtained for 1 h at 100° C as indicated by Cline[3]. The excess of [^{14}C] anhydride and free [^{14}C] acetic acid formed were recovered *in vacuo*, and the [1-^{14}C] acetylcholine washed in ether, weighed, dissolved in water and stored at −15° C until required for use as substrate. Radiochemical yield of the acetylated product was theoretical in terms of choline and sufficient for some 25,000 enzyme assays.

After incubation of the enzyme preparation and labelled substrate under the required conditions a small sample (1–5 μl.) is deposited on a 10-mm cavity microscope slide and 5 μl. of 0.5 N hydrochloric acid immediately admixed. The mixture is then dried in a vacuum desiccator, gently warmed in air for a few seconds and counted for carbon-14 activity under a thin end-window Geiger–Muller tube in the conventional way, geometry being carefully standardized. Under these conditions free [^{14}C] acetate is completely volatile while activity of carbon-14 due to the unhydrolysed acetylcholine is not. Control assays in which the enzyme is absent or known to be inhibited (for example, by 10^{-3} M eserine sulphate) are made simultaneously. The difference in counting rates between active and control samples therefore represents the free acetate resulting from enzymic hydrolysis of the substrate. Alternatively, enzyme samples and substrate may be mixed directly on the microscope slide and incubated in a moist atmosphere. The method can be applied to samples containing weights of substrate of the order of 1 μg. Weights of about 1 mg are required for the conventional Warburg manometric or ΔpH techniques[4].

An error would arise if the labelled acetate liberated enzymatically were further metabolized or underwent isotopic exchange with the formation of labelled metabolites which were not volatile on drying in the presence of hydrochloric acid. For example, [^{14}C] acetate is rapidly metabolized by the adult housefly *in vivo* with the formation of labelled amino-acids, protein, etc.[5] However, tests with diluted cholinesterase-active housefly head tissue extracts (1–2 heads per ml.) showed that all residual carbon-14 activity became acid volatile on pretreatment with 0.1 N sodium hydroxide at room temperature. Cholinesterase activity determined by the method was not significantly affected by the presence of 10^{-3} M cyanide, which would be expected to suppress any oxidative metabolism of free acetate. These observations indicate that residual carbon-14 activity was due entirely to unhydrolysed [^{14}C] acetylcholine and not due to labelled metabolites.

F. P. W. WINTERINGHAM
R. W. DISNEY

Biochemistry Department,
Agricultural Research Council,
Pest Infestation Laboratory,
London Road, Slough, Bucks.

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Repression of β-Galactosidase Synthesis by Amino-Acids in Thymine-starved *Escherichia coli*

THE ability to synthesize β-galactosidase of thymine-less mutants of *Escherichia coli* has been shown to be retained for a prolonged period of thymine starvation even when most of the starved bacteria have lost the viability[1,2]. These observations were obtained by inducing the thymine-starved bacteria in energy-deprived media. When the induction was carried out in the presence of an energy source, such as glycerol, the ability to synthesize β-galactosidase of the thymine-starved cells declined proportionally as the decline in the viability[2]. During the prolonged thymine starvation catabolites derived from the carbon source should have accumulated within the cells. On the subsequent induction with glycerol, these accumulated catabolites should exert 'catabolite repression'[3] on the synthesis of β-galactosidase, which is a catabolic enzyme.

In a previous report[2], it was demonstrated that the longer the duration of the thymine starvation the more rapidly enzyme was made by the starved cells in the induction medium deprived of glycerol. On the contrary, when a medium enriched with casamino-acids was used for the induction in an independent experiment[4], the thymine-starved cells showed a decreased rate of enzyme synthesis even in the absence of glycerol This communication describes the repressive effect of casamino-acids on the β-galactosidase synthesis in thymine-starved cells

E coli 15 *T-PA-* (thymine- and phenylalanine-less)[5] was grown and then starved for thymine in a mineral salts–glycerol medium at 37° with necessary growth factors as described previously[2] Cells which had been subjected for varying times to the thymine starvation were washed and suspended in two kinds of induction media One was a mineral salts medium without glycerol containing 0 001 M methyl-β-D-thiogalactopyranoside (TMG) and 0 0001 M L-phenylalanine The other was the same medium enriched with 500 μg/ml casamino-acids (Difco, certified) L-[1-^{14}C]-Leucine (specific activity 5 15 m c /m mole, New England Nuclear Corp , Boston, Mass) was added at zero time to give a final concentration of 15 μM together with non-radioactive L-leucine (0 0002 M, which is about equal to the L-leucine concentration in the medium enriched with casamino-acids) The non-radioactive leucine was not added to the enriched induction medium During the induction 1 and 2 ml aliquots were withdrawn at different time-intervals for the assay of β-galactosidase[6] and for the measurement of L-[1-^{14}C]-leucine incorporation into the protein of the cells[7], respectively In both induction conditions in the absence of glycerol, no net increase of total protein was observed Thus, the differential rate of enzyme synthesis during a given time of induction was calculated as follows

$$\frac{\Delta\text{Units } \beta\text{-galactosidase synthesized/ml}}{\Delta\text{Counts/min L-[1-}^{14}\text{C]-leucine incorporated/ml protein}}$$

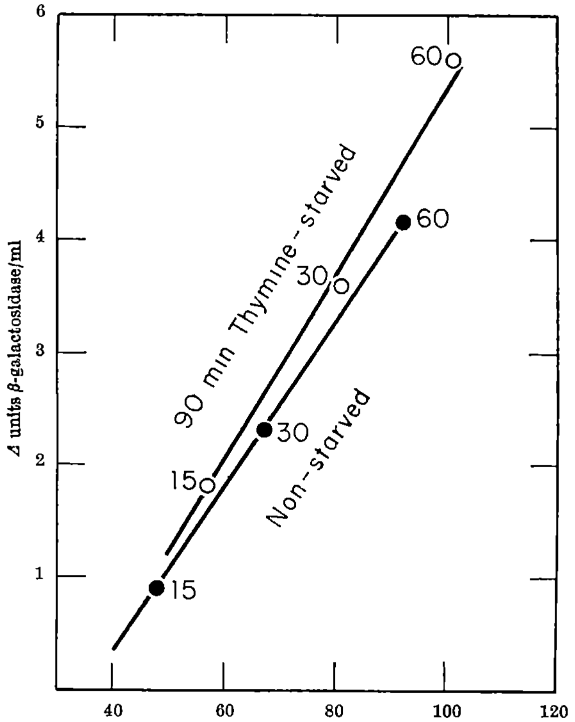

Fig 1 The differential rate of β-galactosidase synthesis by thymine-starved and non-starved *E coli* 15 *T-PA-* in induction media without casamino-acids Numbers in the figure indicate the induction period in min After 90 min starvation for thymine the viability was 3 7 per cent

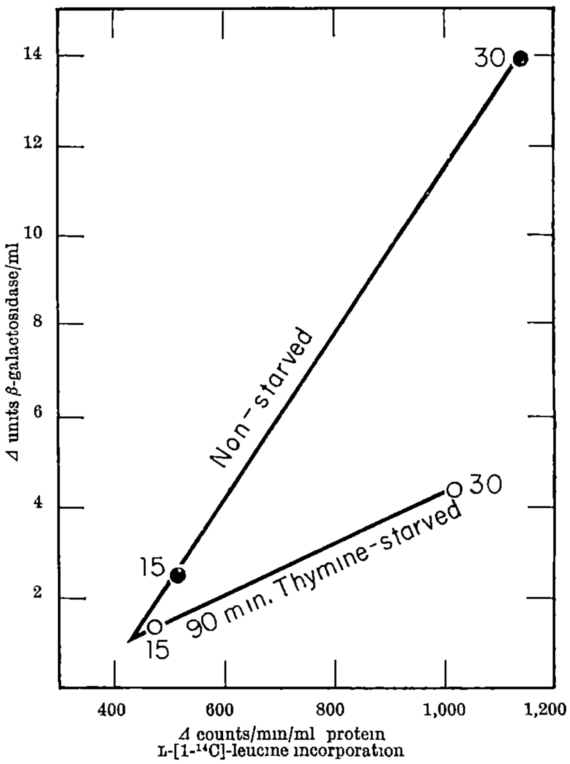

Fig 2 With casamino-acids (*see* legend to Fig 1)

A typical experiment is illustrated in Figs 1 and 2 In the induction medium without casamino-acids, the cells which had been starved for thymine for 90 min synthesized in the same time-interval more β-galactosidase than the non-starved cells The differential rate of enzyme synthesis was essentially the same in both starved and non-starved cells (Fig 1) On the other hand, in the induction medium enriched with casamino-acids a marked decrease of the differential rate of enzyme synthesis was observed in the cells starved for 90 min The amount of enzyme formed in the same time-interval by the previously starved cells was also markedly less than that by the non-starved cells (Fig 2) Some of the amino-acids supplied in the casamino-acids can presumably be metabolized by the cells to produce energy Thus in the previously thymine-starved cells, where the rate of protein synthesis is slow, the catabolites produced by the degradation of the amino-acids accumulate in the cell and repress the induced synthesis of the catabolic enzyme, β-galactosidase

Table 1 DIFFERENTIAL RATE OF β-GALACTOSIDASE SYNTHESIS BY *E coli* 15 *T-PA-* WHICH HAVE BEEN STARVED FOR THYMINE FOR VARYING TIMES

Thymine starvation before induction (min)	Viability (%)	Differential rate of β-galactosidase synthesis*, per cent retained	
		Induction without casamino-acids	Induction with casamino-acids †
0	100	100	100
30	120	106	107
60	13 6	92	51
90	4 1	112	36
120	0 5	101	12

* See text, expressed in per cent of the value obtained by the non-starved cells The values were taken between 15 and 30 min of induction During that time both enzyme synthesis and L-[1-^{14}C]-leucine incorporation showed linear increases

† Induction with casamino-acids generally gave a greater amount of enzyme synthesis than without casamino-acids though the differential rate of enzyme synthesis was smaller

The effect of amino-acids on β-galactosidase synthesis after different periods of thymine-starvation is presented in Table 1 Thymine-starved cells taken at varying times of the thymine starvation did not show any significant change in the differential rate of enzyme synthesis upon induction without casamino-acids, while with casamino-acids they did show a decline proportionally to the duration of the prior thymine starvation

This communication together with the previous reports indicate that the DNA after thymine starvation is good enough to act as primer for further synthesis of DNA when thymine is added back[8], and to give information for the synthesis of new species of protein The cause of the 'thymine-less death', however, is still obscure

This work was supported in part by a grant from the National Institutes of Health, No *CY*-5210, administered by Prof B Magasanik

DAISUKE NAKADA

Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Mass

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Functional Alteration in the Prothrombin Molecule

PROTHROMBIN is the central protein involved in the blood-clotting mechanisms, and is of great importance in normal physiological functions From the point of view of health ordeals, reduced activity is associated with hæmorrhagic diseases and excess with thrombosis As a consequence knowledge about this molecule is of special importance Prothrombin has been isolated from plasma[1], and is the source of two enzymes, namely, thrombin, which clots fibrinogen, and autoprothrombin *C*, which catalyses the activation of prothrombin[2] In addition to these two enzymes at least three intermediates of prothrombin activation are known and have been called autoprothrombin I (ref 3), autoprothrombin II (ref 4) and prothrombin-*R* (ref 5)

Chromatography of the purified prothrombin of Seegers[1] on DEAE cellulose alters the structure of the molecule with respect to the C-terminal amino-acid composition[6] Glycine and tyrosine were found and after chromatography it was serine The *N*-terminal amino-acid alanine was not changed We have now made further comparisons of the original purified prothrombin and the DEAE chromatographed material The latter yielded essentially only thrombin as the end-product of activation whereas the former yielded the two enzymes thrombin and autoprothrombin *C* Furthermore, non-chromatographed prothrombin was added to factor *X* (Stuart–Prower) 'deficient' plasma and prothrombin utilization followed even though the original plasma contained prothrombin which was not consumed However, this prothrombin consumption was not found when the prothrombin was chromatographed on DEAE cellulose before it was added to the deficient plasma nor was it possible to elute anything active from the cellulose column As a consequence of these facts we think that factor *X* does not exist as a separate clotting entity Factor *X* 'deficient' plasma most likely has a prothrombin molecule that is functionally abnormal, much like the DEAE-cellulose chromatographed prothrombin From this point of view Stuart–Prower hæmorrhagic disease is a molecular disease To get prothrombin consumption in the plasma there must be a potential source of autoprothrombin *C*, and since the DEAE cellulose prothrombin does not supply this we are inclined to think that the prothrombin in the Stuart–Prower plasma does not readily yield this enzyme either

In our experiments we prepared the DEAE-chromatographed prothrombin[7] and dissolved it in a 25 per cent (w/v) sodium citrate solution Under our conditions no thrombin activity generated in 12 hr even when some purified thrombin was added at zero time as a catalyst We then added some autoprothrombin *C*, prepared according to procedures previously described[2], and were able to get thrombin rapidly with the use of this catalyst When this thrombin generated from the DEAE-cellulose chromatographed prothrombin there was practically no autoprothrombin *C* produced (Fig 1) such as occurs when non-chromatographed prothrombin is activated in sodium citrate solution[2] From previous work[6] we know that after chromatography on 'Amberlite *IRC*-50' there is no change in *C*-terminal amino-acids and the molecule activates in sodium citrate solution to yield both enzymes[2]

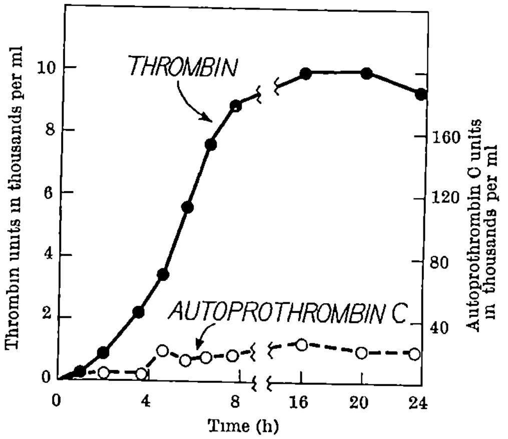

Fig 1 Activation of DEAE cellulose prothrombin in 25 per cent sodium citrate solution at 28° C The prothrombin was dissolved in 25 per cent sodium citrate solution to a concentration of 12,000 U /ml Autoprothrombin *C* (12,000 U /ml) was added at zero time to start the activation Thrombin and autoprothrombin *C* generation was followed

We examined other activation mediums besides 25 per cent sodium citrate solution With tissue extracts, Ac-globulin, and calcium ions, or with 'Stypven' (Russell viper venom, Burroughs Wellcome and Co), Ac-globulin, lipids and calcium ions we were not able to obtain autoprothrombin *C* from DEAE-chromatographed prothrombin These two procoagulant combinations are precisely the ones to which the prothrombin in Stuart–Prower-deficient plasma is resistant The prothrombin of Stuart–Prower plasma does not activate readily in the 2-stage analysis if tissue extracts are used as procoagulant or if 'Stypven' with lipids is used, but activates rapidly with a combination of autoprothrombin *C* and lipids We found the same to be true for DEAE-chromatographed prothrombin. In normal plasma, or in the purified but not chromatographed prothrombin preparations, prothrombin activates rapidly with the 2-stage analytical reagents irrespective of which procoagulant is used.

So far as we know the prothrombin of Stuart–Prower-deficient plasma has not been isolated to see whether it yields autoprothrombin *C*, and there are technical difficulties owing to the limited supply of blood. We are also not aware of any attempt to use purified prothrombin as a therapeutic agent in Stuart–Prower cases. Meanwhile we could try to see which one of our purified prothrombin preparations, if any, would give prothrombin utilization when added to the deficient plasma, and found that the one which can yield autoprothrombin *C* does so while the other one does not.

In our prothrombin utilization studies we used the original method of Brinkhous[8] with the exception that purified autoprothrombin *C* served as the procoagulant in the 2-stage prothrombin analysis in place of tissue extracts. This modification was introduced because Stuart–Prower plasma seemed to contain only 50 u. of prothrombin/ml. when tissue extract was the procoagulant whereas it in fact contained 235 u. when autoprothrombin *C* was used as procoagulant (Fig. 2). The prothrombin utilization of the Stuart plasma was slow and incomplete. This was not improved with the addition of DEAE-chromatographed prothrombin, which is the functionally altered molecule. On the other hand, prothrombin utilization was rapid when purified prothrombin was added. For example, after a delay of 5 min. the prothrombin concentration dropped from 255 to 25 u./ml. in 15 min. (Fig. 2).

As a consequence of considering the facts related to our experiment we offer as our preferred interpretation that Stuart–Prower 'deficient' plasma contains an abnormal prothrombin molecule and that the defect is a molecular disease. This view can be incorporated with the known observations about the genetics involved[9]. The factor *X* which is supposed to be lacking in this plasma really does not exist. It may be that fractions of plasma obtained in different laboratories and which are supposed to have factor *X* activity actually contain autoprothrombin *C*.

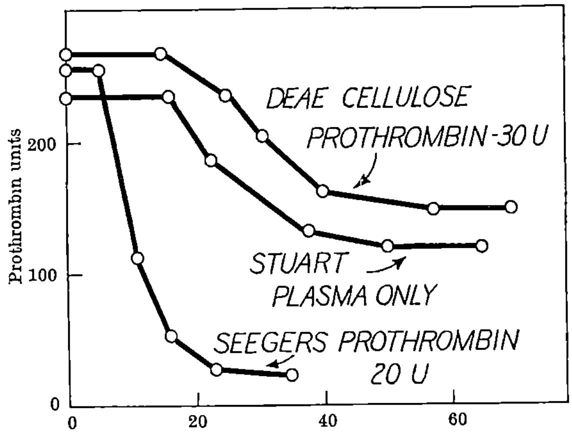

Fig. 2. Prothrombin consumption in Stuart plasma. Following reaction mixture called the control was prepared at 27° C.: Stuart plasma 0.5 ml., 0.4 per cent 'Cephalin' 0.1 ml., 0.05 *M* calcium chloride 0.1 ml. At time-intervals samples of 0.1 ml. were taken for the 2-stage prothrombin determination. A modification of the original two-stage method was used in which the lung extract thromboplastin was replaced by 0.1 per cent cephalin suspension mixed with 500 units of autoprothrombin *C*. Top curve same as control, but with DEAE-cellulose prothrombin added at zero time. Bottom curve same as control, but with non-chromatographed prothrombin added at zero time.

An abnormal prothrombin molecule has been created in our laboratory by changing the one isolated from normal plasma. The known alteration made in the structure is at the *C*-terminal amino-acid location. Here there seems to be a modification that is not noticed on the basis of a complete amino-acid analysis[5]. The chromatography apparently destroys what is built into the prothrombin molecule during its cellular synthesis. Failure to incorporate this specific or related structure could easily account for an abnormal prothrombin molecule in Stuart–Prower 'deficient' plasma. Moreover, this perspective may be applicable for exploration in other abnormalities such as factor VII and IX deficiencies, where prothrombin derivatives are known to be involved[3,4].

This work was supported by a grant *H*-5141 from the National Institutes of Health, U.S. Public Health Service. We thank John B. Graham of the University of North Carolina for a sample of Stuart plasma and also John A. Penner of the University of Michigan for a sample of factor *X* deficient plasma.

EWA MARCINIAK*
EDMOND R. COLE
WALTER H. SEEGERS

Department of Physiology and Pharmacology,
Wayne State University,
Detroit, Michigan.

* Rockefeller Foundation Fellow.

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Role of the Krebs Cycle in Ethylene Biosynthesis

SEVERAL reports have appeared in the literature recently on ethylene biosynthesis in fruit and in the mould, *Penicillium digitatum*. With apple tissue, Burg and Thimann[1] concluded, from the findings of isotope experiments, that ethylene is derived from sugar via pathways unrelated to glycolysis, the Krebs cycle, the pentose phosphate pathway or the glucuronic acid pathway. Phon-Chon-Ton[2] demonstrated that in *P. digitatum* ethylene can derive its carbon skeleton from glucose or glycerol carbon atoms and suggested that a long sequence of reactions may have been involved in the biosynthesis of ethylene from glucose.

The mechanism for the production of ethylene in the mould, *P. digitatum*, has also been studied in this laboratory. Glucose and amino-acids labelled with carbon-14 have been used as substrates to a stationary culture of the mould growing aerobically in 100 ml. of a medium containing the essential salts, 0.2 per cent yeast extract and 1.25 per cent glucose[3]. After three days of incubation at 24° C, the carbon-14 labelled substrate was introduced into the culture, and a stream of sterile air was allowed to flow through the enclosed incubation flask at a rate of 40 ml. per minute.

The carbon dioxide and ethylene produced by the mould were trapped respectively by an ascarite column and a silica gel column cooled in a dry ice–acetone bath. The trapped ethylene was released from the column at 50° C and swept into the gas chromatography column with a stream of helium gas. Pure ethylene fraction separated on the chromatography column, consisting of molecular sieve (Wilkens Instrument and Research, Inc., P.O. Box 313, Walnut Creek, California) and silica gel, was collected in a fraction collector submerged in a liquid nitrogen bath. The radioactivity of the ethylene was determined upon the addition of 15 ml. of ice-cold scintillation solvent (1,000 ml. of toluene, 6 g of 2,5-diphenyloxazole and 75 mg of 1,4-*bis*-2(5-phenyloxazole benzene) to the liquefied ethylene, and the mixture was counted by means of a 'Tricarb' scintillation spectrometer model 314 *EX*. The amount of ethylene was estimated by integrating the area under the curve plotted on the gas chromatograph recorder. The silica gel column was replaced daily and an analysis of the ethylene trapped thereon provided information on the time course for ethylene production by the mould. On the average, 1–1.2 μmoles of ethylene were produced by the mould culture over a period of 24 h with the peak production rate being 6–7 days after inoculation. This observation is in accordance with that reported by Phon-Chon-Ton[2].

With glucose labelled with carbon-14 as substrate, the specific activities of ethylene were found to be 55, 41, 99 and 45 dpm per μmole of ethylene respectively from 5 μc of [1–^{14}C] glucose, [2–^{14}C] glucose, [6–^{14}C] glucose and [U–^{14}C] glucose. The findings are basically similar to those reported by Burg and Thimann[1] with apple tissue. The low magnitude of the specific activity of ethylene derived from [U–^{14}C] glucose, however, leads one to believe that C-3 and C-4 of glucose did not contribute much to the formation of the ethylene skeleton. If one assumes that the contribution of C-5 of glucose to ethylene biosynthesis is of the same magnitude as that of C-2 of glucose, a simple calculation using the specific activities of ethylene observed in the glucose experiments will show that the specific activity of ethylene derived from either C-3 or C-4 of glucose is of the order of $\frac{(45 \times 6) - (55 + 99 + 41 + 41)}{2} = 17$ dpm per μmole. These results, along with the understanding that in *P. digitatum*[4] glucose is catabolized primarily via the glycolysis-Krebs cycle pathway (77 per cent)[4], suggest that the ethylene skeleton may have derived its origin from degradation products of glucose in the glycolysis-Krebs cycle sequence.

In order to verify the foregoing described contention, a series of experiments have been carried out with amino-acids labelled with carbon-14 as the tracing substrates. The amino-acids used were glycine, alanine, aspartic acid and glutamic acid, each is closely related to a member of the Krebs cycle. The use of amino-acids instead of Krebs cycle acids is motivated by the results of preliminary experiments in which it was demonstrated that amino-acids can be readily utilized by the mould in the presence of glucose.

The amino-acid experiments were carried out in essentially the same manner as that of the glucose experiments. Mould was allowed to grow aerobically in salts–yeast extract–glucose medium for three days, at which time approximately 9 μmoles of the labelled amino-acid, having a specific activity of 0.55 μc per μmole, were introduced into the incubation medium. Production of ethylene was followed by daily chromatographic and radiochemical analysis over a period of six days. The specific activities of ethylene observed in these experiments were those totalled for the 6-day period, and they were normalized to equal substrate radioactivity, that is, 5 μc per 9 μmoles of each of the amino-acids.

The specific activities of ethylene derived from the labelled alanine samples are 114, 182 and 799 dpm per μmole of ethylene from C-1, C-2 and C-3 of alanine respectively. This fact is in line with the findings in the glucose experiments, if one visualizes glucose as being converted to 2 moles of pyruvate via the glycolytic pathway with C-1 or C-6 of glucose being equivalent to the methyl carbon atom of the pyruvate. The fact that C-1 of alanine was not incorporated to ethylene to any great extent suggests that the pyruvate may have undergone oxidative decarboxylation, giving rise to acetyl CoA prior to its conversion to ethylene.

The mechanism involved in the conversion of acetyl CoA to ethylene is a complex one since, on one hand, acetyl CoA can be converted to glyoxylate prior to the formation of ethylene or, on the other hand, it can be routed into the Krebs cycle, giving rise to a number of Krebs cycle intermediates which are in turn converted to ethylene molecules. That the glyoxylate is probably not involved directly in the biosynthesis of ethylene is indicated by the finding that the relative specific activities of ethylene derived from C-1 and C-2 of glycine were 13 and 115 dpm per μmole respectively. Although C-2 of glycine was preferentially utilized for ethylene biosynthesis, the extent is much less in comparison with the case of C-3 of alanine.

It therefore appears that acetyl CoA, as derived from glucose via the glycolytic pathway, may have been further catabolized by way of the Krebs cycle, giving rise to a compound which serves as a more direct precursor of ethylene. The nature of this compound is revealed by the findings in the labelled aspartate and glutamate experiments. Specific activities of ethylene derived from aspartate were found to be 304 and 25 dpm per μmole from C-3 and C-4 aspartate respectively. This fact is in line with the findings observed in the alanine experiments, inasmuch as C-3 of aspartate is metabolically corresponding to the C-3 of alanine.

More striking results are observed in labelled glutamate experiments. The specific activities of ethylene derived from C-1, C-2, C-3(4) and C-5 of glutamate were 5, 30, 624 and 8 dpm per μmole of ethylene respectively. These results suggest that C-3 and C-4 of glutamate are closely related to the precursor of ethylene in the mould.

On the basis of these findings, it can be speculated that the conversion of glucose carbon atoms to ethylene followed the route involving the glycolysis and the conversion of acetate to α-ketoglutarate via the Krebs cycle steps. The α-ketoglutarate so formed is presumably converted to succinic acid by oxidative decarboxylation and oxidation of the succinate, giving rise to fumarate. It is possible that the carbon skeleton of ethylene or its precursor is derived directly from the middle carbon atoms of fumarate. It is recalled that earlier Buhler, Hansen and Wang[5] observed that ethylene labelled with carbon-14 can be incorporated into the acid fraction of avocado fruit. The radioactivity was found to be located primarily in the fumaric acid and succinic acid, in particular the carboxyl carbon atoms Although it is not possible to directly correlate the present work and the previous findings with fruit, the intimate relationship between ethylene and the Krebs cycle acids is, nevertheless, indicated by the results in both experiments

This work was supported by the U S Atomic Energy Commission, contract number *AT* (45-1)-573

C H Wang
Albert Persyn
Julia Krackov

Departments of Chemistry and Science Research Institute,
Oregon State University,
Corvallis, Oregon

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Occurrence of *Bis*(β-Amino-β-Carboxyethyl) Trisulphide in the Hydrolysates of Wool and other Proteins

Investigations of acid hydrolysates of wool labelled with sulphur-35, and of non-radioactive wool hydrolysed with ^{35}S-cystine, have shown the presence of unidentified sulphur compounds derived from cystine[1,2] The most abundant of these (I) increased in amount with increasing time of hydrolysis up to 100 hr, when about one-tenth of the original sulphur in wool was present as (I) Somewhat smaller amounts of (I), representing about one-fiftieth of the protein sulphur, were found in acid hydrolysates of insulin, ribonuclease and bovine serum albumin, but none could be detected in hydrolysates of lysozyme and β-lactoglobulin

We have recently obtained 1 4 gm of (I) as a stable, white, microcrystalline powder from 150 gm of wool by the following procedure The wool was hydrolysed in 1 5 litres of 5 7 *N* hydrochloric acid for 100 hr under reflux, and the hydrolysate fractionated by chromatography on a 9-cm × 40-cm column of 'Zeo-Karb 225' (× 4) resin in the H$^+$ form by elution with 2 5 *N* hydrochloric acid[3] (I) was eluted with phenylalanine and tyrosine, and was separated from these by subsequent chromatography on a 4-cm × 15-cm column of starch by elution with 2 1 (v/v) *n*-propanol/0 5 *N* hydrochloric acid[4] Traces of contaminating carbohydrate were removed by re-chromatography of (I) on a 2 5-cm × 5-cm column of 'Zeo-Karb 225' (× 4) in the H$^+$ form eluted with 2 5 *N* hydrochloric acid (I) was isolated from the aqueous solution of its hydrochloride by the addition of pyridine until the *p*H was 4 6

The properties of (I) have led us to conclude that it is the previously unknown trisulphide analogue of cystine, *bis*(β-amino-β-carboxyethyl) trisulphide, $[HOOC\ CH(NH_2)\ CH_2]_2S_3$

Thus, when (I) is oxidized by bromine, with the theoretical uptake of oxygen, cysteic and sulphuric acids are obtained in the molecular proportions of 2 1 On treatment with cyanide under the conditions of Sorbo[5] (omitting Cu^{++} ion), one-third of the sulphur of (I) is converted to thiocyanate Approximately one-third of the sulphur of (I) is released as hydrogen sulphide during reduction with tin or zinc, and hydrochloric acid, and the reduced solution contains cysteine and no other thiol By 'formol' titration (I) has an equivalent weight of 138 (theoretical 136) Elementary analysis gave C, 27 1, H, 4 4, N, 10 0; S, 36 4 per cent ($C_6H_{12}O_4S_3N_2$ requires C, 26 5, H, 4 4, N, 10 3, S, 35 3 per cent)

The compound is stable in the dry state or in strongly acid solutions, but in neutral or alkaline solutions dismutates to give cystine and other products When chromatographed on paper in *n*-butanol/acetic acid/water (4 1 5 v/v), (I) moves as a single spot with R_F 0 09, but analytical amounts (< 0 5 mgm) chromatographed on 0 9-cm × 40-cm columns of 'Zeo-Karb 225' (× 4) resin by the hydrochloric acid elution method are resolved into a double peak, indicating the presence of two stereoisomers

By refluxing a suspension of *N*-acetylcysteine in chloroform containing sulphur dichloride with constant stirring for 40 hr, distilling off the chloroform, and hydrolysing the residue for 4 hr in 5 *N* hydrochloric acid we have obtained, in addition to cysteine, cystine and one other unidentified minor product, a substance the chromatographic behaviour of which is identical with that of (I), but which has not yet been further characterized

The formation of the trisulphide must involve the scission of a carbon–sulphur bond in cystine We have, therefore, examined cystine solutions that had been subjected to a 4 × 10^7 r dose of γ-irradiation from a multicurie cobalt-60 source Traces of (I), about 3 per cent of the initial cystine sulphur, were found, but not sufficient to make this a practicable source of the compound

This work, which is sponsored by the Agricultural Research Service of the U S Department of Agriculture under the authority of Public Law 480, will be published in detail elsewhere

J C Fletcher
A Robson

Wool Industries Research Association,
Torridon, Headingley Lane,
Leeds 6

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An Interpretation of the Transition in Composition of Soluble Lipoproteins

A recent examination[1] of a wide range of soluble lipoproteins has shown that a transition in the proportions of protein, phospholipid and neutral lipid occurs in the region where these three components are present in about equal amounts This transition is clearly evident when the percentage of neutral lipid (Y) is plotted against the proportion of phospholipid in the protein–phospholipid complex (X) Curves fitted to the experimental points for 42 lipoproteins by least squares showed that two linear equations

$$Y = 8\ 54 + 51\ 6\ X \text{ (for } Y < 40 \text{ per cent)} \qquad (1)$$

$$Y = 8\ 73 + 99\ 5\ X \text{ (for } Y > 40 \text{ per cent)} \qquad (2)$$

represented the data more adequately than a cubic equation fitted to all the points However, both types of equation show evidence of the transition since the discontinuity between the linear equations and the point of inflexion in the cubic occur in the same region From other experimental evidence[1,2] it was suggested that lipoproteins represented by equation (1) may have a structural integrity approaching that of a protein molecule, while those conforming to equation (2) evidently have a lipid core A difference in structure, however, does not indicate why this transition occurs where the components are present in equal proportions The balance of non-polar forces between the water-soluble protein–phospholipid complex and neutral lipid was examined and appears to provide a possible explanation for the transition

It was assumed that polar forces are primarily responsible for the interaction between protein and phospholipid, that non-polar forces predominate between neutral lipid and this complex, and that the CH_2 content of each component can be used as an index of the non-polar forces involved, although the availability and interaction potential of each CH_2 group are unknown There is experimental evidence to support some of these assumptions, for example, most of the neutral lipids can be extracted from lipoprotein solutions with ethyl ether, but more-polar lipid solvents are required to extract all the phospholipid

The CH_2 content of the protein moieties of a few lipoproteins was estimated from their amino-acid composition[3] and Waugh's[4] estimates of the CH_2 values for amino-acids, and found to be 17 m moles/gm The CH_2 content of phospholipids and neutral lipids was estimated from their average composition[5,6] and molecular weight Phospholipids had a CH_2 content of 42 ± 2 m moles/gm and neutral lipid ranged from 57 m moles/gm for the predominantly triglyceride types to 63 m moles/gm for the cholesterol-rich low-density fractions of blood serum The lower value was considered more representative of neutral lipids in most lipoproteins These values (17, 42 and 57 m moles/gm) were used to compute the CH_2 content of the lipoproteins represented by equations (1) and (2) since their CH_2 content is determined primarily by the proportions, rather than the composition, of the three components

These figures show that the sum of the CH_2 contents of the protein and phospholipid is essentially equal to that in the neutral lipid when the three components are present in equal proportions, as they are at the transition zone To extend this comparison over the entire range of lipoprotein composition, the CH_2 content of the neutral lipid was subtracted from the CH_2 content of the predominantly polar protein–phospholipid complex, and this algebraic difference is plotted against the total CH_2 content of the lipoproteins in Fig 1, together with a supplementary scale that serves to indicate their total lipid content The solid lines represent the values obtained from the fitted curves described by equations (1) and (2) For comparison, the points represent the average compositions reported[5] for the four main classes of serum lipoproteins, and are independent of the above equations

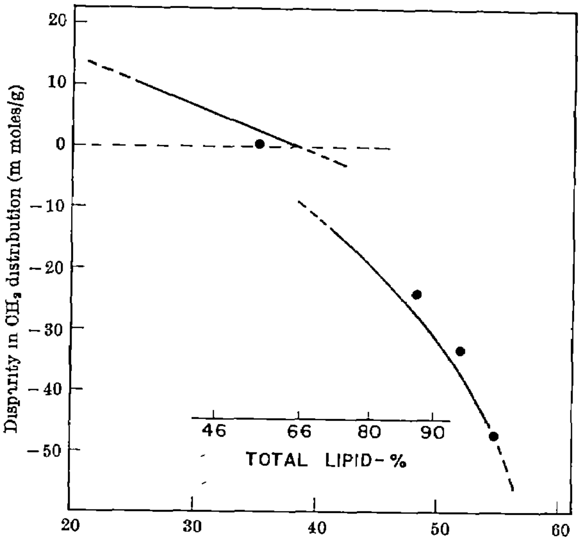

Fig 1 Disparity in CH_2 distribution in lipoproteins of different CH_2 contents Disparity is CH_2 content of protein and phospholipid less that in neutral lipid Total lipid content is indicated on inserted abscissal scale

The slope of the lines and the discontinuity at the transition zone, as shown in Fig 1, are naturally dependent on the equations used for their construction The position of the curves, however. is determined by the CH_2 content of the three components Lipoproteins with positive ordinates have sufficient CH_2 groups in their protein–phospholipid complex to interact with all those in the neutral lipid and the latter could therefore be distributed throughout the volume of the molecule Beyond the discontinuity the CH_2 groups on the neutral lipid predominate (negative ordinates) and, if they interact strongly with one another, as suggested by the abrupt transition, this would produce a lipoprotein with a lipid core A similar conclusion has been reached on the bases of particle size and neutral lipid content[1] Although the agreement with observation may be fortuitous, the present interpretation, based on the relative number of CH_2 groups in the neutral lipid and other components, is consistent with available information on lipoproteins and possibly explains why the transition zone appears where the three components are present in equal amounts

W H Cook

Division of Applied Biology,
National Research Council,
Ottawa, Ontario

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Non-Enzymatic Hydroxylation of Aromatic Compounds

In a recent communication, Dewhurst and Calcutt[1] discussed the hydroxylation of polycyclic aromatic hydrocarbons by the Udenfriend[2] system This system, consisting of a buffer, substrate, ascorbic acid and a metal complex in the presence of oxygen, is capable of hydroxylating aromatic compounds[2,3] However, the system is also capable of oxidizing aliphatic[4,5] and heterocyclic[6–8] compounds, and hence the hydroxylation of aromatic compounds may be regarded as a special aspect of a more general oxidation reaction

The hydroxylation reaction is believed to proceed through two intermediate steps (1) the metal-catalysed oxidation of ascorbic acid to hydrogen and monodehydroascorbic acid free radicals[9], (2) interaction of these free radicals with the substrate[10]

Under the experimental conditions usually adopted in the Udenfriend hydroxylation, the rate-determining step of the reaction is the first This will, in turn, depend on the catalysing power of the metal complex present in the system The availability of the metal ion will then be a function of the stability of the metal complex and the redox potential of the cation It is unlikely, however, that a simple correlation between the extent of hydroxylation and the stability constant of the metal complex can be determined experimentally Under the oxidizing conditions of the reaction the true value of the stability constant of the complex is unknown and may be different from the constant under normal conditions Hence a relationship between the yield of hydroxylated product and the stability constant of the complex will be of only a semi-quantitative nature

We have studied the effect of various conditions on the Udenfriend hydroxylation of aniline with a system consisting of 710 μmoles ascorbic acid, 75 μmoles cation, 400 μmoles disodium salt of ethylenediamine tetraacetic acid, oxygen and 300 μmoles substrate in a phosphate-buffered aqueous solution of a total volume of 15 ml The cation used was ferrous ion, containing ferric ion In view of the kinetics of the reaction, and of our unpublished results and results of the studies of the ascorbic acid-induced oxidations of non-aromatic compounds, we are of the opinion that the ferric state is the significant state for the catalysed oxidation of ascorbic acid and subsequent oxidation of the substrate In general, the higher valence state of the cation is necessary for the catalysis of the reaction

Various ligands were added to the model system, and the effect of the metal complexes on the activity of the system in converting aniline to *p*-aminophenol is shown here (Fig 1) The *p*-aminophenol was determined by Brodie's method[11], while the existence of *o*-aminophenol was established by paper chromatography The results indicate the absence of a simple correlation between the yield of product and the stability constant of the complex

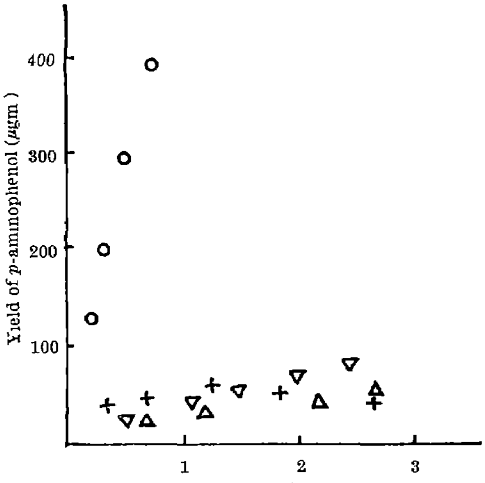

Fig 1 Effect of complexing agents on the conversion of aniline to *p*-aminophenol O Ethylenediamine tetraacetic acid, Δ, tartaric acid, +, acetyl acetone, ∇, oxalic acid

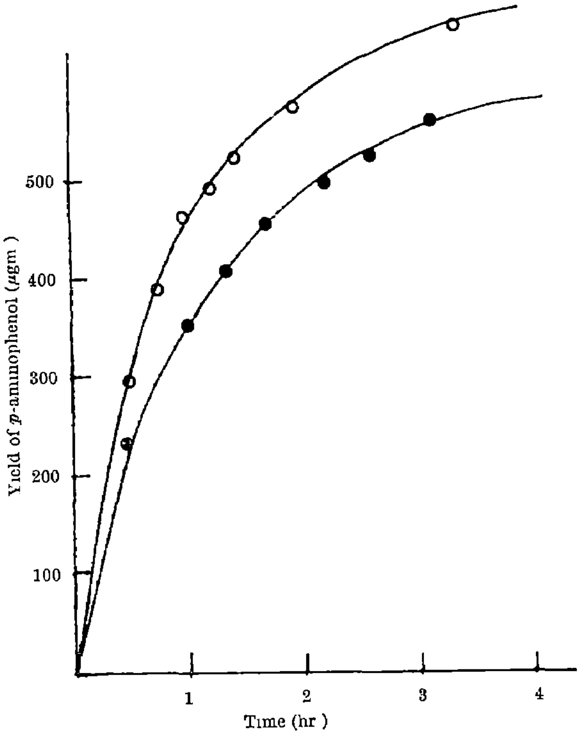

Fig 2 Inhibition by fluoride ion of the conversion of aniline to *p*-aminophenol O, Ethylenediamine tetraacetic acid, ●, ethylenediamine tetraacetic acid plus fluoride

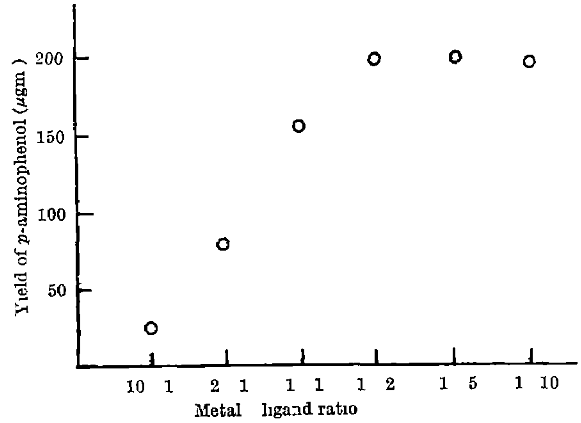

Fig 3 Effect of varying metal ligand ratio on the conversion of aniline to *p*-aminophenol

Dewhurst and Calcutt[1] reported the failure of cyanide to inhibit the system completely This is in agreement with our results for fluoride inhibition shown here (Fig 2) For the iron ethylenediamine tetraacetic acid complex, the yield of product is an exponential function of time, an apparent first order reaction If fluoride is then added to the system the activity of the system is only partially inhibited, and is still of the same form, that is, the yield of product is again exponential with time At this stage no explanation is offered as to this apparent anomalous behaviour

Not only the nature of the metal complex but also the metal ligand ratio is of importance in a consideration of the hydroxylating power of the system Butt[12] found that the metal ligand ratio had marked effects on the autoxidation of ascorbic acid If the assumption is correct that the rate-determining step in the hydroxylation process is the metal-catalysed autoxidation of ascorbic acid, then the metal ligand ratio should have a marked effect on the overall yield of hydroxylated product We have shown this to be correct (Fig 3)

J H Green
Department of Nuclear and Radiation Chemistry
B J Ralph
P J Schofield
School of Biological Sciences,
University of New South Wales,
Kensington, Australia

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PHYSIOLOGY

Excretion of Haptoglobin in Normal Urine

The presence of haptoglobin in normal urine was first suggested by Patte *et al* [1] and was later established by direct immuno-precipitation[2] The haptoglobin found in urine gave a reaction of immunological identity with purified plasma haptoglobin However, it could not be detected in 4 of 12 individuals whose urine was examined

The varying ability of different normal individuals to excrete haptoglobin could be ascribed to the particular genetically controlled haptoglobin type of the individual Thus the low molecular weight of haptoglobin of type 1-1 should be more easily excreted by the kidney than the high molecular weight polymers characteristic of haptoglobin type 2-2 In order to test this hypothesis urine was collected from one individual of haptoglobin type 1-1, six individuals of type 2-1 and two of type 2-2 Twenty-four-hour urine specimens were used The urine proteins were concentrated by means of ultra-filtration as described by one of us[2] Visking dialysis tubing of 8/32 in inflated diameter was used as the ultra-filtration membrane The urines were concentrated about 2,000–3,000 times to volumes of 0 30–0 68 ml Before the final concentration step the urine was dialysed against 0 9 per cent saline

The urine concentrates and sera from the same persons were examined by vertical starch gel electrophoresis in borate buffer at *p*H 8 6 (ref 3) Hæmoglobin was added to the samples prior to electrophoresis After the electrophoresis the gel was cut into two slices The top slice was stained for protein with amido black and the bottom slice for haptoglobin with the benzidine reagent The protein staining showed the presence of distinct albumin and transferrin *C* bands Two or three additional protein bands were observed between the albumin and transferrin bands The benzidine stain showed the presence of haptoglobin in all urines except in the two individuals of haptoglobin type 2-2

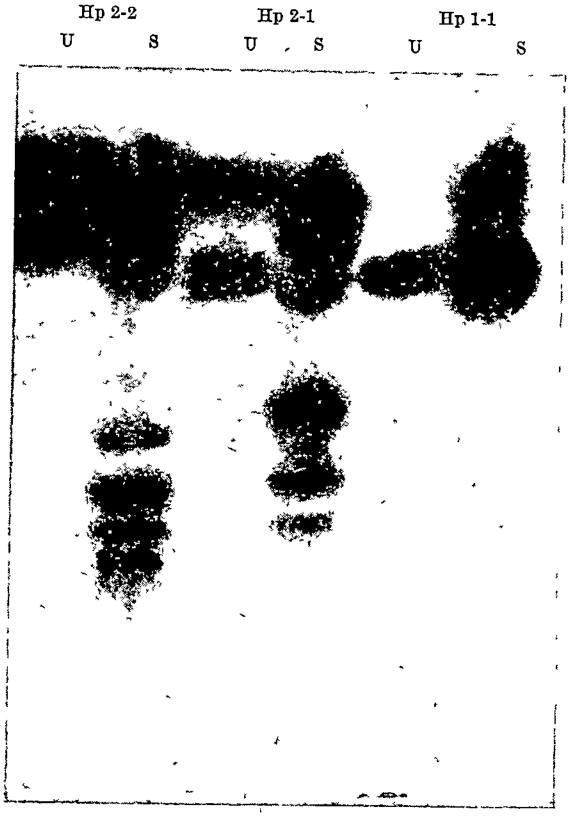

Fig 1 Analysis by starch gel electrophoresis of haptoglobin in sera (S) and urines (U) of normal individuals of the haptoglobin types 2-2, 2-1 and 1-1 Benzidine stain Hæmoglobin was added in excess prior to electrophoresis and bands of free hæmoglobin are also seen

Fig 1 shows a comparison of the patterns of the three haptoglobin types in serum and urine The urine of the individual of haptoglobin type 1-1 had a distinct haptoglobin 1-1 band and this band was also observed in the urines of the six individuals of haptoglobin type 2-1 In addition, the urine from type 2-1 individuals contained small amounts of haptoglobin polymers, as evidenced by one or two weak bands corresponding to the most rapidly moving polymeric bands of the corresponding serum The relative intensities of the haptoglobin 1-1 band and the polymeric bands varied in different urines The urinary 2-1 pattern reproduced in Fig 1 was one with a relatively strong 1-1 band and only one weak polymeric band If the tubular re-absorption of protein is non-selective[4] the observed variation might reflect individual variations in the selectivity of the glomerular filter Generally, the urinary haptoglobin bands showed a somewhat higher mobility than the corresponding serum bands

From this investigation it appears that haptoglobin polymers occur in detectable quantities in the urine from individuals of type 2-1 but not in urine from individuals of type 2-2 Indeed, polymeric haptoglobin would be more easily excreted in urine of type 2-1 individuals than in urine of type 2-2 individuals since part of the polymeric haptoglobin from 2-1 individuals has a smaller molecular size than the polymeric haptoglobin from 2-2 individuals[5] The fact that not even the most rapidly migrating polymer of the 2-2 sera could be detected in the corresponding urine is probably due to the low concentration in serum of this polymer (Fig 1)

It has been shown previously that patients with the nephrotic syndrome belonging to haptoglobin type 1-1 may excrete several grams of haptoglobin per litre of urine[6] Using starch gel electrophoresis, Marnay[7] recently studied the haptoglobin patterns in nephrotic urines of patients belonging to the types 2-2 and 2-1 Haptoglobin was not observed in the urines of patients of type 2-2, whereas the 2-1 urine showed distinct 1-1 bands and two weaker bands corresponding to the most rapidly migrating polymeric bands in serum. The present investigation shows that the haptoglobin patterns of normal urine are similar to those reported for nephrotic urine Rowe and Soothill[8] have presented evidence interpreted as indicating a lower degree of selectivity from the point of view of molecular weight for the excretion of serum proteins by the normal than by many nephrotic kidneys Using a quantitative immunochemical gel diffusion technique, these authors reported approximately the same urine/serum ratios for albumin, transferrin, γ-globulin and ceruloplasmin for normal individuals whereas a high selectivity between these proteins has been reported in nephrotic patients showing minimal renal histological changes[9] The present investigation, in conjunction with the study of Marnay, indicates that the renal discrimination for haptoglobins of differing molecular size is markedly selective and similar for normal and nephrotic kidneys

This work was supported in part by a grant from the National Institutes of Health, U S Public Health Service

I BERGGÅRD
A G BEARN

The Rockefeller Institute,
New York 21.

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Pigment and the Olfactory Mechanism

THE pale-yellow to dark-brown colour of the olfactory epithelium has led many to suppose that pigment is essential to olfaction[1–3], and the finding that there are stores of carotenoids in the olfactory epithelium of cattle[4,5] has given some support to the view that these compounds are involved[6,7] More specifically, Briggs and Duncan[5] have recently proposed that—on analogy with vision—carotenoids are the receptors of energy from odorous molecules The purpose of this note is to present evidence inconsistent with such theories

If carotenoids were as essential to olfaction as they are to vision they should commonly be found in vertebrate olfactory epithelia There seems to be no evidence for this, indeed, Gerebtzoff *et al* [2] found no carotenoids in rabbit or sheep olfactory tissue Are there, then, species differences in the distribution of these pigments ? To test this hypothesis, olfactory epithelia from one cow, 32 albino rats, 29 black rats and three pigs were investigated[8] The heads of the freshly killed animals were sawn in half along the nasal septum to expose the turbinals, and all yellowish or yellowish-brown epithelium was dissected out and weighed After grinding down the tissues to a smooth paste, they were saponified in 80 per cent methanolic potassium hydroxide solution, and then extracted repeatedly with petroleum ether The pooled extracts for each group and a control solution of β-carotene were chromatographed separately on alumina impregnated filter paper[9] in an atmosphere of carbon dioxide The deep yellow bands of pigment separating out from the cow extract, and the single band on the control strip, gave strong positive reactions with antimony trichloride (tha Carr–Price reagent), indicating considerable concentrations of β-carotene and other carotenoids in the cow tissue The same test, however, failed to reveal any carotenoids in the almost colourless extracts from the rat and pig groups

Non-carotenoid pigments present in the saponifiable portions from all groups, and separable into three fractions by paper chromatography, are apparently responsible for the yellowish-brown colour of the rat and pig olfactory epithelia They appear to be either auto-oxidation products of phospholipids[10], or are adsorbed on to phospholipids (predominantly lecithins)[2] In view of widely cited reports that albinos are anosmic (due—it is implied—to the lack of olfactory pigment)[1], it is interesting that no significant differences could be found in the colour, quantity or composition of these pigments in the black and albino rats It seems likely that such claims have arisen through a misinterpretation of the phenomenon of photodynamic sensitization

The results indicate that while there are stores of carotenoids in the olfactory epithelia of cattle, these pigments are absent, or present in only trace quantities. in the epithelia of rats and pigs It appears, then. that cattle are the only species in which carotenoids have been identified in olfactory tissue It is significant that they are also among the few mammalian species known to store primary carotenes in the fatty tissues of their bodies, and among those species which do not are the rat, pig and cat Thus Goodwin's suggestion that the carotenoids in cattle olfactory tissue are waste products without functional significance seems a reasonable one[11]

The appearance of anosmia in rats deprived of vitamin A[6,12], and the return of olfaction in some anosmic patients, have been cited—but cannot be accepted—as further evidence that carotenoids are involved in the olfactory process[5] Responses to odour depend on nasal mucous membrane function[13] and such membranes are highly sensitive to variations in vitamin A concentrations of the body[12,14] Furthermore, the extent to which recovery of 'olfaction' reflects changes in odorant access to the acutely chemo-sensitive 'free' nerve endings of the nasal mucosa is not known

Finally, it should be emphasized that radiation theories of olfaction—which include those which allot a role to carotenoids and other pigment in the olfactory mechanism—have received extensive and important criticisms from Thompson[15] on both theoretical and practical grounds, and appear to be untenable In any event, there exist theories which disregard colour, and which seem more in accord with available evidence[16] The assumption that the pigment is present in the receptors is without support from the great majority of relevant studies[17], while there are reports that the pigment and olfactory areas do not coincide[18], and that it is lacking from the olfactory area of some species with known olfactory powers[2] Thus, until there is much more information concerning the location, chemical composition, and distribution of the pigment complex in representative vertebrate species, any theory implicating these compounds in the olfactory process must be viewed with extreme caution

D G MOULTON

Division of Physiology,
Florida State University,
Tallahassee, Florida

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IN his critical review of recent investigations[1,2] on the possible relationship of carotenoid pigments to olfaction, Dr D G Moulton has made four points that he regards as conclusive against carotenoid pigments being involved in the olfactory mechanism We wish to comment on the validity of these four points

(1) It is claimed that carotenoids do not occur in the olfactory epithelium of all mammals Our work[1,3] has so far been confined to the cow and the dog, both of which possess large amounts of olfactory carotenoids Negative findings for rabbits and sheep have been reported by Gerebtzoff and Phillippot[4,5], for salmon by Wright[6], and now for rats and pigs by Moulton

It is well known[7] that some mammals preferentially accumulate carotenoids If carotenoids are present in the olfactory areas of only this group, as Moulton suggests, then why have no carotenoids been detected in the olfactory areas of sheep (a carotenoid-accumulator[8]) while they have been detected in the olfactory area of the dog (which is not a carotenoid-accumulator[9,10]) ? This evidence renders untenable Moulton's hypothesis for the distribution of olfactory carotenoids

An alternative hypothesis concerns the difficulties of carotenoid analysis and wide differences in tissue concentrations Thus, in the experimental work described in Moulton's communication, detection of carotenoids was by reaction with the Carr-Price reagent on paper chromatograms This is scarcely sufficient to identify carotenoids, for not all react with antimonytrichloride, which in any event is not specific for carotenoids, while some give very transitory colours[11] As spectrophotometric methods can be applied to detect even very small amounts of carotenoids, it is essential that this method be used wherever possible, together with a comparison of chromatographic behaviour, *cis-trans* equilibration, and derivative formation against known standards where quantities available render these tests possible As an example of the difficulties involved in the detection of carotenoids, consider the case of retinene in insects Many investigators[12] have failed to detect this haplocarotenoid in insect eyes, but a re-examination[13] of this question has shown that retinene occurs in most insects, and the insect visual pigments so far obtained[14] are retinene-containing rhodopsins It is quite possible that a similar situation exists with olfactory carotenoids, which apparently show very wide species variations in concentrations and the olfactory tissues are difficult to collect and preserve

(2) Moulton claims that the loss of olfaction by animals raised on a carotenoid-free diet is not evidence that carotenoids are involved in olfaction as the loss is open to other explanations This is undoubtedly true But even if the carotenoid hypothesis of olfaction were based solely on this prediction (which it clearly is not), it cannot be falsified in this manner The nutritional observations are merely supplementary evidence It still remains to be demonstrated whether or not carotenoid-deficient animals are anosmic due to lack of essential olfactory pigment or to the sort of changes mentioned by Moulton

The pathological changes in epithelia occurring in carotenoid-deficient animals are thought to be due to an absence of carotenoids from the epithelial cells Hence changes in the olfactory epithelia in carotenoid deficiency indicates that carotenoids are normal, essential constituents of the healthy tissues

(3) Vitamin A has proved of clinical benefit in the treatment of anosmia The effect is definite and marked in all but a few patients Moreover, the recovery of olfaction is almost always accompanied by the same clinical signs namely, the recovery first of the ability to detect unpleasant odours, followed some appreciable time later by the return of pleasant odours The suggestion of Moulton's that the effects are merely due to an improvement in the status of tissue vitamin A is most unlikely The patients showed no signs of avitaminosis A and there was no clinical evidence of dryness of the nose[3]

(4) Moulton's criticisms of our postulated molecular basis of olfactions do not seem valid There is no doubt that some naturally occurring carotenoids occur in the *cis*-form[15] and there is no evidence either way on the geometrical isomerization of the olfactory carotenoid Some preliminary evidence on extracts from the cow suggests that an appreciable proportion is present as hindered forms[16] The evidence on the presence or absence of pigment in the receptor hairs is inconclusive There is no doubt that most of the pigment is present in the supporting cells and in Bowman's glands, but whether physiologically significant amounts of pigment are present in the receptors is unknown Certainly one group of workers[17] who have studied the histology of the olfactory sensory cells and pigment distribution were struck by the resemblance to the retina Perhaps the carotenoids in the Bowman's gland secretion, which bathes the receptor hairs, are the primary odour receptors

In summary, we would say that, first, the evidence regarding the occurrence of carotenoids in the olfactory area of mammals is insufficient to draw any definite conclusions, and that the difficulties of carotenoid detection, illustrated by the case of insect retinene, should be borne in mind in assessing the evidence Secondly, that our clinical findings on anosmia cannot be ascribed to dietary lack of vitamin A Thirdly, that the histology of the olfactory areas is insufficiently understood to rule out a role for carotenoid-proteins in odour reception

M H Briggs *
Victoria University of Wellington,
Wellington, New Zealand

R B Duncan
403 D I C Building,
Wellington, New Zealand

* Present address Space Biology Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena

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Effect of Environmental Temperature on Conditioning in the New-born Poikilothermic Rat

In the rat a period of poikilothermy exists for approximately the first seventeen days of life[1-2] In this phase of the development action potentials and nerve conduction rate decrease with the lowering of the environmental temperature[3] Temperature may change, therefore, the ability of the new-born rat to learn the conditioned leg withdrawal to a vibrotactile stimulus

Fifty-six new-born Sprague-Dawley rats were used The animals were immobilized and the unconditioned stimulus, a direct current electric shock of 1 0 m amp and 50 msec duration, was delivered to the foot of the right foreleg, which was connected to a motion displacement recorder[4] The conditioned stimulus was a vibration of 128 c/s applied to the rat's chest and preceded presentation of the unconditioned stimulus Trials with the vibration were given all animals to ensure that leg withdrawal did not occur prior to conditioning Forty rats were divided into four equal groups Each group received eighty trials of the conditioned and unconditioned stimulus The four groups differed in the following manner in groups I and II the environmental temperature during conditioning was 28° C whereas in groups III and IV it was 23° C Furthermore, in groups I and III the conditioned stimulus preceded the onset of the unconditioned stimulus by 1,800 msec and for groups II and IV the interval was 900 msec (Fig 1) The two conditioned stimulus values utilized in this investigation were based on previous work demonstrating reliable conditioning in the new-born rat for durations between 600 and 2,400 msec[4] Testing was done in a temperature-controlled room (± 1 0° C) and the body temperature of the subject was monitored by a rectal thermocouple (± 0 1° C) Conditioning commenced when the rectal temperature had stabilized at either 28° C or 23° C For each of the experimental groups a pseudo-conditioning control group ($N = 4$ per group) was tested utilizing the same conditioned and unconditioned stimuli, but with their order of presentation random A conditioned response was defined as any right foreleg withdrawal occurring in the interval from 50 msec after the onset of the conditioned stimulus to its termination

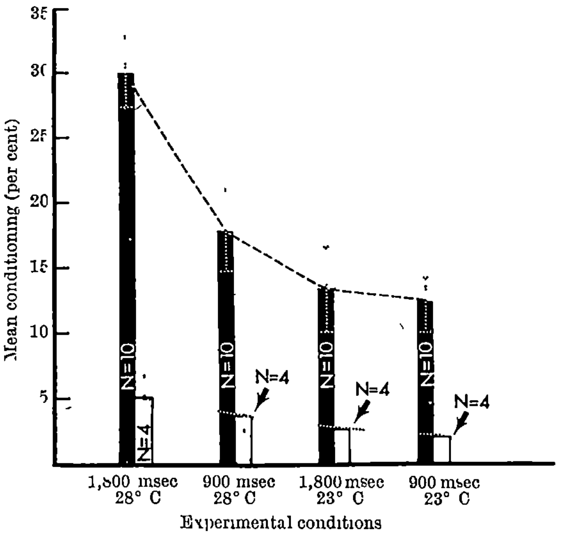

Fig 1 Environmental temperature affects the level of conditioning in poikilothermic new-born rats This variable interacts with the duration of the conditioned stimulus Black bars represent experimental groups, white bars the respective pseudo-conditioning control groups The range of one standard deviation is indicated at the top of each group bar

Fig 1 presents the mean per cent conditioning and standard deviation for the four experimental and control groups based on the eighty conditioning trials

The performance difference between experimental and control groups indicates the validity of the conditioning data Rats in the 1,800 msec, 28° C group manifested the highest performance level (mean per cent = 29 8, *S D* = 5 66) followed by the 900 msec, 28° C group (mean per cent = 17 9, *S D* = 6 63) In turn, both these groups were superior in conditioning to the 1,800 and 900 msec, 23° C treatments (mean per cent = 13 2, *S D* = 6 39, mean per cent = 12 2, *S D* = 3 50, respectively) Although the 1,800 msec, 23° C group was superior to the 900 msec, 23° C condition, this difference amounted to only 1 0 mean per cent

These results clearly indicate an interaction between conditioned stimulus duration and environmental temperature Furthermore, the contention that formation of a conditioned reflex in the poikilothermic new-born rat is significantly altered by environmental temperature is demonstrated by the higher level of conditioning found for the 28° C temperature, as compared with 23° C for both temporal intervals

This work was supported in part by the U S Atomic Energy Commission under contract *AT*-(11-1)-821

D F Caldwell
R Brand
J Werboff

Animal Behaviour Laboratory,
Lafayette Clinic and
Wayne State University,
Detroit

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PATHOLOGY

Pathological Zinc-rich Synapses

Experimental carbon-monoxide hypoxia in the guinea pig, if sufficient in tempo or duration to produce seizure, tends to create nerve-cell soma changes of the 'eosinophilic ischæmic' type relatively selectively in 'region *CA*2' of the hippocampal pyramids '*CA*2' may be defined as the zone of *CA*3-type large neurones the apical-dendrite shafts of which are traversed by the end-bulb of the mossy fibre system[1], and is particularly well delineated in the guinea pig because the 'mischzone' that occurs between *CA*1- and *CA*3-type pyramids in most other mammals is here negligible and the end-bulb is unusually bulbous

In a series of 14 guinea pigs where serial sections of the brain were impregnated by the silver sulphide method for zinc-enzymes[2], after various numbers of exposures of the animals to carbon monoxide, 4 seizure cases in which the '*CA*2 ischæmia' was well marked displayed conspicuous pathology within the end-bulb The 'presynaptic bags'[3] or 'swellings'[4] at the sites of synapses-de-passage of the mossy fibres had here a flattened 'collapsed' appearance, compared with their normal roughly circular contour elsewhere They formed a contiguous series of Morse-code-like 'dashes' outlining the shafts of *CA*2 apical dendrites, giving a railway-track appearance to longitudinally cut shafts In another 6 of the cases (2 without seizures), no ischæmic changes could be detected in *CA*2 somata or processes by Nissl, hæmatoxylin and eosin, PTAH, periodic-acid–Schiff, methylene-blue, luxol-fast-blue or Weil staining, but moderate to severe stages of 'synapse collapse' were present around and along especially soma-proximal parts of shafts within the end-bulb In the remaining 4 (seizure) cases 'collapse' was obscured by 'shrinkage' 7 'controls' with no hypoxia, or with under 30 min survival, and no *CA*2 ischæmia, showed no 'collapse' within the end-bulb in either hemisphere

To test the possibility that such 'synaptic collapse' might be a precursor of ischæmic soma changes wherever hippocampal pyramids synapse with the axons of the granule cells of the fascia dentata, another 7 guinea pigs had ischæmic somata created in 'region *CA*3' by means of other types of hypoxia, such as low oxygen-tension, prolonged ether anæsthesia and circulatory stagnation In none of their 14 hemispheres could a convincing picture be obtained, in varied planes of section, of 'collapse' along the apical-dendrite shaft of even one *CA*3 pyramid Yet in each of the five of these cases in which *CA*2 had no ischæmic somata, the self-same type of 'proximal collapse' was to be seen within the end-bulb as seen in the six non-ischæmic carbon monoxide cases already mentioned (After repeated hypoxic seizures of whatever type, there was a less obvious tendency for 'region *CA*4' to exhibit a sprinkling of ischæmic somata and of 'collapsed' synapses around the soma-membrane of other-than-ischæmic 'pyramids') Presynaptic-bag 'flattened collapse' would therefore appear to be a pathological process essentially confined to the end-bulb and not necessarily spatially related to, though temporally prodromal to, ischæmic soma changes due to hypoxia It may be noteworthy that of the cases showing 'early collapse' but no *CA* ischæmia, one had survived onset of ether anæsthesia by only $3\frac{1}{2}$ h, one had survived the start of low-oxygen-tension exposure by only $1\frac{1}{2}$ h, and one had survived, from the start of exposure to carbon monoxide without seizure, for only 48 min This aspect of the prodromal nature of the synaptic pathology was not under explicit exploration in these experiments

Finally, in 8 guinea pigs experimental seizures were produced by insulin hypoglycæmia Repetitive seizures in 3 created acute non-eosinophilic, but strikingly 'luxolophilic', shrunken-soma cell-changes throughout 'region *CA*1', with no conspicuous changes in other *CA* pyramids In all three of these, synaptic pathology was clearly revealed by the silver sulphide method for zinc Here again it was only within the end-bulb, but it was now characterized by a fairly uniform 'shrinkage' of most of the, still roughly circular, presynaptic bags 3 cases with no detectable *CA*1, or other *CA*, cell-soma changes showed lesser and presumably 'earlier' degrees of 'shrinkage', which tended to be most advanced near the centre of the end-bulb The 2 remaining cases were intermediate in respect of both *CA*1 cell-changes and end-bulb synaptic 'shrinkage' In 4 cases where insulin hypoglycæmia had been combined with carbon monoxide hypoxia, 'shrinkage' seemed to have largely precluded 'collapse'

At the end of my communication dealing with impregnation and histochemistry of the normal zinc-rich synapses[2], I touched on the appearance of a 'diffusion' of heavy-metal substance between the end-bulb and stratum oriens, through *CA*2 After one or two carbon monoxide seizures (5 animals) the intensity of this 'diffusion' was diminished below normal After three or more seizures (7 animals) it had completely disappeared in both hemispheres Within the 12 brains the degrees of 'diminished diffusion' roughly paralleled the degrees of 'synaptic collapse' in related end-bulbs This might be an indication that zinc normally 'overflows' from the mossy fibre system into stratum oriens in the region of *CA*2 (Special features at the 'uncal tail' of *CA*2 and the end-bulb will be described elsewhere) In the brains of two animals under the additional influence of amphetamine during carbon monoxide seizures, the degree of 'synaptic collapse' was, however, patently less than a hypothesis of simple causal relationship to 'diffusion' would predict

As remarked elsewhere[5,6], these pathological observations add to the list of peculiarities of the end-bulb *vis-à-vis* the rest of the mossy fibre system as revealed in ammoniacal silver impregnation and in histochemical enzyme studies of normal guinea pig hippocampi Functional elucidation should be forthcoming soon through the electron-microscopic, and more detailed enzymic, investigation of such experimental pathological states as described above From my further explorations of the effects of varied post-mortem delay in guinea pigs, there would seem to be no reason why the silver sulphide method should not be applied, experimentally or clinico-pathologically, to large mammals provided the brain, or biopsy therefrom, be reduced to room temperature within about 1 h after death

TURNER MCLARDY

The Laboratory,
St. Andrew s Hospital,
Northampton

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Effect of Tobacco Diets on Rodents

THE habit of chewing snuff or tobacco has been associated in human beings with the occurrence of leukoplakia and cancer of the buccal mucosa[1,2] In India, the chewing of dried and earth-cured tobacco along with betel nuts and slaked lime has been linked with oral cancer[3,4] Differences in incidence of cancer for various communities occurs according to the site at which tobacco mass is kept in the mouth The investigations on mice and rats recorded here show the effects of direct contact of tobacco with the buccal mucosa and effects on those organs responsible for its degradation

Male Wistar rats 6 weeks old and male mice 2 months old were fed commercially available tobacco products mixed with ground 'Purina' diet Control animals were fed ground 'Purina' chow without tobacco All animals were weighed monthly The mice and rats were killed after being on the experimental diets for 15 months and 18 months respectively

For the cigarette experiments the cigarette paper was removed and the tobacco dried on metal trays and then mixed with ground 'Purina' chow The ratio, by weight, of tobacco from low nicotine cigarettes to food was 1 25 for mice, for rats it was 1 25 for 3 months, then changed to 1 30 for the remainder of the experiment Rats were also fed tobacco from a mixture of popular brands in a ratio of 1 25

Both mice and rats were fed snuff Fresh commercial snuff was dried in a vacuum desiccator until the 1¼ oz contents of one can weighed 19 g This dried snuff was mixed with finely ground food The rats were fed snuff in a ratio of 1 20, by weight, while the mice were fed snuff in a ratio of 1 4 for one month, 1 9 for 4 months and 1 18 for the remaining 13 months

Table 1 presents the number of animals constituting the various experimental groups as well as the number which remained when the experiments were terminated The final average weights for rats on experimental diets were significantly lower than the controls (0 05 level) and show a dose response pattern, indicating that snuff diet caused the greatest loss in weight followed by regular cigarette mixture and low nicotine cigarette mixture Throughout the experiment, mice on the snuff diet gained less weight than controls The difference in final weight was statistically significant (0 05 level) for *DBA* mice but not the $C_{57}B1$ mice

Table 1 MEAN AND STANDARD ERRORS OF FINAL WEIGHTS OF SURVIVING ANIMALS

Animals	Tobacco diets			Controls
	Snuff	Regular tobacco	Low nicotine tobacco	
Wistar rats	(16/40)* 235 ±13 8†	(17/31) 366±17 4	(22/34) 408 ±11 8	(31/32) 441 ±9 9
DBA mice	(23/34) 24 3± 0 42	—	(44/44) 26 3± 0 30	(17/17) 28 0±0 51
$C_{57}B1$ mice	(16/16) 24 7± 0 69	—	—	(20/20) 26 6±0 73

* Survivors/original group
† Average final weight of survivors

Survival of rats indicates a highly statistical significant trend (0 01 level) in toxicity corresponding to trend of weight reduction Snuff diet had a less-pronounced effect on survival of *DBA* mice, but survival was nevertheless significantly lower than control (0 05 level) These results are similar to those observed among regular smokers in human populations[5]

Animals were autopsied and histological sections of grossly abnormal tissue were studied Few pathological changes were noted Four rats which were fed snuff had interesting changes consisting of one leucocytic leukæmia, one kidney sarcoma and two with superficial ulcers of stomach mucosa Four rats given tobacco from popular cigarettes had chronic passive congestion of the liver Bronchiectasis was found in almost equal incidence in the low-nicotine tobacco group of rats and in control animals This observation had been previously reported and related to the ageing process of rats[6,7] Twenty-three mice, mostly *DBA*, on the snuff diet had nephritis as compared to 2 animals in the control group Calcareous pericarditis was found in 9 *DBA* experimental animals and 1 control, however, this lesion has been reported to occur spontaneously in *DBA* mice[8] Three *DBA* mice had leukæmia involving liver and kidney Tumours are known to occur spontaneously in these animals, but none was observed in the controls for these experiments The most striking abnormality found in *DBA* mice fed low-nicotine tobacco was the lip papilloma in 3 mice No malignancy developed, and all three eventually shed the growth Histological examination revealed that the stomachs of two mice on this cigarette diet showed hyperplastic gastric mucosa, a condition often found in human beings who smoke

In comparison with control groups, rodents fed unsmoked tobacco products reached and maintained significantly lower weight There is a reduction in the amount of food which the animals consume This has also been true of animals exposed to cigarette smoke or nicotine[4] The effect on weights and survival may be due to a combination of toxicity coupled with the possible appetite depressant action of nicotine This latter concept would tend to explain the less-pronounced effect of the low-nicotine tobacco cigarettes which are made from leaves that naturally have a low level of nicotine

JOSEPH A DIPAOLO

Roswell Park Memorial Institute,
New York State Department of Health,
Buffalo 3

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RADIOBIOLOGY

Tumour Development in the Rat induced by Blood of Irradiated Animals

EXPERIMENTS are being carried out to ascertain whether the incidence of tumours in non-irradiated animals can be influenced by the introduction into the body of fluids or tissues from irradiated donors This communication reports preliminary results from a three and a half year study of rats injected with blood plasma or with blood plasma ultra-filtrate from irradiated rats and sheep

Blood plasma was obtained separately from (*a*) lethally irradiated rats, (*b*) normal rats, (*c*) lethally irradiated sheep, (*d*) normal sheep Blood was collected over versene (sodium ethylenediamine tetraacetic acid, Na_2EDTA) under sterile conditions from ether-anæsthetized rats via the cava vein Samples from several animals were pooled and the plasma obtained by centrifugation Sheep blood was obtained without anæsthesia from immobilized animals through jugular venipuncture, collected in closed sterile plastic bags on Na_2EDTA, and the plasma expressed from the bags after being centrifuged The plasma samples of both rats and sheep were freeze-dried and kept at 4° C until used (not more than 4 days) The sheep plasma in addition was ultra-filtered in cold (cellulose dialysis tube, 'Visking') before being frozen Before injection, rat plasma was re-constituted to twice and sheep plasma ultra-filtrate to twenty times the original concentration

Animal species irradiated with cobalt-60 γ-rays were (*a*) adult Sprague-Dawley rats of both sexes, 1,250 r at 7 72 r /min, (*b*) adult grade Hampshire sheep, female or castrate male, 1,610 r at 0 95 r /min

Rats were injected intraperitoneally at 7 days of age with 0 2 ml of concentrated blood plasma from (1) irradiated and (2) normal rats the treatment was repeated on days 9 and 14 Of 126 rats in group 1, 50 died, as did 28 of 79 in group 2 There was no difference in mortality-rate between groups (In further experiments now in course, a much lower mortality was observed in rats injected with blood plasma from irradiated or from normal donors) When concentrate plasma ultra-filtrate from irradiated or normal sheep was used (as was also the case with physiological saline), new-born rats were injected in the heart cavities when less than 24 h old with 0 05 ml and again 7 days later, afterwards they were injected intraperitoneally at 12, 17, and 21 days of age with 0 3 ml each time Of 129 animals receiving intracardial and intraperitoneal concentrated plasma ultra-filtrate injections from irradiated or normal sheep, only 19 died, about the same mortality-rate as when saline was used Survivors weaned at 21 days of age were fed with Purina Laboratory Chow and water *ad libitum* Animals receiving blood plasma or blood plasma ultra-filtrate were observed for tumour development during their life-span, none of these animals was bred (Occasionally some rats in all of the groups developed incisors so long that to avoid death by starvation partial cutting of these teeth was required)

The differences between proportions were estimated using 95 per cent confidence intervals as the criteria to compare the tumour incidence among the groups

Female rats which received either blood plasma from irradiated rats or blood plasma ultra-filtrate from irradiated sheep showed a significant increase of tumours when compared with those injected with normal plasma or with physiological saline (Table 1) Tumour incidence in male rats showed no significant differences between the groups The time in which the first appearance of the tumour was noted was not significantly different between females injected either with plasma or ultra-filtrate and irradiated and normal animals (Table 2)

Table 1 Tumour development in rats injected with blood plasma from irradiated rats (Plasma, *I*), blood plasma from normal rats (Plasma, *N*), concentrated plasma ultra-filtrate from irradiated sheep (Ultrafiltr , *I*), concentrated plasma ultra-filtrate from normal sheep (Ultra-filtr , *N*), and physiological saline (Saline) F, Females, M, males

Plasma characteristics	No of rats		Rats with tumours In the group		Per cent	
	F	M	F	M	F	M
Plasma, *I*	61	15	52*	5	85	33
Plasma, *N*	33	18	18	5	54 5	28
Ultrafiltr , *I*	30	25	30*	6	100	24
Ultrafiltr , *N*	30	25	14	8	47	32
Saline	30	35	8	14	27	40

* Significantly different from normal at a level of $P < 0\ 05$

Table 2 Time at which rat tumours were first observed after being injected with blood plasma (rat) and blood plasma ultra-filtrate (sheep)

Plasma characteristics	No of rats		First tumour observation (range in days)	
	F	M	F	M
Plasma, irradiated	61	15	158–1,007	630–1,213
Plasma, normal	33	18	835–1,118	510–1,057
Ultrafiltr , irradiated	30	25	692–912	787–845
Ultrafiltr , normal	30	25	701–903	751–863
Saline	30	35	716–868	767–831

The histology of the tumours has not shown appreciable difference in the relative frequency of each particular type between the groups injected with plasma or with ultra-filtrate from irradiated or from normal animals, either in females or males In female rats 40 per cent of the tumours of the mammary gland were adenomata, the remainder being about equal proportions of fibroadenoma, fibroma, fibrosarcoma, and adenocarcinoma Tumours other than mammary were rarely observed in the female rat under conditions of the experiment In male rats tumours were mainly squamous papilloma, cavernous hemangioma located subcutaneously in the escapular or thoracic regions, and squamous carcinoma of the malar region with some fibrosarcoma and adenofibroma at various sites

Both incidence of tumour in males and their histological type were not apparently different from those spontaneously arising in the rat colony

Although the number of rats used in these experiments is rather small the incidence figure, as herein recorded, shows that blood plasma from irradiated animals (rats) and plasma ultra-filtrate (sheep) are capable of increasing the frequency of breast tumour in the female rat Explanation for this, other than the presence in the blood of irradiated animals of one or more tumorogenic substances or of factors potentiating the onset of mammary tumours, is difficult to conceive However, in virgin female Sprague-Dawley rats kept in this colony during their life span, the spontaneous incidence of mammary tumours has been about 55 per cent Howell and Mandl[2] found in rats of the Birmingham strain the incidence of spontaneous mammary tumours ranging from 49 per cent in nulliparæ females to 4 per cent in multiparæ Because of this high incidence, it appears that the possibility of other non-specific factors which may increase the number of rats with tumours cannot be excluded in any tumorogenic experiments with the Sprague-Dawley rats Shellabarger *et al* [3] observed a 90 per cent incidence of neoplasia of the mammary gland in female Sprague-Dawley rats exposed to total-body cobalt-60 γ-irradiation at 40 days of age and examined until death Mammary tumours may be induced in a high percentage of female rats by aminofluorene compounds, carcinogenic hydrocarbons, growth hormone, and œstrogens[4] Comparison of mammary tumours developed using blood from irradiated animals and with carcinogens is not possible at this time The incidence and type of breast tumour differ with the nature of the carcinogen, dose and route of administration, age of the animal at the time at which the carcinogen is given, diet, and the hormonal status of the rats[5–7] At the present time we have no adequate explanation for the reduced tumour incidence in female rats injected with saline

Development of mammary tumours in male rats injected with blood plasma of irradiated animals is not significantly different from that in males injected with normal blood plasma Lack of evidence exists, too, regarding male rats treated with carcinogens[6–8]

Tumours as developed in untreated Sprague-Dawley female rats are primarily mammary, other types amounting to only 5 per cent[5] It is not known at present whether tumours other than of the breast could be formed or potentiated by irradiated blood plasma or blood plasma ultra-filtrate in rats of other strains or in other animal species

JOSE SOUTO

Agricultural Research Laboratory*,
University of Tennessee, Oak Ridge

* Operated for the U S Atomic Energy Commission

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IMMUNOLOGY

Immunological Significance of the Thymus of the Adult Mouse

MICE thymectomized in neonatal life have shown a marked deficiency of small lymphocytes in the peripheral blood, spleen, lymph nodes and Peyer's patches[1–3] and a depression of the immune response to both viral and bacterial antigens[4] and to allogeneic[1,2,4,5] and even heterospecific[2,4] skin grafts Mice thymectomized after 3 weeks of age have not shown any significant depression of the immune response to foreign skin grafts nor any marked reduction in the lymphocyte population[1,2,4] These observations have suggested the hypothesis[1] that during embryonal and early neonatal life the thymus produces the progenitors of immunologically competent cells which mature and migrate to other sites, alternatively, it would produce a humoral factor essential for the maturation and proliferation of lymphocytes throughout the body during development Evidence from this laboratory, summarized below, suggests that the function of the thymus in initiating immunogenesis is not necessarily restricted to early life

Table 1 EFFECT OF THYMECTOMY IN ADULT LIFE ON THE FORMATION OF SHEEP RED CELL AGGLUTININS FOLLOWING SUBLETHAL IRRADIATION

Treatment	No of mice in group	No of mice with following log₂ titre agglutinins* 0	1	2	3	4	5	6
Thymectomized, irradiated, injected with sheep red cells	6	2	1	2	0	1	0	0
Sham-thymectomized, irradiated, injected with sheep red cells	8	0	0	1	0	3	3	1
Sham-thymectomized, not irradiated, injected with sheep red cells	12	0	0	1	1	3	4	3
Not thymectomized, not irradiated, not injected	10	10	0	0	0	0	0	0

* Serum titrated 7 days after injection of red cells

Table 2 EFFECT OF THYMECTOMY IN ADULT LIFE ON RECOVERY OF IMMUNE RESPONSE TO SKIN HOMOGRAFTS FOLLOWING SUBLETHAL IRRADIATION

Treatment	No of mice in group	Donor skin	No of mice showing skin graft survival for < 20 days	20–50 days	> 50 days
Thymectomized, irradiated	16	*C3H*	1	0	15
		C57BL	2	10	4
Thymectomized, sham-irradiated	18	*C3H*	18	0	0
		C57BL	18	0	0
Sham-thymectomized, irradiated	16	*C3H*	15	1	0
		C57BL	16	0	0
Sham-thymectomized, sham-irradiated		*C3H*	10	0	0
		C57BL	10	0	0

Twelve-week-old F_1 hybrid mice from a cross between *Ak* and *T*6 were thymectomized or sham-operated and given a single dose of total body irradiation of 350 r two weeks later They were challenged four weeks after irradiation with either sheep red cells or skin grafts from both *C3H* and *C57BL* donor mice The results are shown in Tables 1 and 2 It will be seen that a single dose of 350 r following thymectomy in adult life was associated with an impairment of the ability to recover the capacity for an immune response to both sheep red cells and skin homografts Furthermore, the impairment was long lasting in the case of grafts from donors closely related immunogenetically to the host It is possible that at higher levels of total-body irradiation exposure the impairment would have extended to grafts from donors distantly related to the host The thymus in adult life appears, therefore, to be essential for complete recovery of the immune mechanism following sub-lethal irradiation.

There is one cytological feature common to the thymus in neonatal life and the thymus regenerating in the adult animal after acute involution induced by such agents as irradiation[6] There is in the cortex of the normal thymus of the neonatal mouse a zone of immature, large lymphocytes with a very high mitotic index During the first two weeks of life, the immature cells in this zone differentiate rapidly and by three weeks of age the cortex is composed predominantly of mature small lymphocytes[7] During regeneration of the thymus following irradiation in the adult, the cortex resembles that of the neonatal thymus in showing a striking prevalence of immature lymphoid cells It is perhaps more than a mere coincidence that circumstances which prevent this lymphoid cell differentiation sequence from taking place both in neonatal and in adult life should be associated with deficiencies in the immune mechanism Results obtained with cytologically marked thymus grafts in neonatally thymectomized hosts indicate that some type of host cells (stem cells) invade the thymus graft and find in it an environment suitable for lymphoid cell differentiation and multiplication[4] The thymus-grafted animal eventually regains full immunological competence[1,2,4] Unlike thymus grafts, injection of dissociated thymus cells from neonatal donors into mice thymectomized at birth has failed to establish a properly functioning immune system[2] One therefore wonders whether the thymus initiates immunogenesis, not by actually generating, *ab initio*, the progenitors of immunologically competent cells, but by providing the environment wherein cells can differentiate and acquire the capacity for immunological competence The mechanism whereby this occurs, whether by direct cell to cell interaction, or by the elaboration of some specific humoral-like maturation factor by non-lymphoid thymic cells, is not known

I thank Prof Alexander Haddow for his interest in this work and Dr S M A Doak for help with some of the techniques used The investigations were supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U S Public Health Service They also received special support through a grant from the Tobacco Manufacturers Standing Committee

J F A P MILLER

Chester Beatty Research Institute,
Institute of Cancer Research
Royal Cancer Hospital,
Fulham Road, London, S W 3

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Non-Antigenicity of Synthetic Polyribonucleotides and Apurinic Acid

THE antigenicity of nucleic acids is a subject which has been extensively explored Much of the information available is clouded by the fact that the materials used were contaminated with protein and other cellular constituents so that the specificity of the antigen–antibody reactions observed has been unclear[1] Recently, however, two facts have emerged which leave little doubt as to the ability of nucleic acids to participate in immunological reactions The first is the observation that T_2, T_4 and T_6 phage DNAs, when highly purified, are capable of interacting with antisera prepared by inoculation of animals with whole phage lysates[2] The antigenicity of these DNAs depends upon the presence of glucosylated 5-hydroxymethylcytosine[3,4] Secondly, the sera of certain patients suffering from lupus erythematosus contain antibodies capable of reacting with native or denatured DNAs of diverse source[5,6] The antigenic site for this interaction appears to involve regions of the DNA molecule where thymine occurs in repetitive sequence[7]

In order to explore further the problem of the antigenicity of polynucleotides, attempts were made to immunize rabbits with the synthetic polynucleotides polyadenylic, polyuridylic, polycytidylic, and polyinosinic acids These homopolymers were prepared from the appropriate nucleoside diphosphates using a polynucleotide phosphorylase derived from *M lysodeikticus*[8] and were virtually protein free In addition, attempts were made to immunize rabbits with apurinic acid[9] derived from commercially obtained beef thymus DNA (Sigma Chemical Co) Two animals were used for each polynucleotide An initial injection of material emulsified in complete Freund's adjuvant was followed by 3 weekly series of 3 injections in saline, each animal receiving a total of 7 5–10 mg of polynucleotide The animals were bled 7 days after the last injection, and the sera were examined for the presence of antibody by the following methods Ouchterlony double diffusion in agar gel, Oudin single diffusion in agar gel, complement fixation, and classical immunoprecipitin techniques In all cases the results were entirely negative

Synthetic homopolynucleotides were used in this experiment because they are substances which do not occur in Nature, and it was hoped that, like certain synthetic polypeptides, they would have antigenic properties Highly purified T_2 phage DNA has been shown to be incapable of producing antibodies when used as the immunizing material *per se*[10] Methods are therefore being sought of combining polynucleotides with effective protein antigens such as bovine serum albumin in the hope that such a material will be effective in eliciting an antibody capable of interacting with the pure polynucleotides

S YACHNIN

Argonne Cancer Research Hospital* and
Department of Medicine,
The University of Chicago,
Chicago 37, Illinois

* Operated by the University of Chicago for the U S Atomic Energy Commission

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BIOLOGY

Breakdown and Synthesis of Cell Walls during Growth

PLASTIC extension of the primary wall in enlarging plant cells is thought[1–3] to require relaxation of the interwoven structure of the pre-existing wall as well as deposition of new wall materials It is possible that relaxation of the old wall, which has usually been attributed to fibre re-orientation, could also be facilitated by the breakage of bonds This communication deals with the reality of wall catabolism and the extent to which it and wall anabolism are correlated with growth All work was carried out with sections excised (as in Fig 1) from the third internode of 8-day-old etiolated pea epicotyls (*Pisum sativum* L var Alaska)

In preliminary tests sections were incubated on water, with no sugar supplied in order to minimize wall synthesis, and comparisons were made between changes in fresh weight and in the ethanol- and ether-insoluble dry weight The results after 2 days incubation are recorded in Table 1 Sections cut from the upper part of the epicotyl grew slowly but at the same time lost up to one quarter of their insoluble dry weight, while sections cut 1 cm or more below the apical hook did not enlarge yet gained in dry weight The insoluble fractions contain chiefly wall

Table 1 GROWTH AND INSOLUBLE DRY WEIGHT CHANGES IN PEA EPICOTYL SECTIONS FLOATED ON WATER FOR 48 HR

Section*	Change in fresh wt (%)	Change in dry wt (%)
H	+ 6	−27 4
1	+60	−22 9
2	+40	− 9 2
3	+15	+ 4 9
4	+18	+10 7
5	+ 6	+15 5

*Sections cut and extracted as in Fig 1

polysaccharides and nitrogenous materials Apparently the amounts of the latter increase during the growth of pea epicotyl sections, even on water[4], in which event it must be concluded that breakdown and dissolution of the wall can occur in young tissues, if not in those that have ceased enlarging

In the following experiment, sections were grown for 24 hr on 2 per cent sucrose uniformly labelled with carbon-14 in order to provide a substrate for the wall synthesis that normally accompanies cell expansion Fig 1 shows the insoluble dry weights of sections measured before (curve I) and after (curve II) incubation Under these conditions growth was more rapid than on water and was confined to the basal end of the hook and the apical cm of epicotyl shaft In section 1 length increased by 60 per cent and fresh weight by 105 per cent whereas in section 5 length increased by 4 per cent and fresh weight not at all

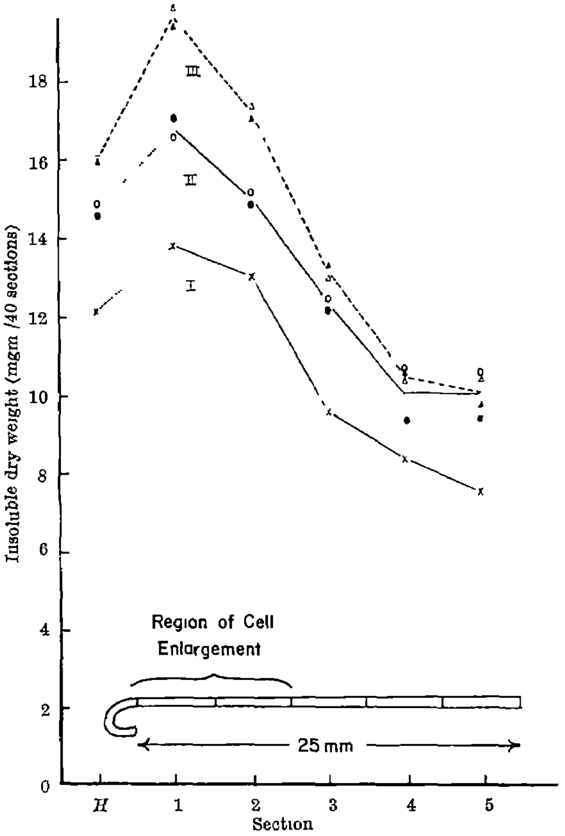

Fig 1 Insoluble dry weights of the hook (*H*) and successive 5 mm sections cut from etiolated pea epicotyls Curve I, weights at zero time, Curve II, weights of duplicates (40 sections each) grown in darkness at 26±1° C for 24 hr on 3 ml 2 per cent ^{14}C-sucrose in 0 01 *M* phosphate buffer, *p*H 6 0, Curve III, weights at zero time plus weights of incorporated ^{14}C-sucrose Weights of sections were measured after 5 half-hr extractions with 15 ml hot 80 per cent ethanol alternating with 5 half-hr extractions with hot absolute ethanol, followed by a 2-day extraction with ethyl ether and a 10-day drying period over calcium chloride *in vacuo* Weights of incorporated ^{14}C-sucrose were calculated as the insoluble c p m divided by specific activity of the substrate (1,990 c p m /mgm)

The growth pattern did not correlate with dry weight increments since these were approximately the same in all sections

Table 2 records the total amounts of radioactivity incorporated into insoluble fractions, as determined by counting powdered and weighed samples in air with a Geiger tube Chromatographic analyses of acid hydrolysates of these fractions revealed that the major radioactive components were aldo-sugars and uronic acids No fructose appeared in any hydrolysate so that none of the radioactivity was present as adsorbed sucrose Accordingly, the levels of radioactivity in the sections closely represented the relative amounts of wall polysaccharide derived from ^{14}C-sucrose Deposition was greatest in those sections that enlarged most, but these were also the sections with the greatest initial dry weights (Fig 1) and, in fact, the extent of wall synthesis was roughly proportional to the amount of pre-existing wall

Table 2 INCORPORATION OF ^{14}C-SUCROSE INTO CELL WALLS AND RESPIRED CARBON DIOXIDE

Section*	Radioactivity in cell walls (c p m /40 sections)	$\frac{\text{Specific activity CO}_2}{\text{Specific activity } ^{14}\text{C-sucrose}} \times 100$
H	8,320	79
1	12,230	79
2	8,780	74
3	7,460	72
4	5,000	63
5	5,290	66

*Details of experiment in Fig 1

Values are plotted in Fig 1, curve III for the initial dry weights of the sections plus weights of the incorporated ^{14}C-sucrose If there had been no breakdown of insoluble material during the experiment these values should have been attained (or exceeded) by the final dry weights (curve II) Curves II and III coincide fairly well in the basal three sections, however, in the apical sections much more wall polysaccharide was formed than could be accounted for by the increments in dry weight For example, the 13 mgm dry weight of section 1 incorporated about 6 mgm of ^{14}C-sucrose, but increased in dry weight by only 3 mgm Evidently, at least one-fifth of the original insoluble material dissolved in the growing regions at the same time as new wall was being formed Dissolution may not have occurred at all in sections that did not grow

It seems doubtful whether any one wall component could account for such extensive dissolution, particularly since the values for wall breakdown indicated in Fig 1 are minimal values Consideration should be given to the decorporation of radioactive wall material, which undoubtedly occurs in such experiments[5-7], and to additional wall synthesis from unlabelled endogenous substrates The extent to which the latter would increase the recorded discrepancies between wall formation and weight increment may be calculated if it is assumed that substrates for wall synthesis and respiration were derived from the same metabolic pool Since respired carbon dioxide was 65–80 per cent as radioactive as the supplied ^{14}C-sucrose (Table 2), the values for total wall synthesis would be greater than those shown in Fig 1 by the reciprocal of these percentages Applied to section 1, this calculation leads to the conclusion that about one-third of the original insoluble material dissolved during the experiment

It is therefore proposed that extensive breakdown of pre-existing wall, rather than synthesis of new wall, be regarded as an essential feature of plasticization in enlarging cells During growth, new wall material is intercalated in amounts which, depending on substrate availability, may or may not replace the materials dissolved

We thank the National Research Council of Canada for financial support

G A MacLachlan
M Young*

Department of Botany,
University of Alberta,
Edmonton, Canada

* Present addresses Department of Botany, McGill University, Montreal, and Department of Botany, Dalhousie University, Halifax

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Nitrogen Status of the Carnation Plant

A low level of nitrogen given under short-day conditions has been shown to delay flower initiation in the carnation[1] Though a slower growth-rate was observed under these conditions, the plants, unexpectedly, did not show typical symptoms of nitrogen deficiency Further work has shown that the nitrogen content of plants grown in sub-irrigated sand culture bears little relation to the level of nitrogen in the nutrient solution Under conditions of low nitrogen supply, considerably more nitrogen was found in the plants than was supplied in the nutrient solution

Experimental details were similar to those described previously[1] The plants were given either short-day (8 h) or long-day (17 h) treatment at two temperature-levels, the higher being maintained at about 15° C and the lower at about 10–12° C The plants were grown in sub-irrigated sand culture with nitrogen levels, supplied as nitrate, of 5 p p m , 15 p p m and 45 p p m Each nitrogen treatment was replicated four times within a daylength-temperature treatment, and the basic unit was two plastic pots 9 in in diameter connected to a single nutrient reservoir holding 4 l of solution One pot of each pair held four cuttings of the variety Puritan and the other held two cuttings of the variety White Sim A single axillary shoot from each cutting was taken to anthesis Estimations of the nitrate content[2] were carried out on the initial solution, and at intervals on the solution in the reservoirs As soon as the solution became out of the range 2–8 p p m for the 5 p p m solution, 12–18 p p m for the 15 p p m solution, and 40–50 p p m for the 45 p p m solution, the reservoir was refilled with fresh solution The total nitrogen content of the whole plants at anthesis was determined by the Kjeldahl method

Despite the low levels of nitrogen supplied, the plants did not show severe nitrogen deficiency symptoms in any of the treatments Table 1 shows that the total nitrogen, expressed as a percentage of the dry weight of the plants, was only slightly affected by the level of nitrogen supplied The growth-rate was primarily affected by daylength and temperature, and to a smaller extent by nitrogen level, as is shown by the ratio of the time to appearance of the flower buds to the number of leaf pairs below the bud (Table 1) The total amount of nitrogen found in the plants is shown in Fig 1 Regardless of the level of nitrogen supply, all plants under long days contained similar amounts of nitrogen, but under short days the amount of nitrogen in the plants increased with an increasing level of nitrogen supply At 5 p p m of nitrogen the plants contained nearly five times as much nitrogen as was removed from the nutrient solution, and at 15 p p m nitrogen approximately twice as much, whereas at 45 p p m nitrogen there was little difference In the calculation of nitrogen uptake no allowance was made for the amount of nitrogen present in the original cutting, but this was approximately compensated by the removal of all but one axillary shoot, this material being equivalent in size to the original cutting No account was taken of other sources of nitrogen, such as ammonia in the tap water These errors are admitted, but they could only account for a very small portion of the treatment differences observed

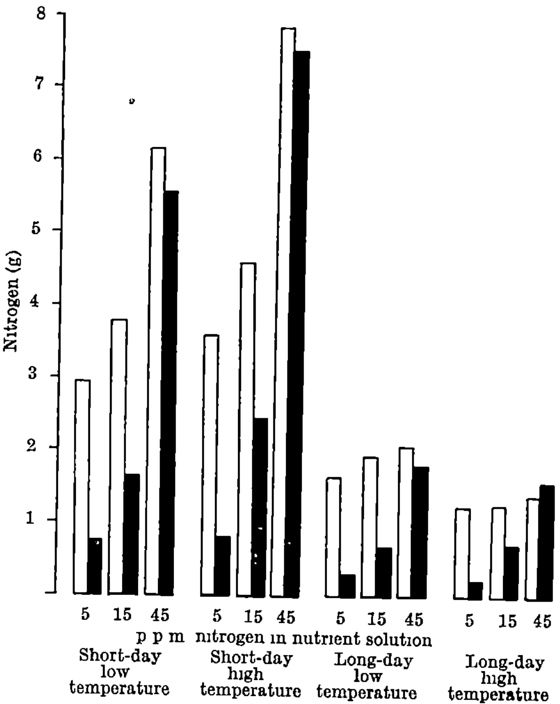

Fig 1 Total nitrogen found in plants (white) and removed from nutrient solution (black) Each histogram represents a total of 24 plants (16 var Puritan, 8 var White Sim)

Despite suggestions that higher plants may be able to fix atmospheric nitrogen[3,4], the results given here are probably best interpreted on the assumption that nitrogen-fixing organisms were present in the sand,

Table 1 EFFECTS OF NITROGEN LEVEL ON GROWTH AND FLOWERING OF CARNATION

Each value is the mean of measurements on 16 plants var Puritan, or 8 plants var White Sim

			Puritan Nitrogen-level			White Sim Nitrogen-level		
	Day-length	Temperature	5 p p m	15 p p m	45 p p m	5 p p m	15 p p m	45 p p m
Total nitrogen as per cent of dry weight	Short	Low	1 02	1 34	1 41	1 64	1 94	4 00
		High	1 16	1 27	1 82	1 45	1 73	2 39
	Long	Low	1 64	1 94	2 25	2 24	2 45	2 69
		High	1 96	2 05	2 37	2 24	2 43	2 58
Weeks to appearance of flower bud	Short	Low	50 0	41 1	38 7	30 3	28 9	25 7
		High	49 7	46 9	45 4	36 6	35 4	34 6
	Long	Low	20 4	18 7	17 0	16 7	15 0	16 9
		High	16 4	16 7	16 6	14 1	15 0	14 9
Leaf pairs to flower	Short	Low	40 2	34 1	33 5	24 7	25 4	24 0
		High	46 9	46 8	46 0	34 6	36 1	34 0
	Long	Low	20 1	19 9	20 5	17 2	16 4	17 2
		High	20 5	20 5	20 5	18 0	18 4	18 5

and that they were active to a proportionately greater extent in the low nitrogen treatment. The results show that in experiments using tap water, and possibly also where distilled water is used, the level of nitrogen present in the plant does not necessarily bear a relation to the amount supplied to the plants in the nutrient solution, and may explain why few workers have been able to detect an effect of nitrogen nutrition on flower initiation.

This work was supported by a grant from the Agricultural Research Council.

JENNET BLAKE*
R. SPENCER†

Horticultural Research Laboratories,
University of Reading.

* Present address: Department of Botany, University College, London.

† Present address: Federal Department of Agricultural Research, Ibadan, Nigeria.

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Promotion of Cell Wall Synthesis by Indolylacetic Acid

It is widely believed that the increase in cell wall synthesis which occurs when growth of plant cells is promoted by auxin is neither a direct consequence of hormone action nor a causal factor in cell enlargement, but is induced indirectly by the occurrence of cell enlargement[1]. A principal reason for this view is that if elongation is inhibited osmotically by mannitol, wall synthesis becomes reduced[2], and promotion of wall synthesis by auxin does not occur[3].

Table 1 compares the effect of 3 mg/l. indolylacetic acid (IAA) on cell wall synthesis in oat coleoptile segments: (*a*) when no inhibitor is present and elongation occurs; (*b*) when elongation is inhibited by 0.25 M mannitol; (*c*) when elongation is inhibited by 0.01 M calcium chloride, which is considered to prevent elongation by making the cell wall more rigid[4]. Segments 8 mm long were cut from coleoptiles of 3-day-old oat seedlings, var. Victory. Groups of eight segments were floated in 1.0 ml. of 0.05 M glucose containing 1.67 μc. of glucose uniformly labelled with carbon-14, on a reciprocating shaker at 120 oscillations per min. in the dark. After 7 h the segments were washed with water, measured and placed in 1 ml. of 80 per cent ethanol to extract soluble radioactivity. Segments were then crushed between glass plates (as described in ref. 5), and extracted with 0.2 per cent crystalline pepsin in 0.03 M phosphate buffer, *p*H 2, for 24 h to remove protein. The insoluble residues, representing cell wall material, were dried on planchets and counted with 25 per cent efficiency. Absorbed activity, as given in Table 1, is the sum of extractable activity and activity incorporated into the cell wall material.

When elongation is not inhibited, IAA causes a large increase in wall synthesis, as has been found previously (ref. 5, and literature cited there). This is associated with an increase in uptake of glucose, but the percentage of the absorbed glucose which is incorporated into the cell wall is nevertheless increased substantially by IAA.

In the presence of 0.25 M mannitol, no promotion of cell wall incorporation by IAA can be demonstrated, in agreement with earlier findings[3].

However, when elongation is inhibited instead by calcium ions, a conspicuous promotion of cell wall incorporation by IAA is observed, associated with no increase in uptake of activity. A similar IAA promotion of incorporation in the presence of calcium ions can be demonstrated in *p*H 4.5 maleate buffer, so the effect does not appear to be due to differences in *p*H.

Table 1. EFFECT OF IAA ON CELL WALL SYNTHESIS

Inhibitor	IAA	Length (mm.)	Cell wall activity (c.p.m.)*	Absorbed activity (c.p.m.)*	Percentage incorporated†	IAA effect‡
None	–	9.2	8,470 ± 820	55,420 ± 3,970	15.3 ± 0.9	
	+	11.8	14,540 ± 410	68,450 ± 4,290	21.2 ± 0.9	1.38
Mannitol	–	8.8	6,000 ± 780	48,540 ± 5,440	12.4 ± 1.2	
	+	8.7	5,460 ± 200	42,830 ± 2,850	12.7 ± 0.8	1.03
$CaCl_2$	–	8.7	7,540 ± 90	43,760 ± 1,480	17.3 ± 0.8	
	+	8.8	10,760 ± 130	43,120 ± 3,210	25.0 ± 1.6	1.44

* Mean and average deviation for 4 replicate samples each consisting of four coleoptile segments.

† Percentage of absorbed activity which was incorporated into cell wall.

‡ Percentage of incorporation in presence of IAA, divided by percentage incorporation in absence of IAA.

The experiment with calcium ions shows that auxin has a promotive effect on cell wall synthesis which is direct in the sense that it is not a result of the effect of auxin on elongation. That IAA promotion cannot be detected when elongation is inhibited by mannitol indicates that mannitol inhibits this action of IAA on wall synthesis.

Relative to uptake of glucose, the promotion of wall incorporation by IAA in the presence of calcium ions is similar to the IAA promotion which is observed in the absence of inhibitor, when rapid elongation is occurring (Table 1, last col.). We presume, therefore, that the same direct IAA promotion is operating during elongation, but is being added to by an indirect effect which may result from elongation and is associated with an increase in uptake of glucose.

The promotion of total wall synthesis by IAA in the presence of calcium ions is, by per cent, comparable with the promotion of hot water soluble pectin synthesis by IAA, which has been emphasized as involved in the action of auxin on elongation[6]. While hot water soluble polyuronides constitute less than 1 per cent of the cell wall, the presently described promotion involves cell wall synthesis as a whole and is, therefore, a gross effect compared with that on pectin. It is due mainly to increases in incorporation of sugar constituents of the cell wall material rather than uronic acids. Detailed information on the effect of IAA on incorporation into individual constituents will be published elsewhere.

The magnitude of the effect of IAA on cell wall synthesis, demonstrable in the presence of calcium ions, suggests that this effect could be causally related to the effect of IAA on elongation. This possibility will be explored.

This work was supported by U.S. National Science Foundation grant *G*-8705.

PETER M. RAY
DAVID B. BAKER

Department of Botany,
University of Michigan, Ann Arbor, Michigan.

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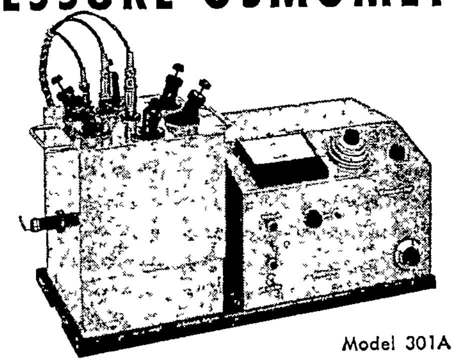

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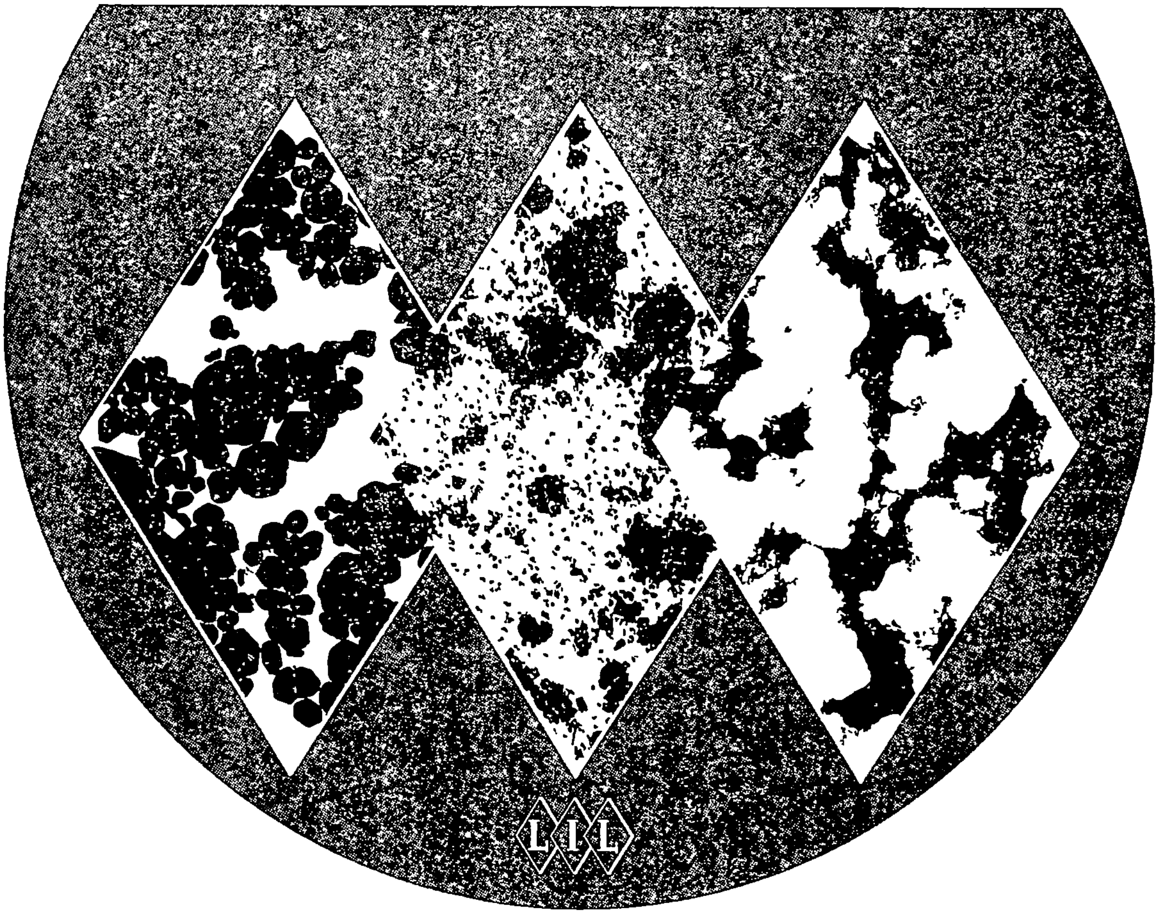

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Does Maternal Immunity to Male Antigen affect the Sex Ratio of the Young ?

Renkonen, Makela and Lehtovaara[1], in a study of human sex ratio, report that the birth of a boy renders a woman less likely to give birth to further boys in the future In other words, the greater the number of boys born previously, the lower is the sex ratio (expressed as the number of boys born for every 100 girls) at a given parity The authors suggest that bearing a male fœtus may immunize some women against male (*Y*) antigen, and that this immunization may harm subsequent male fœtuses The possibility also exists that a female immunized against *Y* antigen might inactivate a proportion of *Y*-bearing sperm before fertilization

I have put to an experimental test in mice the hypothesis that immunization of a female to *Y* antigen will affect the sex ratio of her offspring Female mice of the *C57BL* inbred strain were subjected to one of the following treatments

(1) 3 intraperitoneal injections per week for 7 weeks of half of a *C57BL* ♂ spleen (60–90 million cells per dose) suspended in *tris*-buffered saline, (2) similar injections of one-sixth of a *C57BL* ♂ spleen (20–30 million cells per dose), (3) similar injections of sperm from the vasa deferentia of a *C57BL* ♂ (about 30 million sperm per dose), (4) weekly subcutaneous injections of *C57BL* ♂ spleen in Freund's adjuvant (about 40 million cells per dose), (5) grafted with skin from *C57BL* male

The effect of these treatments on the immune state of the females was tested by grafting them with *C57BL* male skin In untreated females of my *C57BL* colony, survival time of isologous male skin grafts ranged from 14 to 73 days (Table 1) Survival-time was expressed as the time from grafting to the first sign of scabbing of the graft, since the day of final breakdown of the graft is difficult to assess where breakdown occurs slowly Table 1 shows that all treatments, except the first, produced some degree of immunity as judged by accelerated graft breakdown The first (repeated injections of spleen cells, half a spleen per dose) produced a state of tolerance, or immunological unresponsiveness, all grafts surviving for at least five months and showing a vigorous growth of hair That unresponsiveness should have resulted from the repeated injections of relatively large amounts of male spleen cells is not surprising in the light of previous reports[2,3] on the ease with which tolerance to the very weak *Y* antigen can be induced, even in adults On the other hand, the narrow margin between the dosages needed to confer immunity or tolerance is striking

The clear indication of immunity in the two females injected with sperm suggests that sperm contain *Y* antigen The sperm suspensions were contaminated with small numbers of other cell types (erythrocytes, epithelial cells from the vas deferens), but it does not seem likely that these would have been abundant enough to induce immunity

The serum of females injected with male spleen cells was tested for circulating antibody using agglutination tests against *C57BL* male erythrocytes and sperm, indirect agglutination tests (with the aid of an anti-mouse-globulin serum prepared in rabbits) against *C57BL* sperm, and cytotoxic tests[4] against *C57BL* male spleen cells All were negative In contrast, the sera of females injected with sperm showed a high titre (> 1/4,096) of sperm agglutinins, non-specific in the sense that the sera agglutinated sperm from males of other strains as readily as they did *C57BL* sperm Similar findings have been reported previously[5,6]

Table 1 TIME OF APPEARANCE OF FIRST SCAB AFTER GRAFTING *C57BL* MALE SKIN ON TO ADULT *C57BL* FEMALES

Group	No of mice	Time (days)	Immune status
Untreated	16	14 14 14 15 20 22 25 26 26 28 30 34 38 53 59 73	
Repeated injections of ½ ♂ spleen	4	>150 >150 >150 >150	Tolerance
Repeated injections of ⅙ ♂ spleen	6	11 11 11 12 14 14	Immunity
Repeated injections of sperm	2	11 12	Immunity
Repeated injections of ♂ spleen with adjuvant	8	12 12 13 13 13 15 17 19	Weak immunity
Previous skin graft	8	11 11 11 11 12 12 12 14	Immunity

Table 2 SEX RATIO AND MEAN LITTER SIZE OF YOUNG OF TREATED *C57BL* FEMALES

Group	No of litters	♀	♂	Mean litter size
Repeated injection of ½ ♂ spleen	4	11	12	5 7
Repeated injection of ⅙ ♂ spleen	6	24	18	7 0
Repeated injection of sperm	2	2	1	1 5
Repeated injection of ♂ spleen with adjuvant	8	21	19	5 0
Previous ♂ skin graft	8	21	26	5 9
Neonatal injection of ♂ spleen cells	8	27	26	6 6

Two to three weeks after the test grafts had been made all treated females were mated to *C57BL* males The first litter from each was sexed at birth In control matings of comparable age, mean litter size at birth was 6 6 young, and sex ratio did not depart significantly from equality The immune females (the state of immunity of which would have been increased as a result of the test grafting) showed no significant deviations from the control level either in litter size or sex ratio of young (Table 2) with one exception, that is, the two females injected with sperm, both of which had abnormally small litters This raises the interesting possibility that the high titre of sperm agglutinins demonstrated in the serum may have exerted a depressing effect upon fertility

Also included in Table 2 is a group of 8 *C57BL* females rendered tolerant to *Y* antigen by neonatal injection of *C57BL* male spleen cells It has been suggested that the implantation of embryos in the mammalian uterus has an immunological component, involving histocompatibility differences between fœtus and mother In two inbred strains of mice, Fekete[7] found a higher rate of implantation among embryos transferred to the uteri of females of the other strain than among within-strain transfers The existence of inbred strains, isogenic for all known histocompatibility loci, does not necessarily refute this theory, since the mother will always lack *Y* antigen and this may need to be present in the embryos or sperm before an implantation reaction can take place However, since in the present study litter size was normal in the highly inbred *C57BL* females rendered tolerant to *Y* antigen either by neonatal injection or by repeated injections of male spleen cells when adult, it would seem that an immunological reaction of the kind postulated is not necessary for implantation to occur

Although the numbers raised are not large, it is clear from Table 2 that no striking effect on sex ratio occurs in the young of female mice strongly immunized against *Y* antigen, even in a situation (that is, within a highly inbred strain) where no antigenic differences other than that represented by the *Y* antigen would be expected It therefore seems unlikely, though of course not impossible, that immunization against *Y* antigen plays the part postulated for it by Renkonen *et al* [1] in the antigenically complex situation of a human pregnancy in which the degree of immunization would presumably be weaker In addition, studies on mice[8,9] suggest that repeated pregnancies induce unresponsiveness to *Y* antigen, rather than even weak immunity

The more detailed data published by Renkonen *et al* [10] do not convincingly support an immunological interpretation For initiating and maintaining a state of immunity, boys born recently should prove more effective than those born longer ago Yet it appears from their data that the earlier in the family boys are born, the greater is the depressing effect upon sex ratio Where two boys are followed by two girls, for example, the ratio of boys to girls among fifth children is lower than when two girls are followed by two boys This is the reverse of what an immunological hypothesis would lead one to predict

Anne McLaren

Agricultural Research Council,
Unit of Animal Genetics,
Institute of Animal Genetics,
Edinburgh

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Red Water and Mass-Mortality of Fish near Cape Town

Red water, caused primarily by a very high concentration of the dinoflagellate *Gonyaulax polygramma* Stein, associated with another dinoflagellate, *Prorocentrum micans* Ehrb , in smaller numbers, appeared in False Bay, near Cape Town, during March and the early part of April 1962 This bloom resulted in a mass-mortality of fish and marine invertebrates, when the dinoflagellates died in large numbers

Red water is a common phenomenon around the coasts of South Africa and has been shown to be caused by a variety of different organisms, including dinoflagellates and ciliates These different organisms appear to be the causes of several distinct phenomena, including mussel poisoning of man and mass mortalities of marine fauna Sapeika[1] has shown that the pharmacological action of the toxin extracted from the local mussels is similar to that isolated from shell-fish in other parts of the world Mass-mortalities of marine fauna are rare around the coasts of South Africa Some early cases were reported by Gilchrist[2] Off the coast of South West Africa, however, complicated periodic mortalities occur as reviewed by Brongersma-Sanders[3], and later studied by Copenhagen[4] Mortalities farther north in Angola waters have been reviewed by Paredes[5]

In the present case the mass-mortality of fish and invertebrates occurred in limited areas only when the red plankton died and decayed in large numbers The death of fish and invertebrates was apparently caused primarily by the depletion of oxygen in the water aggravated by the release of decay products The living *Gonyaulax* up to the time they died do not appear to have produced any harmful effects on other fauna Healthy patches of red water were found to be supersaturated with oxygen during the day

Prof N Sapeika, of the Department of Pharmacology of the University of Cape Town, carried out toxicity tests on fish, limpets (*Patella longicosta*) and black mussels (*Mytilus perna*) we collected from the affected area The mussels in particular would have become poisonous if there had been any toxic organisms present However, an extract prepared from 12 black mussels by the Dack method produced no neurotoxic symptoms or death in six white mice injected with the material Similar negative results were obtained by an independent medical specialist with other experiments

The *Gonyaulax polygramma* population in this area showed considerable morphological variation as described by Taylor[6] Observations on the non-thecate and cyst forms were similar to those of Sousa e Silva[7] in cultures of *G diacantha* (Meun) Schiller In *G polygramma* it also appeared that multiplication occurred in the non-thecate stage This species was found to be strongly luminescent It is normally considered to be a warm-water species with a wide distribution in subtropical waters, and it only became abundant in the warm surface waters of False Bay The organisms in this population were appreciably smaller than is characteristic for the species

Gonyaulax polygramma was found in small numbers in the cold water south-west of Cape Town at the beginning of March There was an influx of this cold, nutrient rich water into False Bay at this time associated with a period of south-east winds In False Bay the surface-temperature rose, and the *Gonyaulax* multiplied rapidly in these favourable conditions A period of two weeks of calms and northerly winds caused the plankton-rich water to accumulate and eventually to die and decay in certain limited areas where the concentration was greatest The smell of decaying plankton and fish was noticeable in Cape Town 30 miles away After a few days a change of wind direction dispersed the decaying plankton Being largely a surface phenomenon wind was a primary factor in its distribution A further period of calms resulted in a recurrence of the mass decay of plankton and another though smaller mortality of fish two weeks later This case shows a remarkable similarity to mortalities due to *Gonyaulax polyedra* Stein on the coast of California and described by Kofoid[8] and others The only previous recorded case of a mortality due to *G polygramma* was in Japan, where pearl-oyster beds were affected[9]

John R Grindley

South African Museum,
Cape Town

F J R Taylor

Department of Oceanography,
University of Cape Town

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MICROBIOLOGY

Electrophoresis of Colicines in Agar Gel

In 1953 Ludford and Lederer[1] described the electrophoresis of colicines in agar gel, using a simple apparatus deriving its potential from a battery. Experiments were carried out in this department in 1959 using an apparatus similar to that used by Ludford and Lederer. The results were sufficiently encouraging to suggest that the method, suitably modified, could provide information regarding the colicine complexes and might possibly be of use for the differentiation of strains of colicinogenic bacteria. The description and use of the apparatus finally evolved will be described elsewhere. Briefly the method is as follows; a colicine-producing organism was grown as a spot inoculum in the centre of a strip of nutrient agar. The organisms were killed with chloroform, and a potential applied across the strip. The presence of colicine was revealed by areas of inhibition in a carpet of a sensitive indicator organism afterwards grown on the surface of the agar.

Fig. 1 shows the type of picture obtained. This is a photograph of Fredericq's colicines *A* to *I* [2] and shows the variety of inhibitory patterns that can be produced by different colicines acting on the same sensitive indicator organism. Experiment has shown that these patterns are reproducible although, as mentioned later, it is possible to produce variations in the picture.

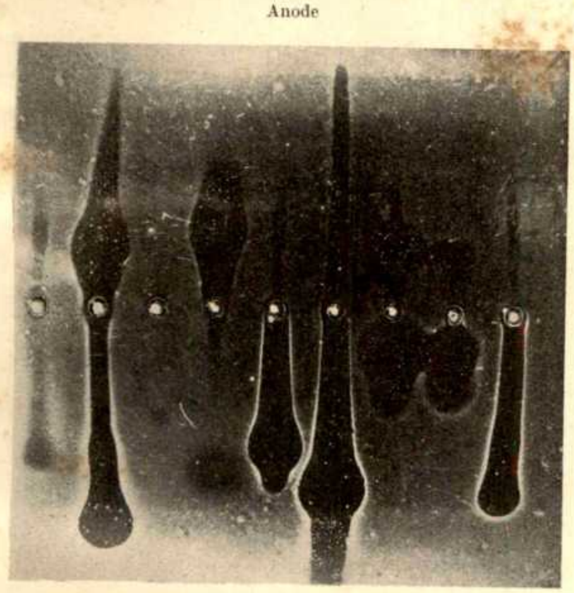

Fig. 1. Electrophoresis of Fredericq's colicines *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H* and *I*, respectively, reading from left to right with the anode end uppermost. Medium was nutrient agar (*p*H 7·4) prepared in this department

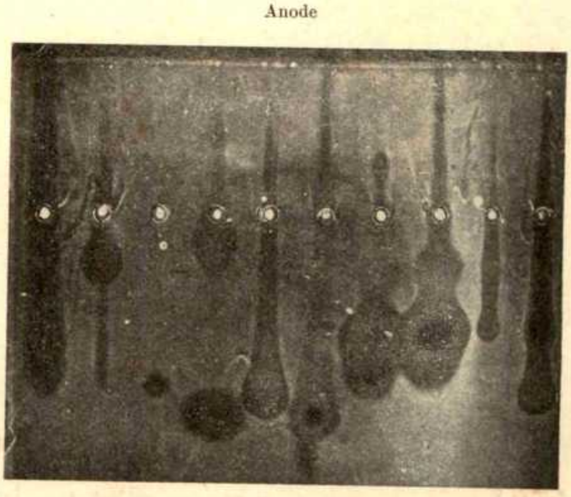

Fig. 2. Electrophoresis of Fredericq's colicines *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *I* and *J* read as in Fig. 1. Medium was nutrient agar (*p*H 7·4) prepared from 'Difco' nutrient broth

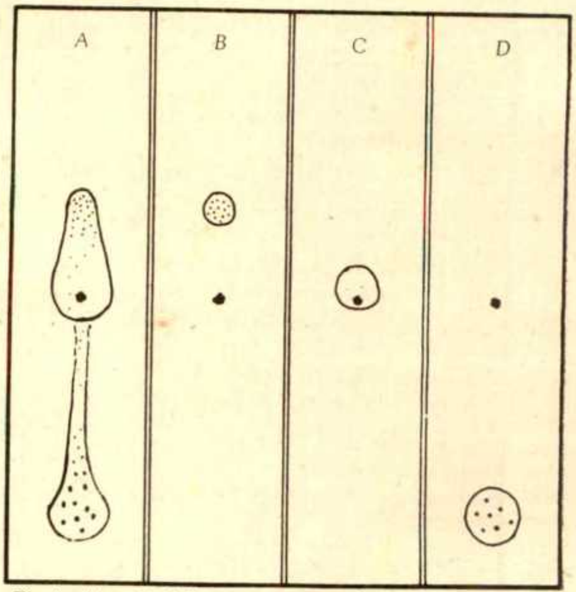

Fig. 3. Theoretical diagram of the breakdown of a colicine complex by the use of several different sensitive indicator organisms, based upon several experiments with different colicine complexes. *A*, Most sensitive indicator available; *B*, *C* and *D*, indicators of differing sensitivity

One fact which is immediately obvious is that there is a large amount of tailing and streaking of the inhibition zones, and in some cases it is difficult to see where one component of the colicine complex begins and where it ends. This tailing and streaking of the zones is due to the type of electrophoresis (frontal) and electro-endosmosis[3]. It was found possible to characterize strains of *E. coli* and *Shigella sonnei* not only by their ability to produce colicine but also by the appearance of the colicine pattern on the electrophoresis plate. Abbott and Shannon[4] and Shannon[5] devised a method for typing *Sh. sonnei* and *E. coli* 0·55: *B*5 respectively, by the patterns of inhibition their colicines produced in a selected group of sensitive indicator organisms. This method is in use in several public health laboratories in Great Britain including the Dysentery Reference Laboratory at Colindale. Strains of *Sh. sonnei* which had been typed by the Abbott and Shannon technique at Colindale were sent to this department, where their colicines were subjected to electrophoresis. There was complete agreement between the two methods where strains from the same outbreak were concerned. However, it is too early to say that the agreement is 100 per cent where strains from separate outbreaks were involved.

The electrophoretic patterns have never varied when the conditions of electrophoresis have remained the same, whereas variation does, sometimes, occur in the inhibition spectrum revealed by the sensitive strains used in the Abbott and Shannon technique. However, variation can occur in the electrophoretic pattern, and this can be caused by changes in the agar medium. In this case the zones sometimes take on a different appearance and may change their direction of movement (Figs. 1 and 2).

That there are several colicines produced by the same organism has been shown by electrophoresis using several different sensitive indicator organisms on the same plate. Fig. 3 is a diagram to illustrate this finding.

These few experiments suggest that the method is worthy of attention, and work is continuing in this Deparment with the view of obtaining a better understanding of the factors controlling the electrophoresis patterns of colicines and also to evaluate the technique for the characterization of colicinogenic bacteria.

This work has been supported by grants from the Department of Scientific and Industrial Research and Medical Research Council.

PAUL J. CHAPPLE *

Department of Bacteriology,
University of Bristol.

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* Present address: Ministry of Agriculture, Fisheries and Food, Infestation Control Laboratory, Virus Unit, Worplesdon, Surrey.

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Production of Yeast Protoplasts by an Enzyme Preparation of *Streptomyces* sp.

Eddy and Williamson[1] obtained structures resembling intact protoplasts from cells of *Saccharomyces* after digestion of the cell-wall by enzymes from the gut of the snail *Helix pomatia*. This report was followed by a number of papers on the structural and physiological properties of the yeast protoplasts obtained by this procedure[2–5].

One cannot expect to find a lytic preparation from any organism able to produce protoplasts from all micro-organisms. Accordingly, it is of considerable interest to obtain preparations from different sources which have a chance of different, though perhaps partially overlapping, characteristics. We are now examining the possibilities of the *Streptomyces* and snail enzymes ; and will eventually examine a mixture of both for the preparation of protoplasts from representatives of other types of micro-organisms.

We have found a species of *Streptomyces* which produces an enzyme able to lyse cell-walls of a number of yeast species and also renders possible the preparation of protoplasts. The *Streptomyces* was isolated by us from the soil, using the plate method with the cell-wall agar media described by Salton[6].

In our investigations, instead of the snail enzyme, we used a lytic preparation produced in the growth media by this *Streptomyces*. The enzyme preparation was obtained after 60 per cent ammonium sulphate precipitation and redissolution in a small volume of distilled water followed by dialysis against distilled water. This preparation was very stable and able to digest isolated cell-walls of *Saccharomyces fragilis*, *Candida utilis*, *S. rossei*, *S. cerevisiae PM*-1 and *SM*-100, *Kloeckera apiculata* and *Torula utilis*, named in decreasing order of susceptibility. A strain of *Torulopsis aedis* has been found to be very resistant to lysis.

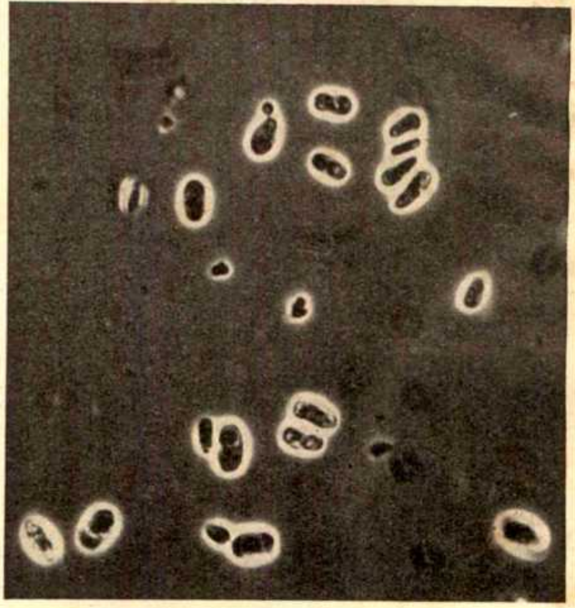

Fig. 1. Young cells of *Candida utilis*. (Phase contrast)

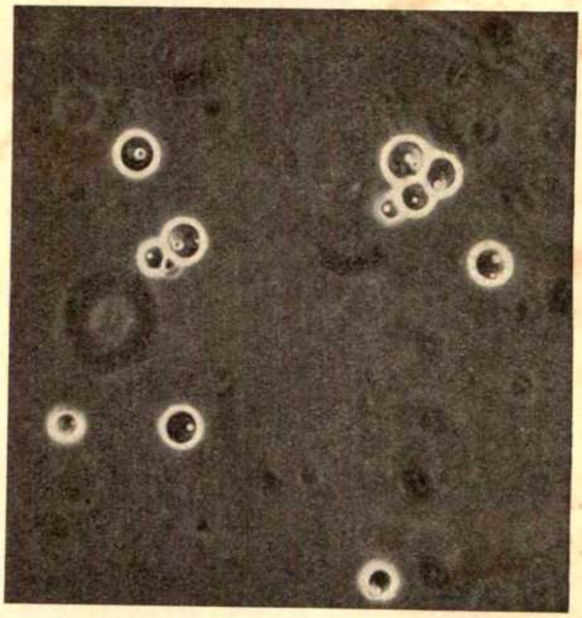

Fig. 2. Isolated protoplasts of *Candida utilis*. (Phase contrast)

Using susceptible species of yeasts, it is observed that it is much easier to obtain protoplasts with young growing cultures. Young cells of each of the aforementioned yeast species were obtained by inoculating a small volume of a 24–28-h old culture into a liquid medium. The initial medium consisted of glucose 2 per cent (w/v) and yeast extract 0·3 per cent (w/v). After a growth period of 18 h at 28° C under vigorous aeration, 20 ml. of this culture were transferred into 80 ml. of the same fresh medium. The cells were collected by centrifugation after about 5 h when a population density of about 5×10^6 cells per ml. was attained. The cells were thoroughly washed in distilled water and were resuspended at a density of about 10^6 cells per ml. in 0·1 M phosphate buffer *p*H 6·8 containing 0·8 M mannitol.

For conversion of the yeast cells to protoplasts, a solution of the enzyme containing 10 mg per ml. was added. The quantities of the enzyme preparation used ranged between 0·2 and 0·5 ml. of dilute solution per ml. of incubation mixture. The digestion of the cell-wall was allowed to take place at 30° C for variable periods of time with gentle agitation. After 2–4 h incubation, the protoplasts began to emerge from some of the yeast cells in the more susceptible species, although protoplast formation was usually complete in 6 or 7 h. The yield of protoplasts, when *Candida utilis* was used, was about 90 per cent. The protoplasts, observed under the phase microscope to contain vacuoles, appeared to be as dense as the contents of the cells from which they came. After the extrusion of the protoplasts a few empty cell-walls could be seen. It is not yet known whether the remaining envelope surrounding the cytoplasmic content is a true protoplast membrane[7] since immunochemical tests relating to wall material in the yeast species used are not yet available nor is the chemistry of the wall material properly known.

The free protoplasts are sensitive to osmotic shock; and lysis was immediately observed under the microscope when they are placed in distilled water. The protoplasts are very stable when stored in hypertonic solution in the cold room for several days. The lytic system is very active on yeasts and fungi, and showed no activity against a number of species of bacteria.

Extensive investigations have been made on the lytic properties of the isolated enzyme[8].

CONCEPCIÓN GARCIA MENDOZA
JULIO R. VILLANUEVA

Instituto "Jaime Ferrán" de Microbiologia,
Velazquez 138,
Madrid 6.

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Protoplasts of the Giant Bacterium, *Caryophanon latum* Peshkoff

CONTRARY to current information, Provost and Doetsch[1] reported recently that *Caryophanon latum* Peshkoff is a Gram-positive bacterium. Evidence cited for this contention, in addition to staining, included susceptibility to egg-white lysozyme and formation of 'protoplasts'. The results of the investigation reported here indicate that protoplasts are formed when osmotically buffered suspensions of *C. latum* are exposed to egg-white lysozyme, and that these bodies possess many characteristics common to protoplasts of other Gram-positive bacteria.

C. latum 1·8 was used in all experiments and criteria suggested by Brenner *et al.*[2] were applied, where possible, in determining that the spherical structures were, in fact, protoplasts.

Surface cultures of *C. latum* were grown on a medium of 1·0 per cent (w/v) 'Hy-case *SF*' (Sheffield Chemical Co., Norwich, New York), 0·5 per cent (w/v) 'Bacto-Yeast Extract' (Difco Laboratories, Detroit, Michigan), 0·5 per cent anhydrous sodium acetate, 1·5 per cent agar (*p*H 7·8), and incubated for 18 hr. at 25° C. Growth was gathered in sterile distilled water, washed twice, and resuspended in diluents the composition of which depended on the nature of the experiment to be undertaken.

When treated with 20 μgm./ml. lysozyme, cell suspensions in 0·05 *M* sodium chloride rapidly decreased in optical density as contrasted to those in a medium of 0·3 *M* sucrose, 0·016 *M* magnesium chloride, and 0·03 *M* phosphate buffer, *p*H 6·7 (Fig. 1). Maximum turbidity reduction occurred

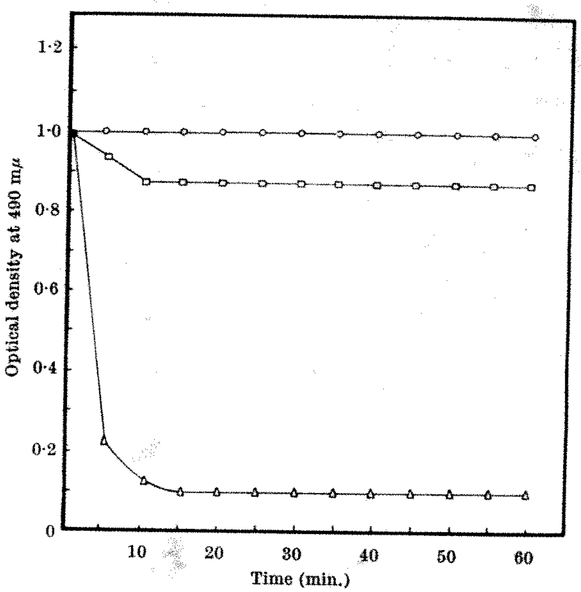

Fig. 1. Effect of lysozyme (20 μgm./ml.) on optical densities of *C. latum* suspensions. ○, Untreated controls; □, cells suspended in 0·3 *M* sucrose, 0·016 *M* magnesium chloride, 0·03 *M* phosphate buffer, *p*H 6·7; △, cells suspended in 0·05 *M* sodium chloride, *p*H 6·7

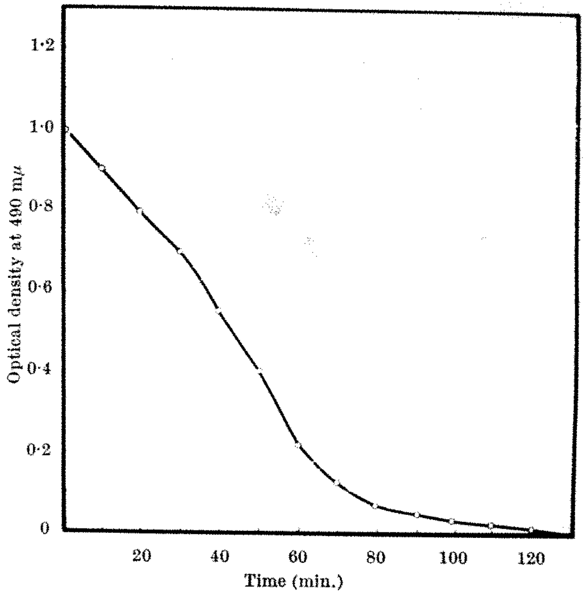

Fig. 2. Effect of lysozyme (20 μgm./ml.) on optical density of *C. latum* cell walls in 0·067 *M* phosphate buffer, *p*H 6·7

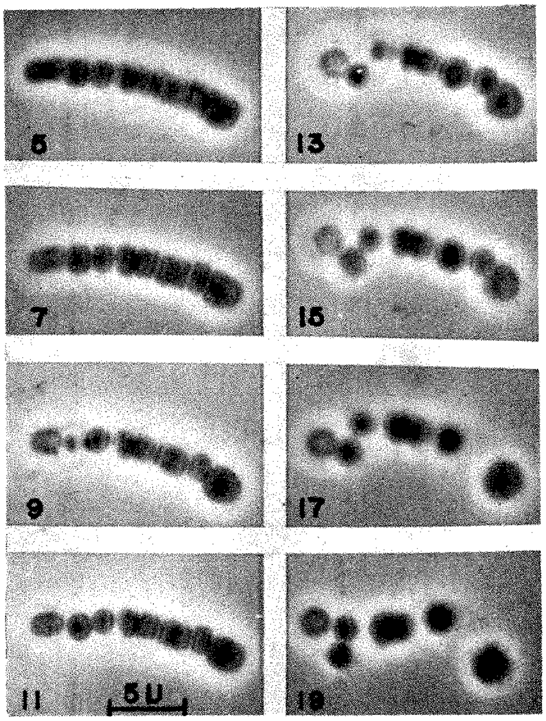

Fig. 3. Bright-phase photomicrographs of *C. latum* being converted to protoplasts upon dissolution of cell walls by lysozyme (20 μgm./ml.). The numbers denote time (min.) elapsed after addition of lysozyme

within 15 min. after addition of lysozyme. Bright phase-contrast microscopic examination of buffered-sucrose suspensions revealed spherical, phase-dense protoplasts and intact trichomes were not observed in such suspensions. Purified cell wall preparations of *C. latum* suspended in 0·067 *M* phosphate buffer (*p*H 6·7) containing 20 μgm./ml. of lysozyme were completely dissolved (Fig. 2) when determined turbidimetrically.

The production of protoplasts by *C. latum* was followed easily using phase-contrast microscopy. Immediately after addition of lysozyme to a bacterial suspension, 1 loopful of the mixture was transferred to a slide, covered with a cover-glass, and sealed with melted 'Petrolatum'. Fig. 3 shows numerous protoplasts emerging from a single trichome. The number of protoplasts derived from individual trichomes varied, and depended on the length and number of cellular units present therein. Fig. 3 clearly depicts the emergence of 7 distinct protoplasts, 1 of which lysed prior to completion of cell-wall lysis. There was definitely a greater number of protoplasts released per trichome from young cultures (14–18 hr.), and older cultures (24–30 hr.) did not possess as many septa per trichome. Not all protoplasts derived from a trichome existed as single spherical bodies. This can be observed in the last photograph in Fig. 3. Continued microscopic observations of such bodies showed them to remain paired. This was interpreted to mean that in many cases cytoplasmic membranes were completely laid down, but that the cross-walls were not. The presence of single protoplasmic membranes where secondary walls were only partially completed was shown by the resulting formation of only 1 protoplast from such structures. Dissolution of *C. latum* trichomes by lysozyme and resultant formation of protoplasts supply additional evidence for the multicellular structure of this organism as envisaged by Pringsheim and Robinow[3].

This work was supported in part by a training grant (*2G*-615) provided by the Division of General Medical Sciences, National Institutes of Health, Bethesda, Md. We thank W. L. Wallenstein for help in the preparation of the figures.

F. J. Tyeryar, Jun.
R. N. Doetsch

Department of Microbiology,
University of Maryland,
College Park, Maryland.

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Lack of Pectic Enzyme Production by Non-Pathogenic Species of *Verticillium*

THE presence of enzymes belonging to the pectic group of enzymes has been demonstrated in a large variety of plant pathogenic organisms. The existence of such hydrolytic enzymes has been shown in culture filtrates of many pathogenic moulds, the enzymes being isolated from those filtrates and their toxic effect on plants demonstrated[1]. A degradation of the pectic material of the cell wall, principal site of attack of these enzymes, could bring about wilting. It thus appears that the formation of pectic enzymes may play an important part in producing the wilting symptoms of many plant diseases.

As a part of the investigations on the lytic activity of a non-pathogenic strain of *Verticillium hemileiae* on uredospores of various rust[2], several species of *Verticillium* have been tested for ability to produce pectic enzymes *in vitro*. Pectolytic activity in fungi has generally been found to depend on a complex of enzymes which may include pectin-methyl-esterase, polygalacturonase and depolymerase enzymes. Forty strains of the following species of *Verticillium* were used in this study: *V. albo-atrum*, *V. tricorpus*, *V. dahliae*, *V. nigrescens*, *V. lateritium*, and *V. hemileiae*, some isolated by us and others received from the Estaçao Agronomica Nacional of Oeiras (Portugal), the University College of Swansea and the Oregon State University. The fungi were grown on a pectin medium[3] for 20 days under static conditions at 28°. Pectolytic activities in the *Verticillium* cultures were determined using the methods described by Waggoner and Dimond[4], and that of the ethanol precipitation[3].

Very high pectolytic activity was found with all the pathogenic species of *Verticillium* whereas no activity was detected in the non-pathogenic strains. No effect was found in the production of pectic enzymes when the substrate was changed. The occurrence of pectic enzymes in all the pathogenic species of *Verticillium* studied was more or less expected and more striking was the lack of pectolytic activity in the non-pathogenic strains tested. These observations might suggest that *Verticillium*, which produce enzyme preparations showing pectolytic activity, are plant pathogens. Smith[3] has found that production of pectin-methyl-esterase enzyme is confined to a small number of pathogenic bacteria. Additional proofs of the role of the pectic enzymes in pathogenesis have also been demonstrated by other workers[4,5].

J. A. Leal
J. R. Villanueva
Instituto "Jaime Ferrán" de Microbiologia,
Velazquez 138, Madrid 6.

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CYTOLOGY

Sex Chromatin in Adult and Fœtal Rat

In 1953, Moore and Barr[1] published the results of an investigation of the occurrence of sex chromatin in neurons from different mammalian species. These authors were unable to show any sex chromatin in rat. However, three years later de Castro *et al.*[2] found typical such in ameloblastic nuclei from young rats aged one day; and in 1959, Wolf-Heidegger and Klinger[3] described sex chromatin in, among other tissues, spinal ganglion cells from adult rats as well as from fœtuses aged 13 and 16 days. Hinrichsen and Gothe[4] and Feiner[5] found a difference in the chromatin distribution between male and female rats. The last-mentioned author, however, found this difference concerning spinal ganglion cell nuclei only in fœtuses and not in adults. In trophoblastic nuclei from rat embryos, Zybina[6] discovered sex chromatin after 6 days.

The rats used by us in our investigation were of the Sprague–Dawley strain. The material was taken from 3 adult males and 4 adult females, 3 male and 3 female fœtuses aged 16 days post-coitum and 12 male and 13 female fœtuses aged 14 days post-coitum. Finally, 30 fœtuses aged 13 days and 41 fœtuses aged 12 days post-coitum were investigated. In the two last-mentioned groups no certain gonad differentiation could be established. Only spinal ganglions were investigated. From adult animals 3–4 ganglia were dissected out, after which these, as well as most of the fœtal material, were fixed in Bouin's fluid. Some fœtuses were fixed in Davidson's fluid. Neither of the fixing fluids, however, proved better than the other in this connexion. Staining in all instances was made in gallocyanin-chrom-alum according to Einarson[7] after previous treatment in 10 per cent perchloric acid for 12 h at + 4° C. 500 ganglion cell nuclei from each of the adults as well as from fœtuses aged 16 days were studied, and 200 or 300 from each of the remaining fœtuses. In the present investigation the occurrence of solely marginally sex chromatin was examined, that is, about 1μ large triangular or plane-convex body situated along the nucleic membrane. During the examination of the ganglion cell nuclei the number of nuclei showing marginally sex chromatin according to the aforementioned definition was determined.

The results from the investigation appear in Table 1, where the average number of nuclei showing marginally sex chromatin in the different stages is given in percentage of the total number of nuclei examined.

Table 1

	Female (per cent)	Male (per cent)
Adult	39·9	3·6
16 days post-coitum	48·4	8·7
14 days ,,	50·9	8·8
13 days ,,	50·2	9·9
12 days	50·4	9·3

Concerning adults and fœtuses aged 16 and 14 days, the nuclear sex identified with the help of marginally sex chromatin in all instances agreed with the gonadal sex of the specimen. In the fœtal groups aged 12 and 13 days it was not always possible to decide definitely the sex of the specimen histologically because testes with delayed differentiation could be misinterpreted as ovaries. In these groups, however, a grouping of marginally sex chromatin around the averages 50·4 and 9·3 per cent (12 days) and 50·2 and 9·9 per cent (13 days) was obtained. Sex identification with the help of marginally sex chromatin in non-gonad differentiated fœtuses agreed in all instances with the results from sex identification obtained by gonad volume determination[8]. No significant difference exists between the averages for marginally sex chromatin in males of different fœtal ages or in females of different fœtal ages. On the other hand, the difference between the averages for adults and fœtuses is significant for males as well as females (male: $\chi^2 = 55{\cdot}86$ for 1 *D.F.*, $P < 0{\cdot}001$; female: $\chi^2 = 70{\cdot}01$ for 1 *D.F.*, $P < 0{\cdot}001$).

John-Gunnar Forsberg
Johannes Lindh
Department of Anatomy,
University of Lund,
Sweden.

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[2] de Castro, N. M., da Silva Sasso, W., and de Góes, M. R., *Nature*, **178**, 1059 (1956).

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Cytological Investigation of Triploid Sterility

The problem of high sterility in triploids is well known since it is easily observed in practice. Most explanations of the cause have been genetical but a cytological follow-up often lends weighty support. So far cytological observations have shown that in triploids the extra (n) chromosome complement per pollen mother cell is shared between the two daughter nuclei at anaphase I in various proportions over and above the division of the basic diploid chromosome number. The movement of the chromosomes is at random so that when the daughter nuclei separate after anaphase II the pollen grains are produced with approximately binomial frequency distribution of the chromosome classes ranging from n to $2n$ chromosomes.

This has been investigated in maize, solanum, *Narcissus*, *Petunia* and tomatoes, and the results thus obtained only give the impression that the formation of a fertile triploid depends on the fusion between pollen and ovule of complementary chromosome numbers, that is, n to $2n$ or $n + 1$ to $2n - 1$, etc. It is true that the chances of such complementary chromosome numbers meeting are not terribly high, but they cannot alone account for the scale of sterility observed in practice.

One way of looking for the answer to the problem lies in observations on the particular chromosomes which make up the chromosomes in the pollen grains and the ovules. But there are very few suitable species in which all the individual chromosomes can be distinguished, such as *Crepis*, maize and *Drosophila*.

I have examined the chromosomes of the bluebell, *Endymion nonscriptus* (L) Garcke, and have succeeded in identifying all of them[1]. This has made it possible to carry out the sort of studies in question[2].

68 pollen grains were examined at pollen mitosis[1] and all the chromosomes in each such grain were identified.

One of the facts which emerged from this investigation was that some of the pollen grains were not pure but either contained all 3 of a particular chromosome (triplicates), or have missing all 3 of a particular chromosome (incomplete grains). The triplicates were more numerous than the complementary incomplete grains and this seems to support the genetical idea that duplications are not as deleterious as deletions. The satellitic chromosome was found to be always present in each pollen grain at least once, which may mean that the absence of this particular chromosome would otherwise be deleterious to the grain.

The presence of impure grains (that is, triplicates and incomplete grains) obviously decreases considerably the chances of proper fertilization since these can lead to the formation of tetrasomic or nullisomic triploids none of which was found among the triploids studied. This cytological analysis provides a more convincing explanation of the scale of sterility found in triploids than has been given by genetical evidence.

J. YANNEY WILSON

Department of Botany,
University of Ghana,
Legon, Ghana.

[1] Wilson, J. Y., *Nature*, **178**, 195 (1956).

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AGRICULTURE

Measurement of Evapo-Transpiration from Large Tanks of Soil

THE direct measurement of evapo-transpiration on any sufficiently large scale requires very expensive equipment. In an attempt to find a cheaper method we have been experimenting with semi-enclosed hydraulic systems involving the use of water-filled, flexible bags on which large tanks can be readily supported (Fig. 1). The change in height of the balancing column of water measures changes in pressure on the system.

It is well known that the factors which determine the volume of water in the system and the height of the balancing column of liquid are pressure and temperature. Since pressure is equal on both sides of the balanced system, no complications arise due to changes in environmental atmospheric pressure; however, changes in temperature, by causing changes in volume of the liquid, affect readings of the water column. In our experiments the flotation bags will be used at depths of soil below 4 ft., where there is little diurnal or even annual change in temperature to affect a system of high specific heat. For example, we have floated a steel tank 8 ft. long by 4 ft. wide and 4 ft. deep, filled with water, and supported on three interconnected water-filled rubber bolsters, each some 9 ft. long and 18 in. wide (Fig. 1).

Evaporation from this tank was compared with evaporation from a series of evaporation pans in use at this station. The results are shown in Fig. 2, which illustrates the cumulative records over a sample 12-day period.

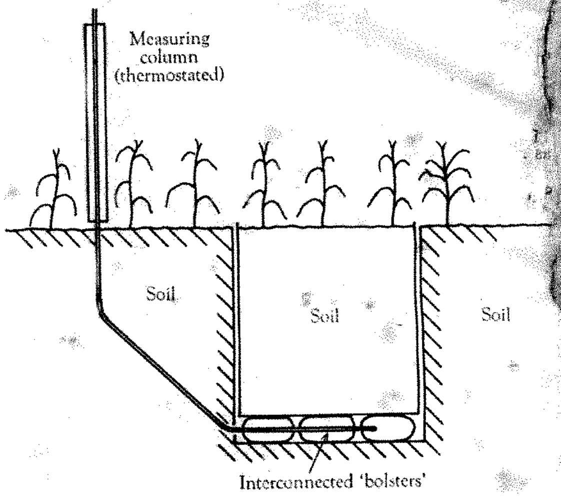

Fig. 1

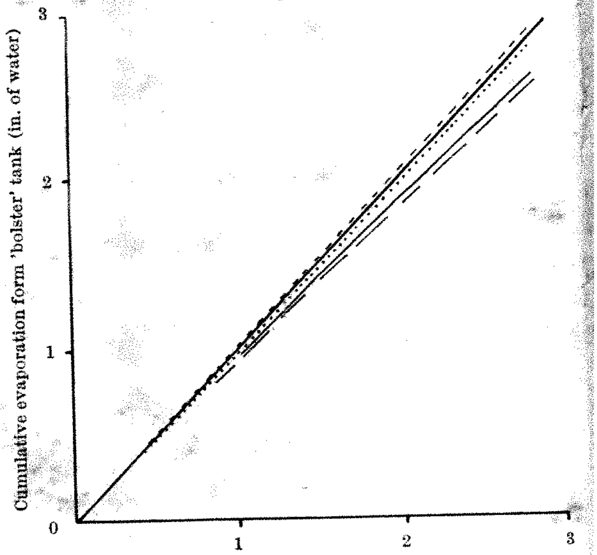

Fig. 2. Cumulative evaporation from Trumble tank (– – –), sunken pan (· · · ·), class *A* pan (—— —— ——) and raised gridded tank (———). Closeness of agreement between the 'bolster' tank and any of the evaporation pans is shown by the nearness of the lines to the thick continuous line at 45° to the axes

Whereas evaporation on rain-free days from all these devices is of the same order, our tank gives results which are more closely correlated on a daily basis ($r = 0{\cdot}995$) with the Trumble tank and sunken pan than with the remainder. The Trumble tank is a water-insulated tank some 35 in. deep, while the sunken pan is air insulated and only 17 in. deep.

The experimental tank has now been filled with soil in place of the water, and the bolsters continue to support it and show changes which are believed to be due to loss in evaporation from the soil surface, since additional amounts of water appear to be accurately reflected by changes in height of the measuring column.

Further tests are to be carried out on a larger scale to study both potential and actual evapo-transpiration under a variety of local crops.

J. GLOVER
J. A. FORSGATE
East African Agriculture and Forestry Research Organisation,
Muguga, Kenya.

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, October 1

Society of Engineers (at the Geological Society, Burlington House, ccadilly, London, W 1), at 5 30 p m —Mr A J Davenport "Plastics a Structural Material"

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Scientific Films

Monday, October 1—Tuesday, October 2

Plant Phenolics Group and the British Mycological Society (at the School of Pharmacy, Brunswick Square, London, W C 1), at 10 a m daily—Symposium on "Fungi and their Metabolites"

Monday, October 1—Wednesday, October 3

British Society of Rheology (at the Hotel St George, Harrogate) —Symposium on "The Rheology of Emulsion"

Tuesday, October 2

Institution of Chemical Engineers, South-Eastern Branch (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 6 p m —Mr M Ruhemann 'Frontiers of Technology—Low Temperature"

Institution of Mechanical Engineers, Internal Combustion Engines Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Rocket Motors"

Tuesday, October 2—Thursday, October 4

Aslib (at Norbreck Hydro, Blackpool)—36th Annual Conference

Wednesday, October 3

Colour Group (Great Britain) (in Lecture Theatre No 2 of the new Physics Department, Imperial College, London, S W 7), at 3 10 p m —Dr R A Weale "The Duplicity Theory of Vision", 4 p m —Dr P H Silver "The Spectral Sensitivity of Animals"

Royal Society of Medicine, History of Medicine Section (at 1 Wimpole Street, London, W 1), at 5 15 p m —Prof J Needham "China and the Origin of Medical Examinations"

British Institution of Radio Engineers, Radar Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Mr G G Roberts "Storm Warning and Wind Finding Radar"

Thursday, October 4

Society of Chemical Industry, Agriculture Group (at 14 Belgrave Square, London, S W 1), at 11 a m —Meeting on "Toxic Products in Groundnuts"

Institution of Mechanical Engineers, Internal Combustion Engines Group (at the James Watt Memorial Institute, Great Charles Street, Birmingham), at 6 p m —Mr S H Henshall "The Turbocharged Loop Scavenge Diesel Engine"

Institution of Mechanical Engineers, Nuclear Energy Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "The Basis of Temperature Limitations"

Society of Instrument Technology (at the Royal Institution, 21 Albemarle Street, London, W 1), at 6 p m —Sir Harry Melville, F R S "The Effect of Instrumental Development on the Progress of Chemistry" (Second Thomson Lecture)

Institute of Metals (at 17 Belgrave Square, London, S W 1), at 7 p m —Mr W K B Marshall "The New Welders" (Chairman's Address)

Thursday, October 4—Friday, October 5

Cement and Concrete Association (at Church House, Great Smith Street, London, S W 1), at 10 a m on Thursday and 9 30 a m on Friday —Conference on "Housing from the Factory"

Thursday, October 4—Saturday, October 6

Council for the Preservation of Rural England (at Lowestoft, Suffolk)—Twenty-third National Conference—"Post-War Developments in the Countryside"

Friday, October 5

Biochemical Society (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W C 2), at 10 a m —Colloquium on "Radioautography" (Chairman, Mr A J Hale), and original communications

Institute of Metal Finishing (at Quaglino's, Bury Street, St James's, London, S W 1), at 2 30 p m —Annual General Meeting 3 30 p m —Dr T P Hoar "On the Electrochemistry of Nickel" (Hothersall Memorial Lecture)

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Prof J H Turnbull "Enzymes, Drugs and Antibodies, some Chemical Common Factors"

Saturday, October 6

Nutrition Society (at the Medical School, Guy's Hospital, London, S E 1), at 10 30 a m —Symposium on "Fluoridation"

Monday, October 8

Plastics Institute, Reinforced Plastics Group (at the Wellcome Building, Euston Road, London, N W 1), at 4 p m —Symposium on "Design Considerations in Three Different Fields"

Institute of Biology (at the French Institute, Queensberry Place, London, S W 7), at 5 15 p m —Prof F Morel (French Centre for Nuclear Studies, Saclay) "Some Aspects of Kidney Function as Revealed by Tracer Studies" (Third Louis Rapkine Memorial Lecture)

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 30 p m —Mr J R Lucas "The One Concept of Probability"

Institution of Mechanical Engineers, Applied Mechanics Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m Discussion on "Testing Machines"

Royal Institute of Chemistry, London Section (joint meeting with the Ewell County Technical College Chemical Society, at the Ewell County Technical College, Reigate Road, Ewell), at 7 p m — Dr. H J Cluley "Complexometric Analysis"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Computing Assistant (with a good honours degree in mathematics or in a pure or applied science) in the Electronic Computing Laboratory—The Registrar, The University, Leeds, 2 (October 6)

Principal Scientific Officer or Senior Scientific Officer (honours graduate in animal husbandry or in another appropriate science, with considerable research experience) to work as the senior member of a team undertaking research into the nutrition of hill sheep and associated problems—The Secretary, Hill Farming Research Organization, 48 Palmerston Place, Edinburgh, 12 (October 6)

Scientific Officers (2) (with a good honours degree in chemistry (physical, inorganic or organic) or in biochemistry, and preferably some postgraduate experience) at the National Research Station, Wellesbourne, in the following Sections (1) Chemistry to study plant nutrients in soils and their effects on the growth of vegetable crops, (2) Weeds to work on problems associated with the absorption, movement and persistence of residual herbicides in soils—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (October 6)

Lecturer (preferably with special qualifications in symbolic logic) in Philosophy at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, October 8)

Lecturer (with a good honours and preferably a higher degree, and a special interest in structural geology, mineralogy and petrology) in Geology—The Secretary, Sir John Cass College, Jewry Street, London, E C 3 (October 10)

Assistant Lecturer, Grade B (with a good honours degree in mathematics of a British university, and prepared to lecture in mathematics to the level of first year degree or Diploma in Technology in engineering and science) in Mathematics—The Clerk to the Governors, Woolwich Polytechnic, London, S E 18 (October 12)

Computing Assistant (preferably with a knowledge of physics and mathematics to degree level) to assist in measurement and analysis of results obtained in optical maser experiments—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (October 12)

Departmental Graduate Assistant (with a degree in physics or electrical engineering, or a comparable qualification, and special training in electronics) in the Department of Chemistry, to help in the design and construction of electronic equipment to be used in gamma-ray spectrometry as applied to an investigation of the Mossbauer Effect—The Registrar, King's College (University of Durham), Newcastle upon Tyne, 1 (October 12)

Research Assistant (preferably with a good honours degree in engineering or physics, and preferably some experience in automatic control) in the Department of Mechanical and Marine Engineering for duties which will include research on control systems—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne, 1 (October 12)

Lecturer or Demonstrator in Chemical Pathology—The Registrar, The University, Leeds, 2 (October 13)

Lecturer (preferably with a degree in mathematics or science, a professional qualification in education and some teaching experience in schools) in Education—The Secretary, The Queen's University, Belfast, N Ireland (October 13)

Lecturer in Applied and Industrial Microbiology in the Department of Chemical Engineering—The Secretary, University College, Gower Street, London, W C 1 (October 15)

Lecturer in Education at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, October 15)

Reyrolle Student in Electrical Power Engineering—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne (October 15)

Senior Lecturer in Geology (Petrology) at the University of Natal, Durban South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, October 15)

Manchester Royal Botanical Society Lecturer in Horticulture—The Registrar, The University, Manchester 13 (October 19)

Official Fellow and Tutor in Mathematics—The President, Magdalen College, Oxford (October 22)

Lecturer in Genetics in the Departments of Botany and Zoology in the Faculty of Science—The Registrar, The University, Manchester, 13 (October 26)

LECTURER (with a wide interest in the subject so as to be able to lecture to students of both departments) IN GENETICS IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY IN THE FACULTY OF SCIENCE—The Registrar, The University, Manchester, 13 (October 26)

READER (with a Ph D in physics or a research record of equivalent level, and preferably teaching experience, or experience in leading research workers) IN PHYSICS at the University of Queensland, Brisbane—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Brisbane and London, October 27)

SECOND CHAIR IN EDUCATION (qualifications in educational psychology or experimental techniques in education desirable but not essential)—The Secretary and Registrar, The University, Southampton (October 27)

LECTURER (preferably with qualifications in clinical and abnormal psychology) IN PSYCHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London S W 1 (Australia and London, October 29)

LECTURER (with appropriate qualifications in experimental psychology or physiology and preferably research experience in a relevant field) IN THE DEPARTMENT OF PSYCHOLOGY—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W C 1 (October 30)

LECTURER or ASSISTANT LECTURER IN MEDICINE, LECTURERS (2) IN SURGERY, and a LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Sauare, London, W C 1 (October 31)

LECTURER or ASSISTANT LECTURER (preferably with a special interest or experience in automatic control or in dynamics) IN MECHANICAL ENGINEERING—The Registrar, The University, Leeds, 2 (October 31)

LECTURER or ASSISTANT LECTURER (medically or non-medically or veterinary qualified candidate) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref CV/179 (November 17)

LECTURER IN PURE MATHEMATICS at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 19)

ACAROLOGIST (with a science degree) at the National History Museum, Adelaide, South Australia, for the care and control of collections of acarina and original research thereon, and routine activities relative to other arachnida—The Agent-General for South Australia, Strand, London, W C 2

ASSISTANT EXPERIMENTAL OFFICER (preferably aged over 22, with a pass degree in physics or an equivalent qualification) for measurement of physical properties of food stuffs—The Secretary, Low Temperature Research Station, Agricultural Research Council, Downing Street, Cambridge

BIOCHEMIST (graduate), for research on gastric proteinases in man—Mr J G Murray, Department of Surgery, King's College Hospital Medical School, Denmark Hill, London, S E 5

BIOCHEMIST (non-medical) for the Group Laboratory, Guildford—The Hospital Secretary, St Luke's Hospital, Guildford, Surrey

BIOLOGIST, to participate in radiopathological research—The Director, Experimental Radiopathology Research Unit Hammersmith Hospital, Ducane Road, London, W 12

HEAD OF THE MATHEMATICAL DEPARTMENT—The Rector, Glasgow Academy, Glasgow, W 2

ELECTRONIC ENGINEER (with experience of constructional and measurement techniques in the UHF band), to assist development of interesting new scientific apparatus for fundamental research—Prof A Charlesby, Royal Military College of Science, Shrivenham, Swindon, Wilts

LABORATORY TECHNICIAN (with two passes in an experimental scientific subject at advanced level G C E or Associateship of I M L T, or equivalent qualifications) to assist in biochemical research on the biosynthesis of proteins—Dr P N Campbell, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W 1

LECTURER OR ASSISTANT LECTURER IN PHYSICS at the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1

POSTDOCTORAL BIOCHEMIST or CHEMIST, to work with a group on the kinetics and mechanism of action of proteolytic enzymes—Dr D T Elmore, Queen's University, Belfast, N Ireland

RESEARCH ASSISTANT (honours graduate in physics, preferably with some initial knowledge of electronic circuits), to take part in investigations of cosmic rays, using counting techniques for high energy particles—Head of the Department of Physics, Northern Polytechnic, Holloway Road, London, N 7

RESEARCH BURSARY or a RESEARCH ASSISTANTSHIP IN THE CHEMISTRY DEPARTMENT, for work on diffusion and sorption of vapours in polymer films—Dr J A Barrie, Chemistry Department, Imperial College of Science and Technology, London, S W 7

TECHNICAL ASSISTANT (preferably with a knowledge of photography), for research work in the Department of Electron-microscopy at Maida Vale Hospital—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W C 1

TECHNICIAN at Pollards Wood Research Station, Chalfont St Giles Bucks, for work on tissue culture—The Secretary, Institute of Cancer Research—Royal Cancer Hospital, 34 Sumner Place, London, S W 7, quoting Ref 301/B/111

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

General Register Office Studies on Medical and Population Subjects No 14 Morbidity Statistics from General Practice Vol III (Disease in General Practice) By the Research Committee of the Council of the College of General Practitioners Pp v + 144 (London H M Stationery Office, 1962) 16*s* 6*d* net [38

London and Home Counties Regional Advisory Council for Technological Education *Bulletin of Special Courses in Higher Technology* including Management Studies and Commerce 1962–63, Part I Autumn Term Pp 113 (London London and Home Counties Regional Advisory Council for Technological Education, 1962) 3*s* 6*d* [3

Ministry of Education General Studies in Technical Colleges Pp ii + 23 (London H M Stationery Office, 1962) 2*s* net [7

Committee of Privy Council for Medical Research Report of th Medical Research Council for the year 1960–1961 (Cmnd 1783) Pp viii + 372 (London H M Stationery Office, 1962) 21*s* net [78

Durham University Appointments Board Sixteenth Annual Report 1960–61 Pp 12 (Durham The University Appointments Board, 1962) [78

Building Research Station Digest (Second Series) No 25 Lightweight Aggregate Concretes—Materials and Properties Pp 4 (London H M Stationery Office, 1962) 4*d* [78

Petroleum Chemistry U S S R, Vol 1, No 1, 1962 Translation Editor D L Samuel Published quarterly Annual subscription rate £30 (85 dollars) Pp 1–218 (London and New York Pergamon Press, 1962) [178

Other Countries

United States Department of the Interior Fish and Wildlife Service Bureau of Commercial Fisheries Fishery Bulletin No 201 Studies on *Parorchis acanthus* (Trematoda Digenea) as a Biological Control for the Southern Oyster Drill, *Thais haemastoma* By Nelson R Cooley Pp iv + 77–91 (Washington, D C Government Printing Office, 1962) 20 cents [78

Proceedings of the California Academy of Sciences Fourth Series Vol 32, No 2 Scientific Results of the Galápagos-Expedition 1953–54 of the International Institute for Submarine Research, Vaduz, (Leichtenstein), Leader Dr Hans Haas Corals from the Galápagos and Cocos Islands By J Wyatt Durham Pp 41–56 Vol 32, No 3 Mating Behavior and Activity-Rest Periodicity in *Protoclythia californica* (Diptera Platypeziade) By Edward L Kessel and Berta B Kessel Pp 57–58 Vol 32, No 4 Two Gnaphosid Spiders from Arkansas By Harriet Exline Pp 79–85 Vol 32, No 5 Distinctive "Congrid Type" Fish Otoliths from the Lower Tertiary of the Gulf Coast (Pisces Anguilliformes) By Don L Frizzell and C Kurt Lamber Pp 87–101 Vol 32, No 6 The *Herpetology of Nepal*—a History, Check List and Zoogeographical Analysis of the Herpetofauna By Lawrence W Swan and Alan E Leviton Pp 103–147 (San Francisco California Academy of Sciences, 1962) [78

Mental Health Book Review Index, Vol 7, 1962 (Whole No 12) Compiled by the Editorial Committee and Contributing Librarians Sponsored by the World Federation for Mental Health and others Pp xiii + 66 Annual subscription 3 dollars (Flushing, N Y Miss Lois Afflerback, Paul Klapper Library, Queen's College, 1962) [78

Stanford Ichthyological Bulletin, Vol 8, No 1 The Osteology of *Brycon meeki*, a Generalized Characid Fish, with an Osteological Definition of the Family By Stanley H Weitzman Pp 77 (Stanford, Calif Division of Systematic Biology, Stanford University, 1962) [78

University of California Publications in Public Health, Vol 4 Epidemiology of the Arthropod-borne Viral Encephalitides in Kern County, California, 1943–1952 By W C Reeves and W McD Hammon, in collaboration with W A Longshore, Jr, H E McClure and A F Geib Pp viii + 257 (Berkeley and Los Angeles University of California Press, London Cambridge University Press, 1962) 6 dollars [78

Food and Agriculture Organization of the United Nations FAO Agricultural Studies No 54 The Control of Ticks on Livestock Prepared by S F Barnett Pp vii + 115 (Rome Food and Agriculture Organization of the United Nations, London H M Stationery Office, 1961) 5*s*, 1 dollar [78

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CERN, European Organization for Nuclear Research Annual Report for 1961 Pp 153 (Geneva CERN, 1962) [78

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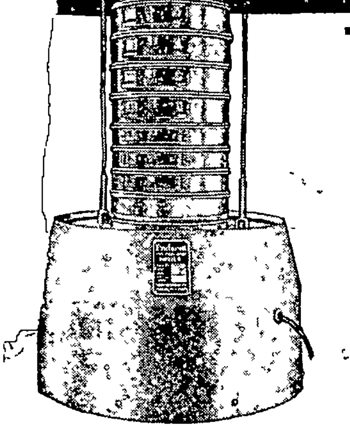

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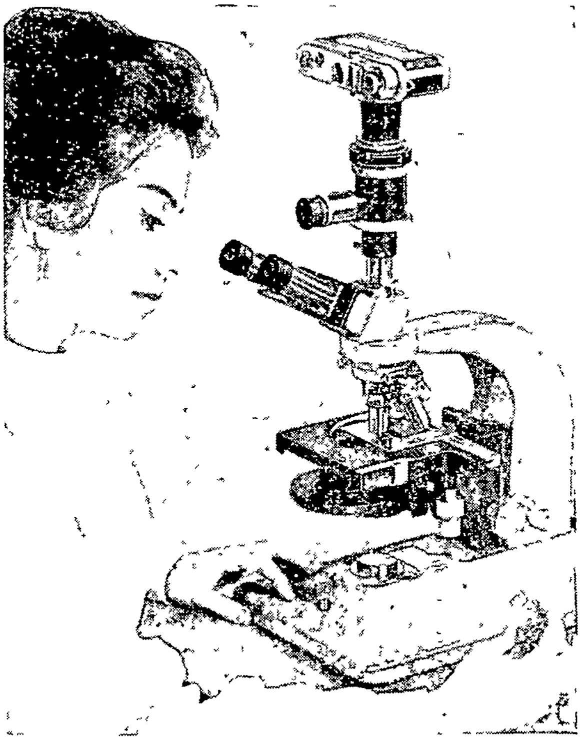

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